ELECTRON-MICROSCOPIC STUDY OF DAMAGE TO CELL ORGANOIDS BY HERPETIC INFECTION IN VIVO AND IN VITRO

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Comparative electron-microscopic investigations of the cytopathology of experimental herpes in vivo (albino mice) and in vitro showed that, besides destruction, in both cases hypertrophy and hyperplasia of individual organoids of the cell took place and was accompanied at certain stages of the infection by the formation of new structures, not found in normal cells. Differences in the pattern of injury to the cell organoids were also discovered in the virus–cell systems investigated.

The diverse manifestations of the action of viruses on cells and specific lesions thus produced in the cells can be studied adequately only by comparing changes in the cells of an animal susceptible to the infection and in cells artificially cultivated in vitro and thus freed from the influence of the regulatory systems of the body (exclusion of neuro-humoral regulation, factors of immunity and inflammation, and possible changes in the sensitivity and reactivity of the cells).

The object of this investigation was to study the cytopathology of experimental herpetic encephalitis and of an infected culture of human embryonic fibroblasts with the electron microscope.

EXPERIMENTAL METHOD

Strains L-2, ELA, and Tolstova of herpes simplex virus (HSV) were used. The dose of virus for intracerebral inoculation into albino mice was 100 LD_{50}/0.03 ml, and the dose to infect a primary culture of human embryonic fibroblasts (HEF) was 0.1-1.0 LD_{50}/cell. The albino mice were sacrificed 12, 24, 48, and 72 h after infection. An incision was immediately made on the exposed brain surface in the cerebral cortex in the immediate vicinity of the hippocampus, and a small volume of fixing solution was injected through it. A piece of brain was then removed, placed on a slide in a drop of fixing solution (1% buffered solution of osmium tetroxide prepared by Palade's formula [4]) by Vanag's method [2], and cut up into thin pieces. The incisions were made along planes parallel to the transverse section through the hippocampus. The pieces of brain were fixed for 2.5-3 h at 4°C. After the end of fixation, they were washed with 30° alcohol and specimens for electron microscopy were then prepared in the usual way. The infected HEF culture was fixed at the same time with 1% osmium tetroxide solution, dehydrated in alcohols of increasing concentration, embedded in methacrylates, and cut into sections on the KB-4800 microtome. The cell sections were shadow-cast with uranyl acetate and additionally with lead monoxide [3]. The electron-microscopic specimens were examined in the JEM-5γ microscope under an accelerating voltage of 80 kV and with an instrumental magnification of 10,500-40,000×. Virus was titrated intracerebrally in albino mice and the results calculated by the method of Reed and Muench.

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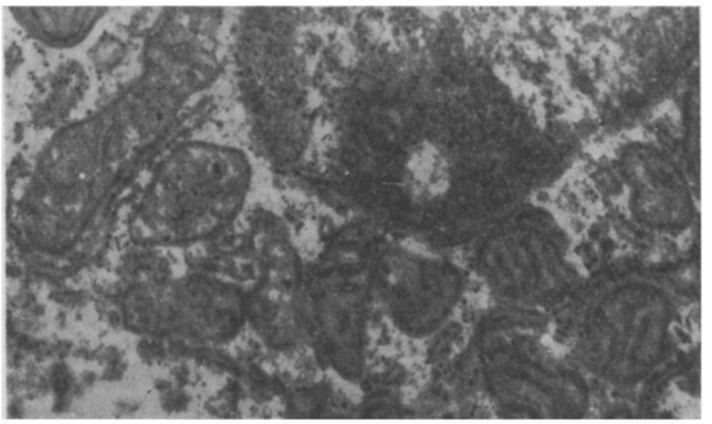

Fig. 1. Strain Tolstova HSV. Large collection of mitochondria in cytoplasm of hippocampal neuron, 52 h after infection, 50,000 ×.

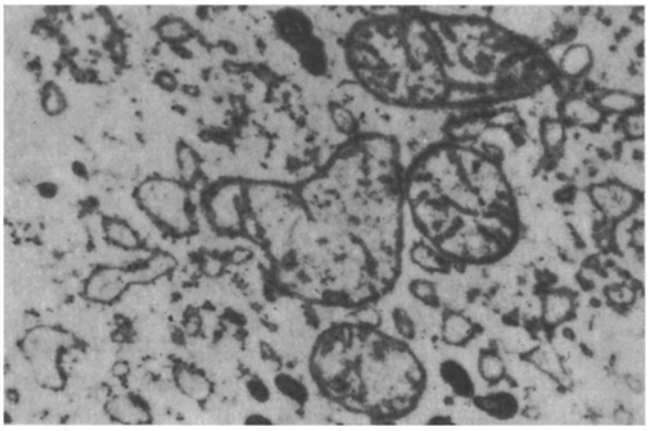

Fig. 2. Strain Tolstova HSV. Destruction of mitochondria in neuron, 72 h after infection, 60,000 ×.

