THE SYMPLAST-FORMING ACTIVITY OF THE VIRUS OF VENEZUELAN EQUINE ENCEPHALOMYELITIS

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About twenty viruses are known possessing the property of forming symplasts following infection of cells of a number of primary and transplantable tissue cultures. These viruses belong to different systematic groups (myxoviruses, herpes viruses, pox viruses, and so on), but they are similar to each other in their mode of proliferation, as a rule they possess a membrane, and they contain large quantities of lipids.

The ideal method of determining the mechanism of symplast formation is by microfilming, which enables the dynamics of the process to be reproduced repeatedly and its features studied. The use of this method has shown that the formation of symplasts during the action of viruses is associated with destruction of the cell walls and subsequent fusion of the cells, or else they appear as a result of amitosis unaccompanied by plasmotomy [3-6].

In the present investigation microfilming, together with certain cytochemical investigations, was used to study the symplast-forming ability of a member of the group A arborviruses – Venezuelan equine encephalomyelitis virus (VEEV).

EXPERIMENTAL METHOD

The strain of VEEV that was used was obtained from the United States and put through three passages in mice in the author's laboratory and twenty-one passages in chick embryonic fibroblasts. The titer of the virus was $5 \cdot 10^{8-9}$ plaque-forming units (PFU)/ml.

Primarily trypsinized cultures of skin and muscle tissue of human and mouse embryos, kidney tissue of monkeys and cows, and transplantable HeLa, KB, HEp-2, L (mouse fibroblasts) SOTs, and A-1 (human embryonic amnion) cells were used. The cells were grown in strips on glass slides to form a monolayer, after which they were infected with massive doses of VEEV (5-10 PFU/cell). After various intervals of time the glass slides were withdrawn, and the preparation washed, fixed, and stained with acridine orange, with phosphine 3R or immune globulin, and conjugated with fluorescein isothiocynanate. The technique of staining and of luminescence microscopy have been described elsewhere [1].

For microfilming, the cells were first grown in a Milyutin's glass chamber, and after infection, photographs were taken with a miniature camera (MKU-1) using a phase-contrast adaptor, on A-2 film with a speed of 1 frame per 12 seconds, so that during projection the process was accelerated approximately 300 times.

RESULTS

In Fig. 1a-g frames from one experiment of time-lapse photography of HeLa cells infected with a massive dose of VEEV are illustrated. It is clear that a small symplast has been formed from the cells in the right part of the frame. The first changes in the cells were observed 3-6 h after infection; at this time the borders between the cells gradually became obliterated and disappeared, leading to fusion of the cytoplasm of neighboring cells into a single mass (Fig. 1b, c). The process of symplast formation took place slowly and was not complete until after 9-12 h (Fig. 1d, e), i.e., it coincided in time with the reproductive cycle of VEEV [2]. For some time rotary movements of the

