CYTOCHEMISTRY OF HERPETIC INFECTION OF A CULTURE OF HUMAN EMBRYONIC FIBROBLASTS

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UDC 616.523-053.13-008.9-092.18

Stimulation of DNA activity in the cell nuclei, an increase in the RNA content in the nucleoli in the early stages of infection, and a decrease in cytoplasmic RNA activity in the late stage are observed in a culture of human embryonic fibroblasts infected with herpes virus. The glycogen content in the cytoplasm of the cells is reduced, and fatty infiltration of the cells is observed.

The results of studies of the metabolism of cells infected with herpes virus described in the literature are contradictory [1, 2, 8, 13].

The object of the present investigation was to study the cytochemistry of nucleic acids and of glycogen and lipids in a cell culture infected with herpes virus.

EXPERIMENTAL METHOD

The Tolstoi strain of herpes simplex virus used in the investigation was kindly supplied by T. M. Maevskaya [1].

A primary culture of human embryonic fibroblasts was infected with cerebral varieties of viruses in doses of 0.1-1.0 LD_{50} for albino mice per cell. After intervals of 3, 6, 12, 24, 48, 72, and 96 h the cover slips with the culture were removed, fixed in Carnoy's mixture and in formalin, and the following reactions were then carried out: Feulgen for DNA, Brachet for DNA and RNA, McManus for lipids [4], and Shabadash for glycogen [6]. At the same times specimens were stained with aniline dyes by Unna's method, and the dynamics of reproduction of the virus was studied by the methods of fluorescent antibodies and biological titration. The specific globulin fraction was labeled by the method of Skvaril and Tranger [5]. At each time of investigation an appropriate number of specimens from uninfected cultures also was studied.

EXPERIMENTAL RESULTS

The first pathological changes were observed 6 h after addition of the virus, and they consisted of an increase in the intensity of the oxyphilic granulation of the nuclei and in the pyroninophilia of the cytoplasm, more especially in syncytia.

Later (24-48 h), deformation of the nuclei was observed, with the formation of large clumps of chromatin and the marginal arrangement of its conglomerations. The intensity of staining with fuchsin (DNA) was increased over-all, vacuoles appeared, and the pyroninophilia of the cytoplasm was slightly reduced. The intensity of staining of the nuclei with fuchsin varied from one group of cells to another.

At the height of development of the cytopathic action of the virus, 72-96 h after infection, in the period of maximum accumulation of the virus, disintegration of the monolayer took place, many of the cells be-

D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. M. Zhdanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditinsy, Vol. 69, No. 6, pp. 110-113, June, 1970. Original article submitted June 17, 1969.

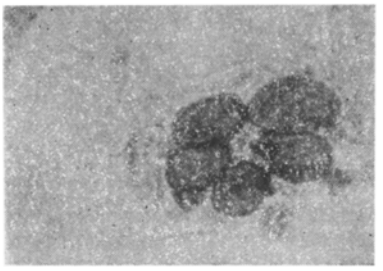

Fig. 1

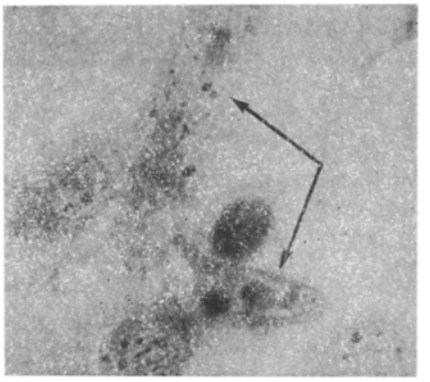

Fig. 2

Fig. 1. Intranuclear inclusions of Cowdray's type A in a syncytium 96 h after infection. Culture of human embryonic fibroblasts. Feulgen reaction, 1000×.

Fig. 2. Intranuclear herpetic inclusions. Unna's stain, 960×.

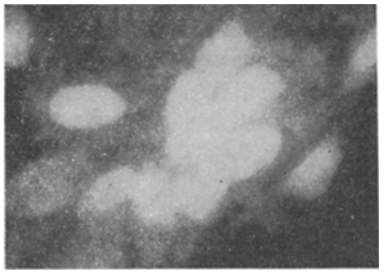

Fig. 3. Total fluorescence of nuclei in a syncytium 24 h after infection. Coons' method, 600×.

came round and shrunken, and were totally stained with fuchsin in the Feulgen reaction or with methyl green in the Brachet reaction (nonspecific adsorption of dyes by the dying cells). In focii of infection, degeneration of the cells took place in two ways: lysis with loss of RNA or pycnosis with a high content of RNA.

In many cells the nucleoli underwent lysis (Brachet), and the nucleus was filled with a weakly Feulgen-positive mass, sometimes separated from the condensed membrane by a light zone and staining less strongly with fuchsin than the chromatin (Fig. 1). These structures corresponded to the oxyphilic intranuclear inclusions revealed by Unna's method (Fig. 2). The intensity of staining of the inclusions varied in different cells.

