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**Abstract**

The normal mitotic dog kidney cell division and the multinucleated giant cell formation and degeneration of the dog kidney cells infected with measles virus were observed by the phase-cinematography. It took only five minutes for the mitotic cell division. The cell assumed a spherical shape before mitosis, and the two divided cells grew to the flat cells on the bottle wall. The giant cell formation was definitely the result of cell fusion. The cellular contents of the multinucleated giant cell were exposed after buddings, and the cell itself died.

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# PHASE CINEMATOGRAPHIC OBSERVATION ON THE MULTINUCLEATED GIANT CELLS INFECTED WITH MEASLES VIRUS

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In 1954 ENDERS and PEEBLES[1] isolated measles virus that always shows cytopathogenic effects (CPE) on cultures of human embryonic and monkey kidney cells and it is neutralized by convalescent sera of measles patients. Thus they have established a tissue culture method of measles virus. Since then various kinds of culture cells have been observed of their sensitivity to measles virus and it has been indicated that the appearance of multinucleated giant cell is one of the morphologic changes specific to measles virus infection.

As for the mechanism of the formation of multinucleated giant cell in the virus infection there seem to be two possible ways, i. e. as in the case of giant cell formation in other causes, one is the result of repeated mitosis without being accompanied by cell division or abnormal karyokinesis and the other is the fusion of cytoplasm of individual cells. Therefore, the author has tried to decide which way is actually taken for the giant cell formation in the case of measles virus infection. In general the cultured host cells show a monolayer cell sheet and have no specific space surrounding the cells while the multinucleated gaint cells formed after the virus infection always show a certain space around the cell. This fact seems to suggest that the cell fusion may occur with the infected cells located at this region.

In an attempt to clarify this mechanism and the behaviors of the multinucleated giant cells infected with measles virus the phase-cinematographic observations were done, and the following results were obtained.

## MATERIALS AND METHODS

The cultured cells used were the dog kidney cells isolated by the author and the measles virus was Edmonston strain being passed successively in the dog kidney cells. At first, these cells were cultured for 2—3 days in a flat culture bottle of 15 ml in volume at 37°C, and after confirming the formation of suitable monolayer cell sheet in the YLE medium containing 20 per cent calf

serum, the cells were infected with measles virus 1,000 to 10,000 $TCID_{50}$ measles virus. While being incubated at 37°C, serial cinematographs of a frame for 30 seconds are taken for 7 consecutive days.

