CYTOPATHIC ACTION OF UNA VIRUS ON BHK-21 CULTURES

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Cytopathic changes produced by the Una arbovirus in a BHK-21 culture can be detected in stained specimens about 24 h sooner than in unstained native cultures. BHK-21 cultures can be used as a system for the accumulation of Una virus, and also as an object for making an early diagnosis.

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Una virus, belonging to the Group A arboviruses in Casals' classification was isolated in 1959 by workers at the Belem Laboratory in Brazil [2] from mosquitoes. The cytopathic action of the virus on a culture of a transplantable line of baby hamster kidney cells (BHK-21) was demonstrated in native unstained cultures [3].

The object of the present investigation was to study the course of cytologic changes reflecting the cytopathic action of Una virus on BHK-21 cells. The biological activity of Una virus in infected cultures was determined in parallel tests by intracerebral injection of the culture fluid into noninbred albino mice weighing 6-7 g.

EXPERIMENTAL METHOD

Una virus was obtained from the State Collection of Viruses at the D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR [1]. A 10% brain suspension from infected newborn mice was used as the source of virus. The suspension was made up in physiological saline and centrifuged for 20 min at 3000 rpm. LD_{50} of the virus, determined by intracerebral inoculation of mice, was $10^{-4.6}$.

For the cytologic study of infected and control cultures of transplantable baby hamster kidney cells (BHK-21), the cells were seeded into tubes and penicillin flasks with cover slips. The cell suspension, containing about 200,000 cells/ml, was added to the tubes in a volume of 1 ml and to the flasks in a volume of 2 ml. The experimental and control cultures on cover slips were fixed for staining with Shabadash's neutral mixture 6, 19, 24, 48, and 72 h after inoculation of the virus. Specimens were stained by Feulgen's method or with hematoxylin-eosin. The cytopathic activity of the virus was determined not only from the cytopathic affect, but also by intracerebral inoculation of mice with the culture fluid.

EXPERIMENTAL RESULTS

The first signs of the cytopathic action of Una virus on the BHK-21 culture in native unstained specimens appeared 48-72 h after inoculation. Granules appeared in the cell cytoplasm. In some specimens, a decrease in density of the cell layer was observed. Destruction of the greater part of the territory of the cell layer was observed 72-96 h after inoculation. The titer of virus in the culture was increase 144 h after inoculation to reach $10^{-6.5}$, after which it remained unchanged.

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 O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 12, pp. 99-101, December, 1969. Original article submitted October 24, 1968.

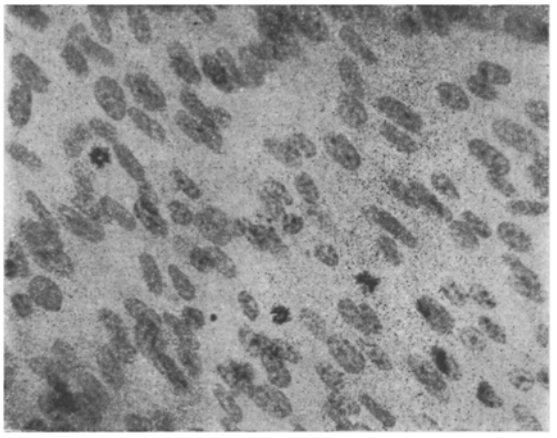

Fig. 1. Uninfected control BHK-21 culture. Mitoses can be seen. Feulgen, 200 ×.

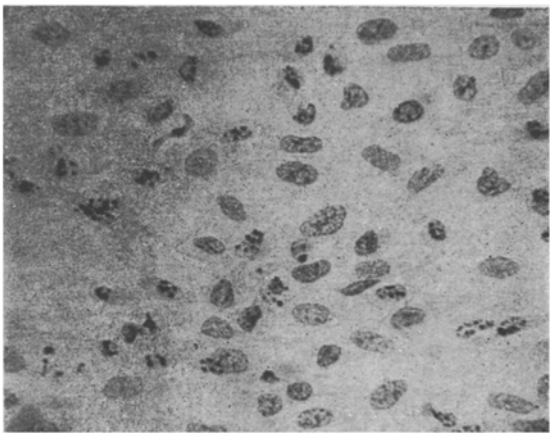

Fig. 2. BHK-21 culture 24 h after inoculation with Una virus. Changes in structure of nuclei. Feulgen, 200 ×.

When stained specimens were studied (dilution of virus 10^{-1}) 6 h after inoculation, no changes were observed in the infected cultures compared with the controls. In some cells 19 h after inoculation rounding of the nuclei was present accompanied by changes in the chromatin structure (margination of the chromatin in some cells and coarsening of the chromatin in others). These changes occupied a considerable part of the cell layer 24 h after inoculation (Figs. 1 and 2). In some parts of the monolayer, furthermore, nuclei with evidence of advanced destruction were observed (with large conglomerations of chromatin, vacuolated and pycnotic nuclei). A picture of karyorrhexis was found in many areas. Meanwhile changes took place in the cytoplasm (marked eosinophilia, degeneration with the formation of large droplets, shrinking of the cell body, plasmorrhexis).

Gross degenerative changes occupied the greater part of the layer 48 h after infection. Areas which had undergone degeneration became detached from the glass. After 72 h the changes in the cell layer were even more pronounced. If virus was used in a dilution of 10^{-3} to infect the cell cultures, after 6 and 19 h no changes were observed in the structure of the cell layer compared with the control. After 24 h disturbances were found, corresponding to those observed 19 h after infection with a high dose of virus. The cytopathic effect after 48 h was visible in the greater part of the cell layer, and it corresponded almost entirely to the changes observed at the same period after inoculation with virus in a dilution of 10^{-1}. Detachment of the degenerated areas occurred by 72 h.

No cytopathic effect was observed during the first 24 h after inoculation of the culture in dilutions of 10^{-5}, 10^{-7}, and 10^{-9}, but 48 h after inoculation with virus in a dilution of 10^{-5} the initial changes were seen in the nuclei and cytoplasm, and 72 h after inoculation gross degeneration of the nuclei and cytoplasm was observed in most cells. Changes in the structure of the nuclei (margination of chromatin, coarsening of the chromatin network) were observed 72 h after infection of the culture with a dilution of 10^{-7}. Finally, when virus was used for inoculation in a dilution of 10^{-9} the changes in nuclei and cytoplasm were very slight and did not appear until 72 h later. No cytopathic changes were observed when a dilution of 10^{-11} was used.

The study of stained specimens thus enables the cytopathic action of Una virus (dilution 10^{-1}, 10^{-3}) on BHK-21 cells to be detected by the end of the first 24 h, i.e., approximately 24 h sooner than by the use of unstained living cultures.

Albino mice, infected intracerebrally with the culture fluids, died in every case when even minimal signs of the cytopathic effect were detected in the corresponding BHK-21 cultures. This indicated reproduction of fully effective virus particles in the BHK-21 cells.

Consequently, the BHK-21 culture was more sentitive in the experiments to detect Una virus than albino mice weighing 6-7 g.

LITERATURE CITED

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