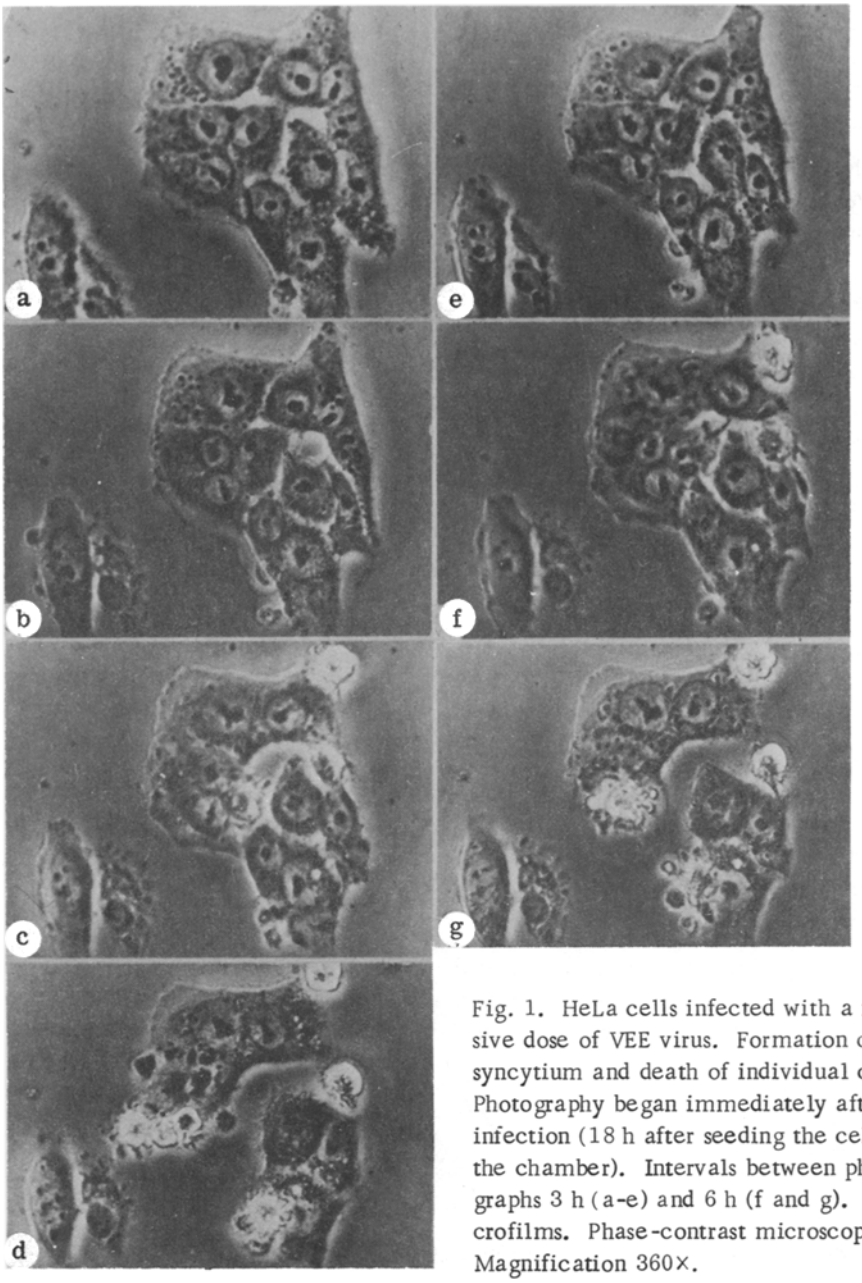

Fig. 1. HeLa cells infected with a massive dose of VEE virus. Formation of a syncytium and death of individual cells. Photography began immediately after infection (18 h after seeding the cells in the chamber). Intervals between photographs 3 h (a-e) and 6 h (f and g). Microfilms. Phase-contrast microscope. Magnification 360×.

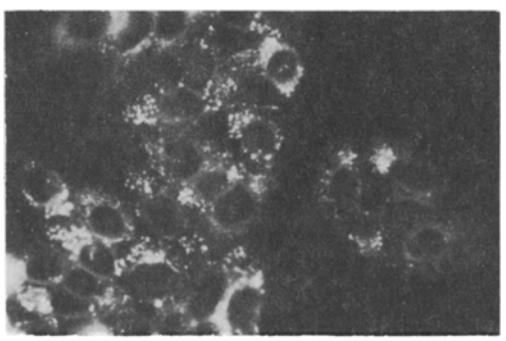

Fig. 2. HeLa cells 12 h after infection with VEE virus. Lipid granules in the cytoplasm of the syncytium. Luminescence microscopy. Fluorochromed with phosphine 3R. Magnification 240×.

nuclei and active displacement of the cytoplasmic granules, characteristic of living cells, were observed for some time in the symplasts. Destructive changes developed 18-20 h after infection; retraction of the symplast took place, and dark, vesicular projections of the cytoplasm appeared at its periphery (Fig. 1f, g). Subsequently the symplast became thicker and optically denser, and finally it became detached from the glass.

At the end of the reproductive cycle of VEEV, some of the cells of the population died, and the number of dying cells progressively increased (Fig. 1e-g). At first the movements of the nuclei and of the cytoplasmic granules gradually became slower, and vacuolation of the cytoplasm and pycnosis of the nuceli developed; the process ended with the rounding and rapid contraction of the cells, leaving in their place a mass of shapeless debris which remained for a long time. After 18-24 h, most of the separate cells had died, and also part of the cells forming the symplast.

The processes of slow symplast formation and of acute destruction of the cells, described above, also took place in other transplantable tissue cultures after massive infection with VEEV. It should be emphasized in particular that only living, functionally normal cells took part in symplast formation, and this process could be delayed or prevented by immune serum added to the culture fluid immediately after infection.

Following infection of the primarily trypsinized cultures, in no case were the multinucleated structures observed to appear, but only acute destruction, characterized by the total death of all the cells of the infected culture.

As a result of histochemical investigation of the infected cells, an appreciable increase in the intensity of fluorescence of RNA in the cytoplasm of symplast could be seen after 3 h. Later, with the development of degeneration of the symplasts, the fluorescence of the RNA progressively diminished. No changes in the DNA of the nucleus could be seen during the first 10-12 h after infection.

When the cells were stained with phosphine 3R, large numbers of tiny lipid granules appeared in the symplast and also in the separate cells. They were scattered throughout the cytoplasm and clearly outlined the borders of the nuclei (Fig. 2).

By means of the method of fluorescent antibodies it was possible to determine the cytoplasmic localization of the virus antigens. This was first detected 9-12 h after infection and later it accumulated until the destructive changes began to take place in the symplasts.

No description of the symplast-forming activity of VEEV or of any other member of the arborviruses could be found in the literature. The reason for this may be that the process of slow symplast formation, evoked by the viruses of this group, is usually balanced by the acute destruction of the cells, and it may remain unnoticed. It is interesting that VEEV, like other symplast-forming viruses, contains large quantities of lipids, which probably come into contact with the cytoplasmic membrane of the cells, and may easily destroy their structure and disturb their function [7].

Cytochemical methods showed that the symplasts remain viable for 10-12 h after formation, and may continue to synthesize virus antigen.

SUMMARY

Time-lapse microcinematography and fluorescence microscopy were used to study the symplast-forming activity of the Venezuelan horse encephalomyelitis virus with regard to a number of primary and transplantable tissue cultures. Experiments revealed sluggish symplast formation in massive infection (5-10 PFU/ml) of transplantable culture cells. The formation of symplasts is based on destruction of cell walls and merging of the cytoplasm of the adjoining cells into a single mass. During 10-12 h after their formation symplasts retain their viability and may support virus reproduction. The process of symplast formation is intercurrent with acute destruction of cells, both types of the cytopathogenic action of the virus being distinctly correlated to the VEEV reproduction cycle.

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