ACUTE DESTRUCTION OF INFECTED CELLS AS SHOWN BY MICROFILMING

(UDC 616-018.1-022.1-003.8-091.18)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 6,
pp. 111-113, June, 1966
Original article submitted November 28, 1964

By means of microfilming it is possible to make a vital study of the behavior of cells infected by a virus from the moment of infection until death, and thus to judge the morphological expression of the process of interaction between viruses and cells. The possibility of the repeated reproduction of the process under investigation, speeded up tens and hundreds of times, makes this an ideal method for the study of the dynamics of the cytopathic action of viruses. Different types of reactions of cells to the action of cytopathogenic viruses can be conventionally subdivided into acute (total), subacute (subtotal), and focal destruction [1, 4].

In the present investigation, using a microfilming method, the morphogenesis of the cytopathogenic action of Venezuelan equine encephalomyelitis (VEE) virus causing acute destruction of cells was investigated.

EXPERIMENTAL METHOD

The strain of VEE virus used had been put through two passages in mice and 18 passages in chick embryonic fibroblasts.

Used were primarily trypsinized cultures of skin and muscle tissue of human, mouse, and chick embryos, kidney cells of monkeys and cows, and transplantable cultures of HeLa, SOTs, KV, L (mouse fibroblasts), APO, KEM-1, RES, A-1 (human embryonic amnion), PKB, and CA-SV_{40}-63-1 (a line obtained from tumors of Syrian hamsters caused by virus SV_{40} [2]). The cells were grown in a Milyutin's glass chamber, and immediately after infection with a massive dose of VEE virus (5-10 PFU per cell) photographs were taken with the MKU-1 microcamera under phase contrast, with a speed of 1 frame in 12-20 sec, so that projection gave an acceleration of the process of approximately 300-500 times.

EXPERIMENTAL RESULTS

The frames of one of the experiments of time-lag microfilming of HeLa cells infected with VEE virus are illustrated in Fig. 1, a-f. The photographs were taken for 20 h until destruction of the population of cells was complete.

During the first 3-4 h after infection no changes were observed other than a slight increase in the clarity of the outlines of the cells and nuclei (Fig. 1a). Characteristic rotary movements of the nuclei and nucleoli and an active displacement of the dark and light cytoplasmic granules, evidently lipid inclusions, mitochondria, and pinocytotic vesicles, were seen on the screen. By the 6th-8th hour death of the cells began to take place, and the number of dead cells progressively increased (Fig. 1a, b). Death was preceded by some increase in the number of cytoplasmic granules, slowing of the movement of the nuclei, vacuolation of the cytoplasm and, as a rule, pycnosis of the nuclei. The process ended with the rounding off of the cytoplasm and with the rapid shrinking (contraction) of the cells, which lost all the structural differences between the nucleus and the cytoplasm and began to refract light sharply (Fig. 1, b-f).

In the chosen experiment a rare phenomenon was found. One of the cells divided between the 6th and 7th hour after contact with VEE virus (in Fig. 1b it can be seen in the metaphase stage), and the daughter cells died immediately after their formation (Fig. 1c).

