# Herpes Simplex Virus in Mice: Electron Microscopy of Neural Spread

Abstract. *Herpes simplex virus rapidly infected the trigeminal nerves of mice after intranasal inoculation. Centripetal neural spread was suggested by histologic evidence of encephalitis in the area of attachment of the trigeminal nerve. Furthermore, electron microscopy revealed virus replication primarily within Schwann cells of the trigeminal nerve, and neurons of the gasserian ganglion.*

Goodpasture (*1*) suggested more than 40 years ago that herpes simplex virus may spread along the trigeminal nerve to produce encephalitis in experimental animals. Previous investigators have considered axonal cylinders, neural lymphatics, and tissue planes between axons as possible routes of neural spread of this virus to the central nervous system (CNS) (*2*). Johnson (*3*), after making an immunofluorescence study of murine herpes simplex neuritis, reported specific viral antigen primarily within Schwann cells and possibly, to a lesser extent, other endoneural cells of the trigeminal nerve after intranasal inoculation with the virus; he concluded that herpes simplex virus may reach the CNS by propagating within these endoneural cells. The questions remained, however, of whether endoneural cells produced infectious virus, and whether the antigen merely reflected virus, either infectious or uninfectious, centripetally moving within the involved nerve. We have confirmed Johnson's view (*3*) in that infectious virus is produced in the trigeminal nerve. Furthermore, direct visualization by electron microscopy revealed viral invasion and replication of both herpes virus strains KOS and XIII primarily within Schwann cells of the trigeminal nerve.

Approximately 300 newborn Swiss mice were inoculated intranasally with virus strains KOS and XIII; both were strains passaged in tissue culture not adapted to mouse CNS. Most observations were made from mice inoculated with the KOS strain; a blunt needle was placed in the nasal cavity, and a small quantity (about 0.02 $cm^3$) of inoculum was injected until some of it appeared in the mouth. Most animals either died or were killed between 1 and 4 days after inoculation.

Virus assay studies (Fig. 1) were done on groups of two to three mice killed daily between days 1 and 4. Pooled specimens of the trigeminal nerve, olfactory bulbs, the remaining portion of brain, and blood were frozen pending assay. The trigeminal nerve was dissected along the floor of the skull, from near its origin in the brain into the nasal region. The possibility of contamination from surrounding tissues was considered. Histologic sections of the specimen of trigeminal nerve revealed predominantly peripheral nerves and usually some ganglia, with only a minimum of surrounding tissue. The titer of virus in the specimen of trigeminal nerve rose rapidly and was markedly elevated to $10^7$ plaque-forming units (PFU) per gram as early as the 2nd day after inoculation; it remained at this high level through day 4. Virus in lower titer was also present in the olfactory-bulb portion of the brain by day 2, but the titer increased to $10^6$ PFU per gram by day 4. Virus was not detectable in the remaining portion of the brain until day 3; by day 4, titer in the remaining portion of the brain increased to slightly below $10^5$ PFU per gram. Virus was not detectable in the blood throughout the 4 days of observation.

Parasagittal sections of the head (including face, oral and nasal cavities, nasal sinuses, trigeminal nerve, gasserian ganglion, calvarium, and brain) from 18 mice killed between 1 and 4 days after intranasal inoculation were examined by light microscopy. Tissues were fixed in 10-percent neutral buffered formalin, and sections stained with hematoxylin and eosin were prepared. Parasagittal sections taken at day 1 showed only slight focal necrosis in the nasal mucosa. By day 2, extensive necrotic zones of nasal mucosa were apparent; the necrotic zones extended into underlying cartilage and adjacent nasal sinuses. On days 3 and 4, well-defined foci of necrosis were seen in the trigeminal nerve and gasserian ganglion; foci of necrosis were also present in the brain in the area of attachment of the trigeminal nerve. The olfactory bulbs on days 3 and 4 contained foci of necrosis.