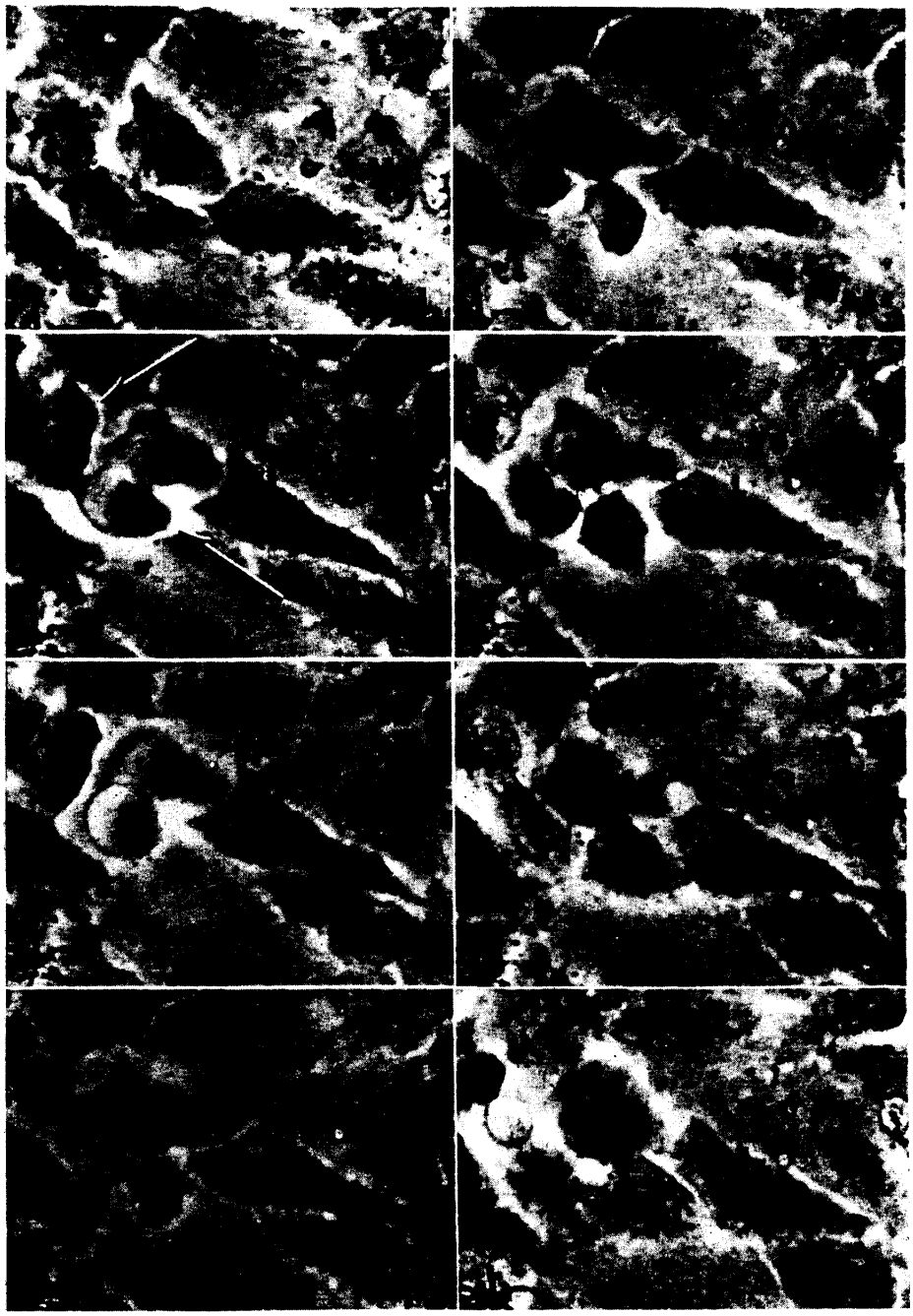
## RESULTS

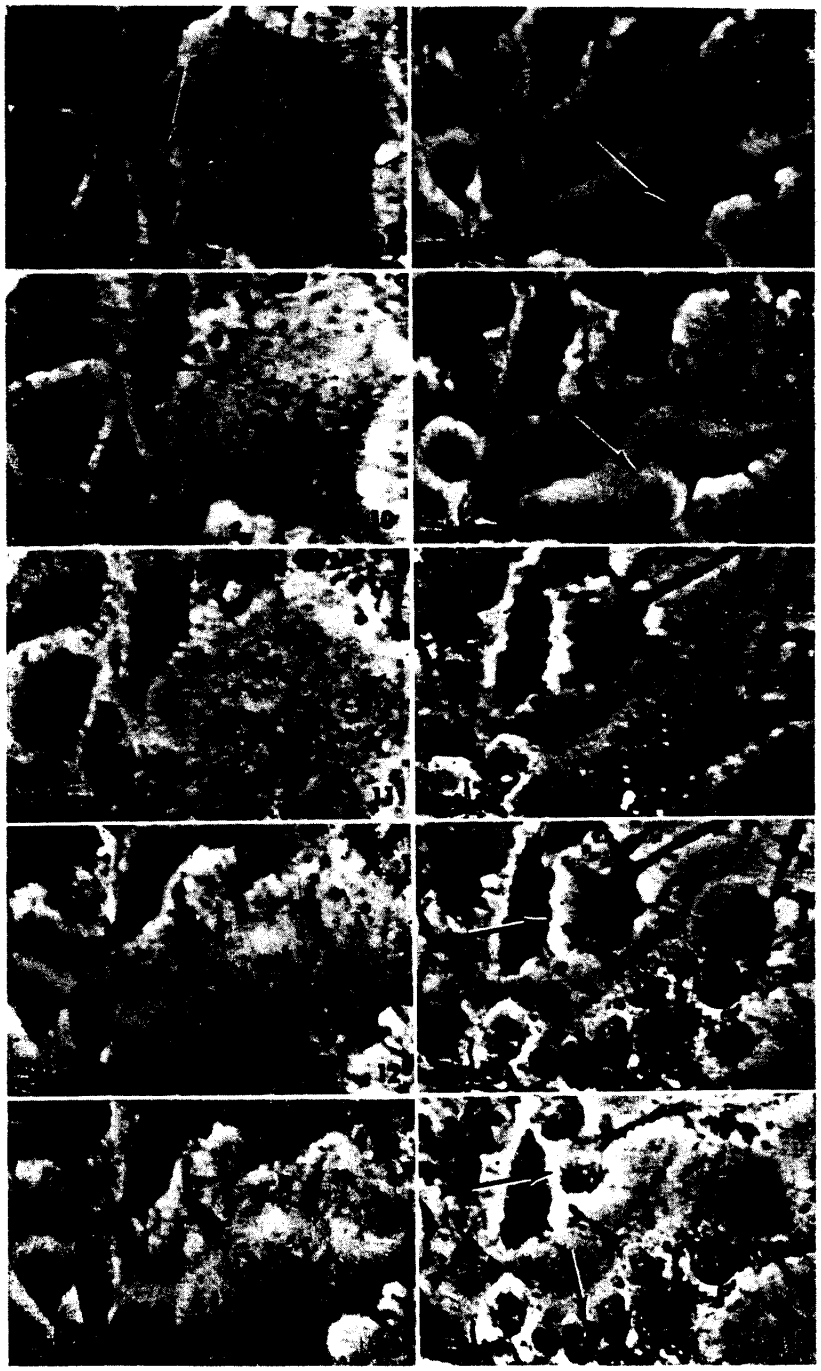
When 4 ml of the dog kidney cell suspension containing $10-15\times10^4$ cells in 1ml of YLE medium are added in a culture bottle and incubated at 37°C, 24 hours after incubation the picture as illustrated in Fig. 1 is obtained. All the cells are epithelial cells, and they are of polygonal, rather round cells in close contact with each other, being arranged like pavement stones. The cytoplasm of these cells reveals a relatively big volume as compared with the nucleus. Their nuclei are usually oval and located in the center of the cell, having one or several nucleoli. Generally the cytoplasm appears rather transparent under the light microscope but under the phase-contrast microscope it contains many spherical granules.

As illustrated in Fig. 2 and subsequent figures the processes of mitotic division and multiplication of these normal dog kidney cells can be observed. In Fig. 2 which is the picture taken five minutes after that in Fig. 1 those cells pointed out by arrows gradually contract themselves and become spherical. Later the cells assume a spherical shape and show no longer the nucleus and other intracellular structures (Fig. 3). There now appear spaces between the adjacent cells and the focus here is not sharp owing to the cells projecting into the culture medium away from the culture vessel wall. In Fig. 4, although the cell is still connected by cytoplasm, it is practically divided into two, and in Fig. 5 the cell is completely divided. By the picture of Fig. 6 the cells have divided completely into two, each of which adheres to the bottle wall and grows to a flat cell. By the time of the picture in Fig. 7 each divided cell shows its nucleus again, and in Fig. 8 the cell matures to the same extent as other cells and they now constitute monolayer cell sheet. The mitotic time of cell division in Figs. 2—8 was nine minutes and thirty seconds.