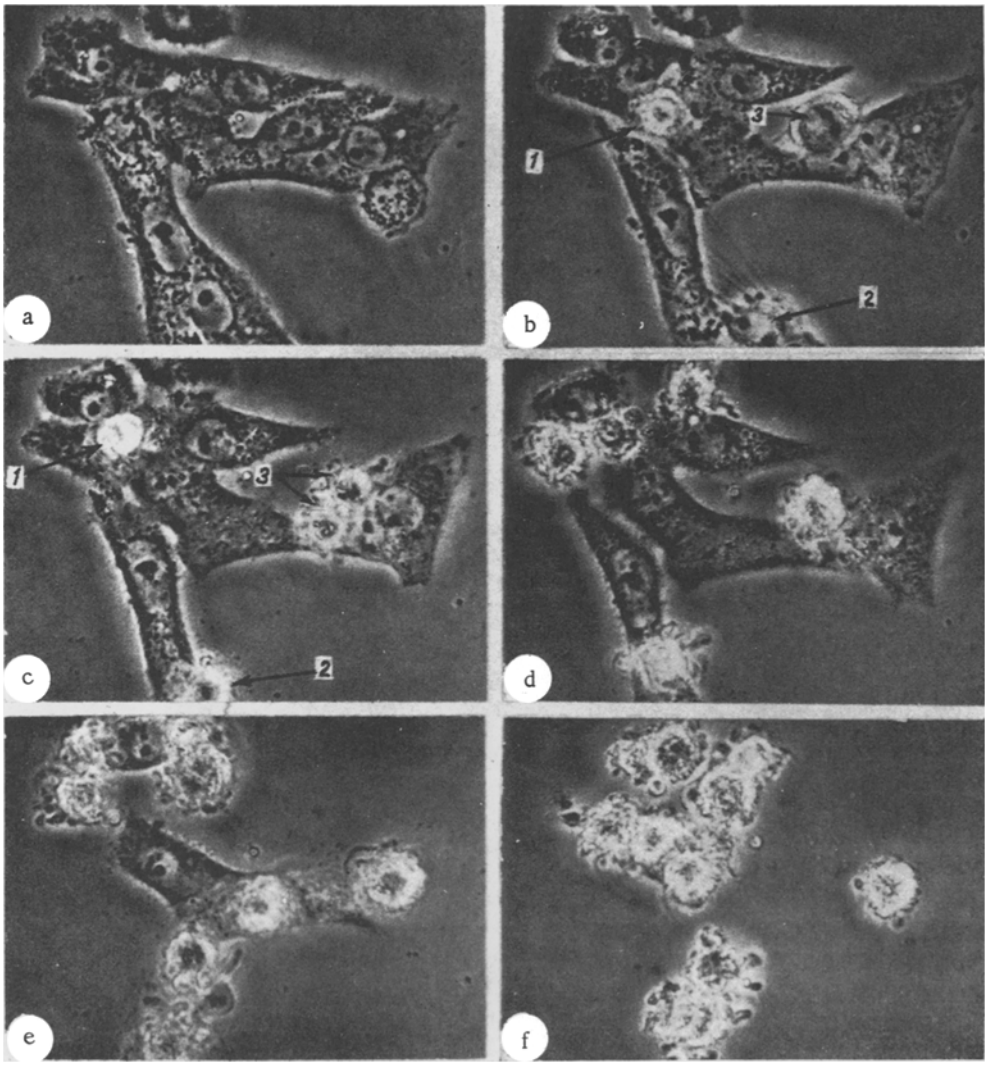

Fig. 1. Frames of time-lag microfilms of HeLa cells (72-h growth) infected with VEE. a) 4 h after infection. Cells unchanged; b) 6 h after infection. First signs of destruction: 1) a dead cell, 2) onset of death of a cell (rounding off), thin fibrils are clearly seen, 3) dividing cell (stage of metaphase); c) 8 h after infection. 1, 2, 3) dead cells; d) 12 h after infection. Increase in number of dead cells; e) 16 h after infection; f) 20 h after. Total destruction of infected cells.

Where the cells had died a mass of amorphous debris remained for a long time, attached to the glass by fibrils of cytoplasm.

The process of acute destruction of HeLa cells described above was also observed after infection of all the other tested primary and transplanted tissue cultures with VEE virus. Some slight individual variations were noted, associated with the origin and character of growth of the culture. For instance, after infection of the cells of the transplanted cultures, in some cases a sluggish process of syncytium formation took place, based on destruction of the cell walls and fusion of the cytoplasm of neighboring cells into a single mass.

After infection of a monolayer of chick embryonic fibroblasts (Fig. 2a), death of the cells was accompanied by a very characteristic process of attrition of the layer, with the formation of many empty spaces (windows). The dead cells were connected by long fibrils of cytoplasm, forming a type of reticulum (Fig. 2b).

At the periphery of the dying cells of the human embryonic amnion (A-1), transparent vesiculiform protrusions were formed as a rule from the cytoplasm, and sometimes they attained a considerable size (Fig. 2d).

