

ISOLATION OF FIVE STRAINS OF HERPESVIRUSES FROM TWO SPECIES OF FREE LIVING SMALL RODENTS

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In November 1976, during field work on the ecology of arboviruses on the territory of Czechoslovakia (close to Bratislava city), small rodents were trapped and investigated also for the presence of viruses from the family *Herpesviridae*.

Newborn laboratory mice were inoculated intracerebrally (i.c.) with 0.03 ml of 10 % brain suspensions or a pool of 10 % suspensions from different organs (lung, spleen, heart, liver and kidney) of the trapped and exsanguinated animals. The same material was inoculated on BHK-21 and rabbit lung (ZP; 1) cell line monolayers incubated at 37 °C in Basal Eagle's Medium with 5–10 % calf serum.

Nineteen suspension samples from two species of murine rodents (*Apodemus flavicollis* and *Clethrionomys glareolus*) were tested. Inoculated groups of animals developed after the 1st (1 group) 2nd (3 groups) or 3rd (1 group) successive mouse-to-mouse i.c. passages severe or fatal encephalitis. From the brains of sick or dead animals altogether five virus strains were recovered (3 from *C. glareolus*). Isolation in 3 consecutive passages in cell cultures was unsuccessful. But brain suspensions from fatal mouse infections gave on BHK-21 and ZP cell cultures a positive cytopathic effect (CPE) in the 1st (one), 2nd (two) or 4th (two) successive passage. The CPE appeared on the 3rd–5th day, in one case on the 8th day p.i. The CPE resembled that caused by herpesviruses (rounding of cells, swollen nuclei or presence of intranuclear inclusions of Cowdry A type in some type of cell cultures).

Because of unequal and rather low virus titres in the media of BHK-21 and ZP cell cultures (10^6 and 10^3 TCID₅₀/ml, respectively), 16 different primary or established cell cultures were tested to reach higher virus yields. Both fibroblast and epithelial types of cell cultures of human and animal origin were employed. Consecutive passaging succeeded in all types of cell cultures tested. BHK-21, L, Vero, rabbit embryo fibroblast (REF) and guinea pig tongue (2) cell lines proved to be the most suitable for obtaining highest titres after 15, 30, 12, 4 or 20 successive passages, respectively, ranging from 10^3 – $10^{7.5}$ TCID₅₀/ml.

Preliminary electron microscopic study (kindly provided by Dr. F. Čiampor, Institute of Virology, Slovak Academy of Sciences, Bratislava) of virus partially purified by differential ultracentrifugation and of ultrathin sections of infected REF cells provided evidence that the viruses isolated from the two species of murine free living rodents belong to the family *Herpesviridae*. Whether the isolates belong to the subfamily *Alphaherpesvirinae* or *Betaherpesvirinae* and what is the degree of their relatedness to known members of these subfamilies, especially to the murine cytomegaloviruses studied thus far (3, 4) remains to be elucidated.

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