A study of the dynamics of the lipids showed a considerable increase in their content in the cytoplasm in the late stages of infection (72-96 h). Droplets of fat were localized around the nuclear membrane, in the cytoplasmic processes, and the impression of diffuse infiltration was frequently produced. The degenerating cells stained totally with Sudan black.

The activity of glycogen, revealed as granules localized in the cytoplasm, decreased as the infection developed, but in the late stages of infection, besides cells containing hardly any polysaccharide, others were seen which contained masses of it. No glycogen could be detected in the nuclei.

The immunofluoresence method showed that 3-6 h after infection of the culture fluorescence of most cells was virtually absent. By 24 h after infection foci of fluorescent cells had appeared. The fluorescence was mainly nuclear, and in most cells, especially in syncytia, it affected the whole nucleus (Fig. 3). Luminescence was negligible in the cyotplasm of the fibroblasts. At the stage of 48 h after infection, when the cytopathic action of the virus began to manifest itself, the focal fluorescence of the monolayer changed to diffuse, and spread into the perinuclear space and into the cytoplasm. The degenerated cells exhibited total fluoresence.

The slight general increase in intensity of the Feulgen reaction (for DNA) in the course of development of herpetic infection correlates with the results of biochemical and microspectrophotometric investigations showing that the synthesis of virus DNA began 5-6 h after infection, and that the DNA content was increased by 40% after 0-12 h, and doubled after 72 h [12, 15, 18].

The increase in pyroninophilia of the nucleoli in the early periods of infection was probably connected with the increase in protein synthesis in the initial phase of reproduction of the virus, because preliminary protein synthesis in the cell is known to be necessary for the synthesis of virus DNA [12], and protein syn-

thesis is closely bound up with nucleolar material. Interesting results from this point of view were obtained by Sydiskis and Roizman [16, 17], who found that between 4 and 8 h after herpetic infection an increase in protein synthesis takes place as the result of stimulation of the synthesis of cytoplasmic polysomes. The decrease in pyroninophilia of the cytoplasm observed in the present experiments in cells at the late stages of infection confirms the observations of Newton and Stoker [11], who showed that at these times, when well marked cytopathic changes were present, RNA could not be detected at all or it was present in much smaller quantity than in the control. This period evidently coincides in time with the 3rd phase of protein synthesis described by Sydiskis [17], namely, a period of progressive decline on account of the cessation of synthesis of viral structure proteins.

Many viruses, including the virus of herpes simplex [10], require increased consumption of glucose by the cells for their own reproduction [7, 9]. The decrease in the glycogen content in the present experiments was probably due to expenditure of the polysaccharide as glucose as a result of stimulation of the energy metabolism of the cells [3]. The isolated collections of glycogen formed in the late stage of infection are evidence of the distortion of cell metabolism and of paralysis of one of the more important physiological functions in the degenerating cells – their ability to utilize nutrients.

The gross disturbance of metabolic processes in cells infected with herpes virus is indicated by their progressive fatty infiltration, which is presumably the result of the disturbance of one link in the lipoprotein chain [5].

The results are thus evidence of profound changes in the metabolism of a cell infected with herpes virus, affecting the principal manifestations of its vital activity.

LITERATURE CITED

1. R. M. Bikbulatov, Cytomorphology of Experimental Herpetic Infection Caused by Antigenically Different Strains of Virus. Candidate Dissertation [in Russian], Moscow (1967).
2. N. N. Nosik, in: Problems in General Virology [in Russian], Moscow (1966), p. 109.
3. N. N. Nosik and G. L. Linitskaya, Vopr. Virusol., No. 3, 279 (1968).
4. F. V. Sushkov, in: Morphology of the Cytopathogenic Action of Viruses [in Russian], Moscow (1963), p. 106.
5. A. L. Shabadash, Problems in the Histological Investigation of Glycogen of the Normal Nervous System [in Russian], Moscow (1949).
6. H. Eagle and K. Hubel, J. Exp. Med., 104, 271 (1965).
7. T. Fischer and E. Fischer, Virology, 13, 308 (1961).
8. R. Koppelman and E. Evans, Progr. Med. Urol., 2, 73 (1959).
9. V. Lewis and L. Scott, J. Bact., 83, 475 (1962).
10. A. Newton and M. Stoker, Virology, 5, 549 (1958).
11. A. G. E. Pearse, Histochemistry [Russian translation], Moscow (1962).
12. B. Roizman and P. Roane, Virology, 22, 262 (1964).
13. R. Rosan, A. Nahmias, and S. Kibrick, J. Histochem. Cytochem., 13, 717 (1965).
14. F. Skvaril and I. Tranger, Zh. Gig. Epidemiol. (Prague), 9, 459 (1965).
15. V. Stoker and R. Ross, J. Gen. Microbiol., 19, 250 (1958).
16. K. Sydiskis and B. Roizman, Science, 153, 76 (1966).
17. K. Sydiskis and B. Roizman, Virology, 32, 678 (1967).
18. P. Wildy, C. Smith, A. Newton, et al., Virology, 15, 486 (1961).