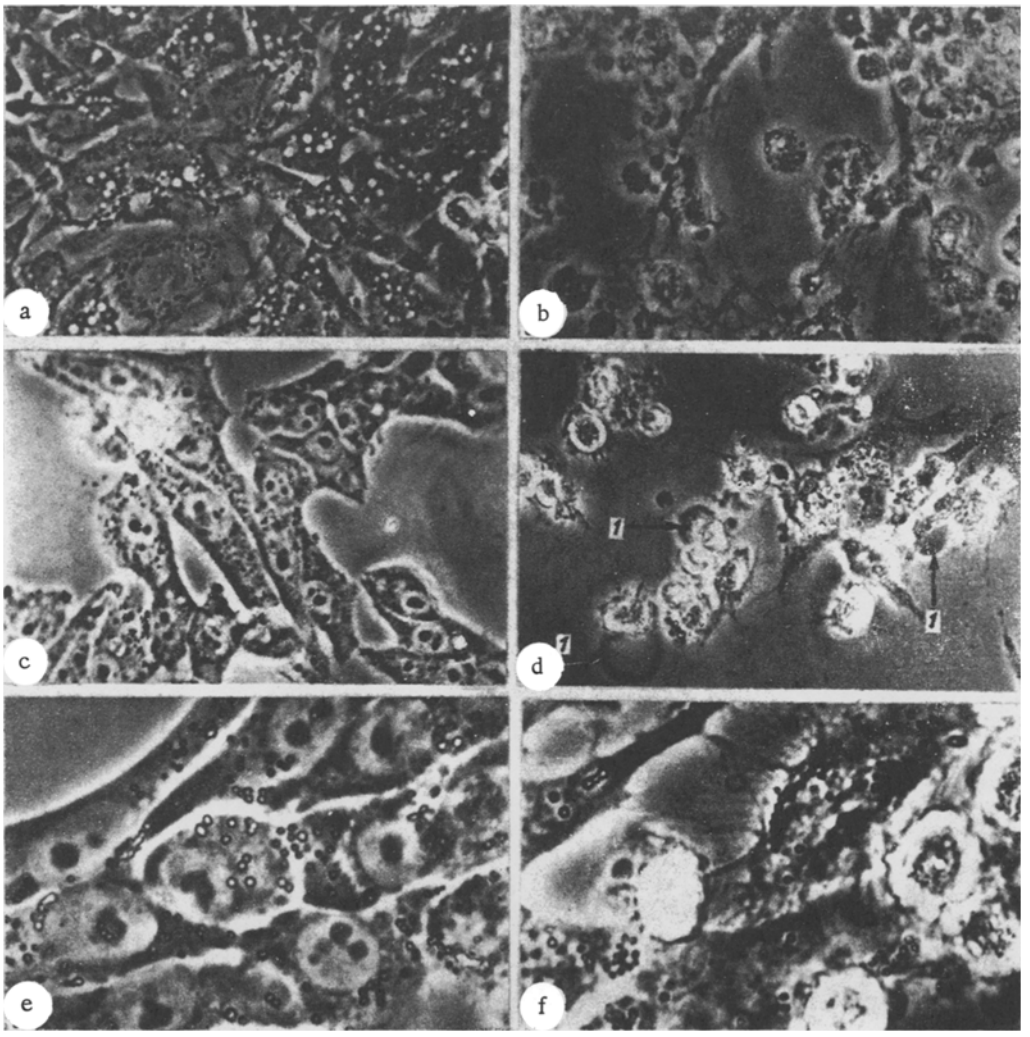

Fig. 2. Destruction of different types of cells infected with VEE virus. a-f) Chick embryonic fibroblasts (20-h growth): a) immediately after infection; b) the same cells 21 h later. Acute destruction, characteristic reticular structure; c, d) A-1 cells (human embryonic amnion), 48-h growth; c) immediately after contact with virus; d) the same cells 33 h later. 1) Vesicular invaginations of the cytoplasm; e, f) SOTs cells (4-day growth). Magnification 900×; e) immediately after infection. The cytoplasmic granules are clearly visible; f) 25 h after. Shrunken and "fixed" cells, "agglutinates," attenuation of layer.

Following massive infection of the SOTs and L cells, only some of the infected cells (about 30-40%) became rounded, while in the remainder the movement of the nuclei and displacement of the ectoplasm ceased, the cytoplasmic granules were crowded together at the periphery of the cells, and characteristic "agglutinates" were formed (Fig. 2e, f). The cells began to look like fixed preparations, preserving their typical morphology for a long time.

In all the cultures studied, the process of death of the cells usually began 6-8 h after contact with the virus and ended after 18-30 h. With a decrease in the multiplicity of infection, death and destruction of the infected cells stretched over a period of 48 h. It may be emphasized, in particular, that after infection with VEE virus in every case the mitotic activity was appreciably reduced—in some cases mitosis ceased completely.

The results of the comparative microfilm study of the principles governing the cytopathogenic action of VEE virus revealed the uniform character of death of the cells of 15 different primary and transplantable tissue cultures. In every case a process for acute destruction and disintegration of the infected cells was observed, characterized by the slowing or even the complete stopping of the characteristic rotary movements of the nuclei, by vacuolation of the cytoplasm, and by the subsequent rounding and rapid shrinking of the cells. With a massive infecting dose the process of total death occupied 18-30 h in the different cultures.

The authors showed earlier that the cycle of proliferation of VEE occupies 6-8 h [3]. It follows from the findings described above that the onset of death of the cells of the various tissue cultures coincides with the end of the cycle of proliferation of the virus, and after destruction of all the cells of the population a halt was found in the further growth of the titer of VEE virus. This was evidently associated with the cessation of synthesis of the newly formed virus in the dead cells.

LITERATURE CITED

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