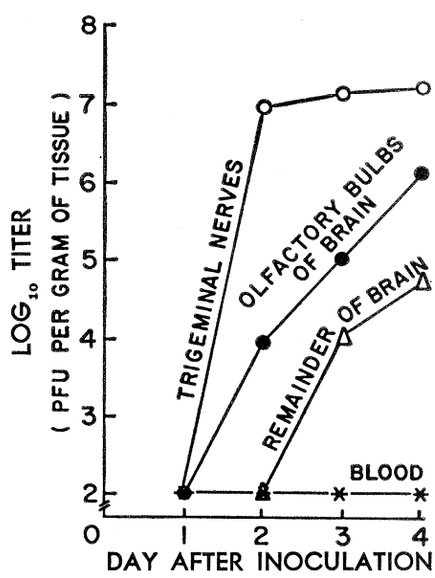


Fig. 1. Neural spread of herpes simplex virus (strain KOS) in newborn mice. Each point represents the mean concentration of virus in a pooled specimen from two or three mice; trigeminal nerves were usually associated with ganglia. Asterisks indicate no detectable virus (asterisk is placed at lowest level the method is sensitive).

By electron microscopy, herpes nucleocapsids were directly visualized, primarily within nuclei of Schwann cells of the trigeminal nerve, as early as 53 hours after inoculation. However, infected Schwann cells were much more apparent by electron microscopy on days 3 and 4. Intranuclear herpes nucleocapsids were also seen in neurons of the gasserian ganglion. In some infected Schwann cells, intranuclear virus particles were sparsely scattered (Fig. 2), while in others aggregates of herpes nucleocapsids appeared (Fig. 3). Nuclear cytopathic change was characterized by loss of nucleoplasm, and prominent margination and clumping of chromatin. Disruption of the nuclear membrance was accompanied by spillage of nuclear contents and virions into the cytoplasm. Enveloped herpes particles were seen intracellularly and also in the extracellular space. Nonspecific cytoplasmic changes consisted of swelling of mitochondria and dilation of the endoplasmic reticulum. More severe changes with cell necrosis were seen, particularly in Schwann cells, which contained disintegrating myelin sheaths.

Viral encephalitis in man and experimental animals usually results from direct viral invasion of the CNS. For viruses to gain access to the CNS from extraneural sites, they must be either transported by the blood stream or spread centripetally along peripheral nerves. Herpes simplex can apparently reach the CNS by either pathway (*2*). Our electron-microscopic study supports the contention that propagation of the virus, primarily within Schwann cells of the trigeminal nerve, accounts for the neural spread of herpes simplex virus

to the gasserian ganglion and adjacent CNS, as has been suggested (*3*).

Acute herpetic encephalitis is a well-known clinical entity in man, and diagnosis of this disease has been confirmed by electron microscopy and by isolation of herpes simplex virus in brain tissue from both surgical (*4*) and autopsy material (*5*). The gasserian ganglion of the trigeminal nerve in man may be involved in recurrent herpetic infections (*6*). Spread of herpes virus to the gasserian ganglion in man may be expected to follow primary oral or perioral herpes infection as a result of centripetal infection primarily within Schwann cells of the trigeminal nerve. In this regard, the gasserian ganglion may act as a viral reservoir, with reactivation of the virus resulting in such clinical entities as trigeminal neuralgia, herpetic perioral fever blisters, and even recurrent encephalitis (*7*).

Herpes simplex virus has not yet been recovered from the gasserian ganglion in man, but attempts have been few (*7*); its chronic multiplication has been demonstrated in the lacrimal and salivary glands of man, and these tissues may well be important in recurrent herpetic disease (*8*).