These monolayer cell sheet are used for the experiment of the phase-cinematographic observation on the multinucleated giant cells infected with measles virus. Further on the morphological changes of multinucleated giant cell infected with virus as shown in Figs. 9—18 are observed. In Fig. 9 is presented a picture of the cell on the fourth day after inoculation, and on the right side of

Figs. 1~8 show the mitotic cell division of the normal dog kidney cells. The cells (arrows in Fig. 2) have projected into the culture medium away from the culture bottle wall before division, and then the cell have divided into two, each of which adhered to the bottle wall and grew to a flat cell.





the picture there is a giant cell with several nuclei, and there is a mononuculeated cell (arrow) on its left side. However, between these two cells there is still a space, proving those to be two independent cells. Later on this cell gradually approaches the giant cell (Fig. 10) as to have contact between the cytoplasm, and further on the nucleus of this mononucleated cell is absorbed into the giant cell (Fig. 11). Now the giant cell has one extra nucleus as shown in Fig. 12. By the fifth day after inoculation, each cell grows in bulk presenting a globular shape and the entire cell body begins to contract (Fig. 13). With further lapse of time buddings of cytoplasm (arrow) occur repeatedly at several places within a short period of time (Fig. 14). When these buddings or broken (Fig. 15) cellular contents are exposed, the giant cell itself dies. Ultimately there can be recognized some nuclei thrown out in the debris of dead cell (Figs. 16, 17). The dense material that surrounds the nuclei in the dead giant cell is found to be eosinophilic cytoplasmic inclusion bodies (arrow) by the staining with hematoxylin-eosin solution (Fig. 18). These dead multinucleated giant cells present the same view of morphology both in Figs. 17 and 18, but other mononucleated cells reveal a variety of shape (arrows), signifying that they are still alive.

This is the life of the multinucleated giant cell infected with measles virus.

## DISCUSSION

The behaviors of the mitotic cell division of normal dog kidney cells and the multinucleated giant cell formation which is one of the specific CPE of the dog kidney cells infected with measles virus were observed by the phase-cinematography. There are reports of the phase-cinematographic observation of mitotic cell division on the human fibroblasts by ITO (1959)[2] and on the chick fibroblasts by KAWARAI and ISETANI (1959)[3], but there is no report of the living cell division of the dog kidney cells. In the experiments with human amnion cells FRANKEL *et al.* (1958)[4] recognized the diminution in the multinucleated giant cell formation by adding glutamine to the culture medium. ODDO *et al.* (1961)[5] likewise observed an identical phenomenon with HeLa cells and KB cells in culture, and by changing infection titers of the inoculum they found that CPE could be altered somewhat but when the cultivations were successively continued, CPE would return completely to the original level. In other words, a generalization may be made on the time interval relationship of the onset of

Figs. 9~18 show the morphological changes of multinucleated giant cell infected with measles virus. Mononucleated cell (arrow in Fig. 9) was adsorbed into the giant cell, and the giant cell had one extra nucleus. The cellular contents were exposed after buddings (arrows in Figs. 14 and 15), and the giant cell dead (arrows in Figs. 17 and 18).

CPE because it always differs according to experimental conditions, but no definitive conclusion can be drawn. On the report by ROIZMAN (1962)[6] altered membranes of the infected cell fuses with the membranes of uninfected cells giving rise to a propolykaryocyte. It has been possible to verify that the formation time of multincleated giant cell with measles virus is due to the experimental conditions and the mechanism of formation of the multinucleated giant cell can readily be understood by the cinematographic observations, proving that the multinucleated giant cell formation is definitely the result of cell fusion.

## SUMMARY

The normal mitotic dog kidney cell division and the multinucleated giant cell formation and degeneration of the dog kidney cells infected with measles virus were observed by the phase-cinematography.

It took only five minutes for the mitotic cell division. The cell assumed a spherical shape before mitosis, and the two divided cells grew to the flat cells on the bottle wall. The giant cell formation was definitely the result of cell fusion. The cellular contents of the multinucleated giant cell were exposed after buddings, and the cell itself died.

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