Acta virol. **13** : 343—345, 1969

PERORAL INFECTION OF SUCKLING MICE WITH HERPES SIMPLEX VIRUS

J. RAJČÁNI, A. SABÓ, D. BLAŠKOVIČ

Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia

Received February 12, 1969

Summary. — The distribution of herpes simplex virus was followed by the fluorescent antibody method and by infectivity titration in suckling mice after peroral infection. Specific fluorescence of viral antigen was detectable in the nasal and oral mucosa, lungs, cranial nerves, thoracic vegetative nerves and ganglia and subsequently in the brain and spinal cord. Groups of fluorescing cells were occasionally seen in the spleen, liver, adrenal cortex and renal medulla.

The intraperitoneal or intranasal routes of inoculation were mainly used in previous studies dealing with the pathogenesis of herpes simplex virus in mice (Johnson, 1964; Yamamoto *et al.*, 1965). According to our assumption, the peroral administration of the virus should better simulate natural conditions, resembling the primary infection in man.

Inoculation of 0.02 ml of virus in tissue culture medium (2×10^3 $TCID_{50}$) into 4 days old baby mice elicited encephalitis and frequently also severe pneumonitis in approximately 66% of the animals between the 5th and 7th day after inoculation. Materials were taken starting from the 2nd until the 7th day from a total of 36 suckling mice for infectivity titrations and histological and immunofluorescence examinations. For fluorescent antibody assay, the materials were cut in a cryostate, fixed in acetone and stained by the indirect method (human immune serum and antihuman conjugate).

The results of infectivity assay, performed in a stable line of rabbit lung fibroblasts, are shown in Fig. 1. The virus was regularly reisolated from the oral and nasal tissues and from the lungs starting with the 2nd and from the brains starting with the 3rd day after infection. Positive isolations from the spleen, liver and other abdominal organs were less regular and attempts to reisolate the virus from the blood were unsuccessful. Bright specific fluorescence of the viral antigen was found in the oral squamous epithelium and subepithelial connective tissues starting from the 5th day after infection (Fig. 2). Histological examination revealed typical intranuclear inclusion bodies. In the epithelial cells of the nasal olphactory mucosa, fluorescence of some cells was already positive starting with the 2nd day. At later time intervals, specific fluorescence occurred also in the subepithelial tissues and large groups of antigen-containing cells lay desquamated in the lumen (Fig. 3). Starting from the 4th day, the viral antigen was detectable in the Schwann cells of some nerve bundles (fila olphactoria, nervus trigeminus and glossopharyngicus), one day later also in the bulbus olphactorius and semilunar ganglion of the trigeminal nerve (Fig. 4). Simultaneously, positive

fluorescence of the virus was found in the glial cells and neurons of the brain stem. By the 6th day, the distribution of virus-containing neurons and glial cells was more widespread. In the cerebral cortex (Fig. 5), cerebellar cortex, thalamic and caudate nuclei, the majority of cells showed specific fluorescence.

The respiratory movements enabled a quick transmission of the virus into the lung soon after infection. In the alveolar epithelial cells and alveolar macrophages, the viral antigen was already detectable on the 3rd day and

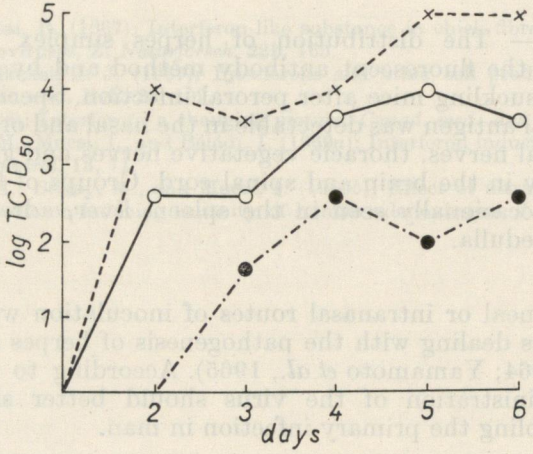

Fig. 1.

Virus titres in the organs and tissues of suckling mice inoculated per os with herpes simplex virus
Abscissa: days after inoculation; ordinate: virus titre in log $TCID_{50}/0.1$ g of tissue.
× Lungs
○ Tissues of nasal and oral cavities
● Brains

starting from the 4th day widespread bright fluorescence of interalveolar septa was seen. This process of interalveolar travel of the virus was accompanied by a typical histological picture of interstitial pneumonitis. Viral antigen was traced by the fluorescent antibody method from the lung tissue along various vegetative nerve trunks (n. vagus and truncus sympathicus) and through the thoracic vegetative ganglia and rami communicantes toward the thoracic cord.

The immunofluorescent findings in the Schwann cells of the nerve bundles are in good agreement with recent electron microscopic observations of virus particles in the nuclei (uncoated particles) and in the cytoplasm (enveloped particles) of these cells and in the intercellular tissue spaces around them (Rabin *et al.*, 1968).

Our experiments confirmed the view that the neural transmission was the most important route of herpesvirus spread into the central nervous system. The occasionally positive findings of viral antigen and reisolation of virus from the spleen, liver, suprarenal gland and kidney (fluorescence of the distal

tubuli) indicates that viraemia should develop but, in view of the negative isolation attempts with blood, it seems to be rather transient. The relation of viraemia to the occurrence of the virus in the central nervous system remains uncertain. On the other hand, the distribution of the first positive immunofluorescent findings in the brain stem, olphactory bulb and thoracic cord could be well explained by spreading of the virus via the neural route. Similar conclusions were also drawn in our previous works on pseudorabies virus in piglets (Sabó *et al.*, 1968*a*) and in cats (Sabó *et al.*, 1968*b*) after peroral infection.

References

Johnson, R. T. (1964): The pathogenesis of herpes virus encephalitis. I. Virus pathways to the nervous system of suckling mice demonstrated by the fluorescent antibody method. *J. exp. Med.* **119**, 343—356.

Rabin, E. R., Jenson, A. B., and Melnick, J. L. (1968): Herpes simplex virus in mice: electron microscopy of neural spread. *Science* **162**, 126—127.

Sabó, A., Rajčáni, J., and Blaškovič, D. (1968*a*): Studies on the pathogenesis of Aujeszky's disease. I. Distribution of the virulent virus in piglets after peroral infection. *Acta virol.* **12**, 214—221.

Sabó, A., Rajčáni, J., Raus, J., and Karelová, E. (1968*b*): Untersuchungen zur Pathogenese der Aujeszkyschen Krankheit der Katzen. *Arch. ges. Virusforsch.* **25**, 288—298.

Yamamoto, T., Otani, S., and Shiraki, H. (1965): A study of the evolution of viral infection in experimental herpes simplex encephalitis and rabies by means of fluorescent antibody. *Acta neuropath. (Berl.)* **5**, 288—306.

Explanation of Photomicrographs:

Specific fluorescence of herpes simplex virus antigen in organs and tissues of suckling mice after peroral infection.

Fig. 2. The oral cavity on the 5th day after inoculation. Viral antigen in the squamous epithelium and subepithelial connective tissue. × 100.

Fig. 3. The nasal cavity on the 5th day after inoculation. Bright fluorescence of the viral antigen in the sensory nasal epithelium. × 100.

Fig. 4. A branch of the nervus trigeminus on the 6th day after inoculation. Viral antigen in numerous Schwann and endoneural cells. The fluorescence of the lamina elastica in the vessel wall near the nerve bundle is nonspecific. × 100.

Fig. 5. The brain cortex on the 7th day after inoculation. Bright specific fluorescence of viral antigen in numerous neurons and glial cells and in the meninges. × 100.