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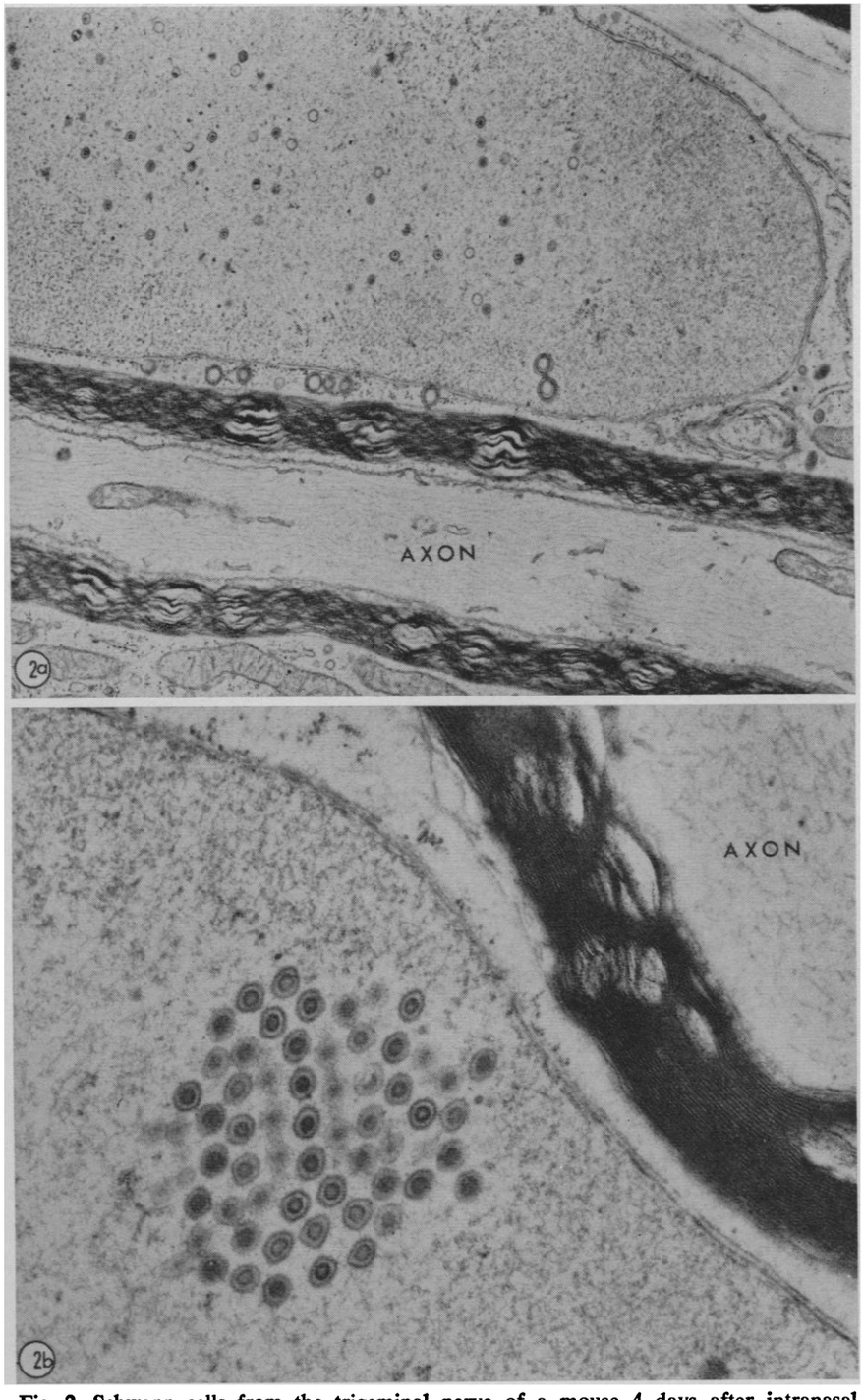


Fig. 2. Schwann cells from the trigeminal nerve of a mouse 4 days after intranasal inoculation with herpes virus. (a) Nucleus of a cell contains scattered complete and empty particles; note the enveloped herpes particles, many of which appear empty (× 18,400). (b) Nucleus of another cell contains an aggregate of herpes nucleocapsids; note the myelin sheath of the cell, enveloping the axon (× 53,600). Virions were not observed within axonal cylinders.

**References and Notes**

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## Human Prothrombin Activation: Immunochemical Study

Abstract. *Antiserums to human prothrombin contain antibodies directed against at least two different antigenic sites. During blood coagulation, prothrombin is cleaved into two major antigenically distinct fragments—thrombin and a "pro" fragment. The latter is present in normal serum, whereas thrombin loses its immunologic reactivity, presumably because of its combination with the natural thrombin inhibitors in blood.*

The mechanism of the conversion of the zymogen prothrombin to the active enzyme thrombin is poorly understood. Important observations have been made of prothrombin conversion in 25 percent sodium citrate solutions (*1*), with tissue extracts (*2*), and with activated factor X preparations (*3*). These studies indicate that major cleavages occur in the prothrombin molecule during thrombin formation. I now report studies of