EXPERIMENTAL RESULTS

Several similar morphological changes were detected in the two herpes virus–cell systems (mouse brain neurons and HEF cells). Twelve hours after infection (titer of virus in 1/log LD_{50}/0.03 ml was 1.5 in the brain and 1.0 in the HEF cells) a marked decrease in the density of chromatin was observed in the center of the nucleus, with its conglomeration and accumulation at the periphery. At this time the nuclear membrane still remained double, but the nucleoli were dense and varied in shape, and occupied their usual central position. Subsequently a marked increase in the number of mitochondria to many times the normal value was observed, followed by their swelling and destruction (Fig. 1). Fragments of cristae and DNA fibrils were irregularly arranged inside the mitochondria, and under high power of the microscope (60,000×) they appeared to be connected to the inner mitochondrial membrane (Fig. 2). A few considerably thickened cristae were observed in those mitochondria which were relatively intact, and the mitochondrial membranes were usually undamaged. In the brain tissue and cell culture infected with herpes virus the mitochondria were usually close to the nuclear membrane, and in the opinion of some investigators this is highly characteristic of certain pathological states of the cell. Other features observed were vacuolation of the cytoplasm and a marked increase in area of the perinuclear space, in which collections of membranous structures and virus particles could be seen.

In the late stages of the investigation 72 h after infection, severe deformation of the nuclei, accompanied by the formation of small syncytia, each containing four or more specifically modified (titer of the virus in the brain and HEF cells 5.1 and 5.3 respectively), was found in the hippocampal neurons from

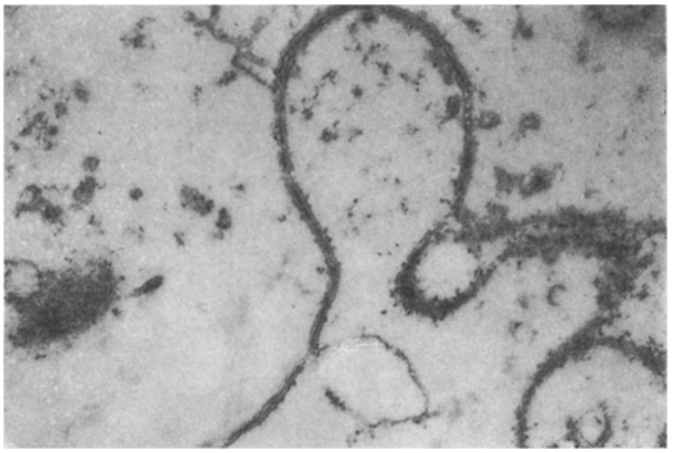

Fig. 3. Strain ELA of HSV. HEF culture. Bulging and deformation of nuclear membrane, 12,000×.

mice with the clinical picture of the disease and at the height of cytopathic action in the cell culture. Differences in the cytopathic picture of experimental herpetic infection were also detected in vivo and in vitro. For example, marked deformation and destruction of the nucleoli and the formation of numerous intranuclear specific inclusions and of syncytia were frequently observed in the HEF culture. In the brain tissue these changes were found less constantly, although morphological structure similar to inclusions could be seen under the electron microscope in the nuclei of the neurons. Because these structures are difficult to interpret, their further study is essential.

Particularly characteristic features of the course of infection in the HEF cell culture, distinguishing it from infection of neurons in herpetic encephalitis, were intensive proliferation of membranous structures with bulging, proliferation, and reduplication of the nuclear membrane (Fig. 3) and the appearance of "concentric membranes," consisting of several layers of electron-dense material resembling the section through an onion or a fingerprint. These structures are composed of the intensively proliferating inner nuclear membrane. However, some workers [5] consider that they are produced by proliferation of the ergastoplasm. Sometimes the impression of proliferation of all membranous structures of the cytoplasm was created. Frequently, in the early stages of infection, very long and very curiously shaped mitochondria were found. This was evidently the state preceding division and reproduction of the mitochondria.

In both HSV–cell systems studied, not only destruction was thus observed, but also hypertrophy and hyperplasia of individual cell organoids and, at certain stages of infection, the formation of new structures not found in normal cells. Further changes in the cell ultrastructures were similar in experimental herpes in vivo and in vitro, but certain differences in the picture of the cytopathic action of the virus in these two systems also were observed. Besides special features distinguishing the infected systems, another cause of these differences was the multiplicity of infection, because a dose of 0.1-1.0 LD_{50} of virus per cell was required on the average to infect the human embryonic fibroblasts, whereas the quantity of virus per brain tissue cell, containing billions of neurons, was much smaller. Another point to be noted was that, whereas the action of the virus in tissue culture is more polytropic, i.e., it has a cytopathic action on cells of many different tissues [1], in animals and man in vivo sensitivity to the action of the virus is strictly limited to certain cells in which the changes characteristic of virus infection can be detected.

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