Histol Histopath (1992) 7: 63-74

Histology and Histopathology

Axonal and transsynaptic (transneuronal) spread of Herpesvirus simiae (B virus) in experimentally infected mice

Georg Gosztonyi[1], Dietrich Falke[2] and Hanns Ludwig[3]

[1]Institut für Neuropathologie, Freie Universität Berlin, Berlin, [2]Abteilung für Experimentelle Virologie, Institut für Medizinische Mikrobiologie, Johannes-Gutenberg-Universität Mainz, Mainz, [3]Institut für Virologie, Freie Universität Berlin, Berlin, FRG

Summary. In order to study the pathogenesis of B virus infection of the nervous system, newborn and young mice were inoculated by four different routes: 1. Intramuscular (i.m.) in the forelimb; 2. I.m. in the hindlimb; 3. Subcutaneous (s.c.) in the abdominal wall; 4. Intraperitoneal (i.p.).Spread of virus was followed by immunohistochemical demonstration of viral antigen in tissue sections of the peripheral and central nervous system. Three distinct patterns emerged: 1. After i.m. limb inoculations, virus progressed along the ipsilateral dorsal column, the bilateral spinothalamic and bilateral spinoreticular systems and along central autonomic pathways. 2. After s.c. inoculation, the dorsal column was spared, otherwise the spread was similar to that following i.m. inoculations. 3. After i.p. inoculation, virus spread in the spinal cord bilaterally, mainly along spinothalamic and central autonomic pathways. The peripheral motoneurons were conspicuously spared, even in the i.m. inoculation mode. In the brain stem, B virus antigen appeared bilaterally, at multiple sites. In the cerebrum, virus infected cells appeared first in the thalamus, hypothalamus and the motor cortex. The mode of spread from spinal levels was mainly orthograde along the ascending systems (dorsal columns, spinothalamic, spinoreticular tracts), but also retrograde along descending systems (pyramidal tract, central autonomic pathways). Oligosynaptic systems transmitted virus more quickly than the polysynaptic ones. In the involvement of various neuronal systems in virus spread, a certain selectivity, sparing the peripheral motoneuron and the cerebellar systems, could be assessed.

Offprint requests to: Dr. Georg Gosztonyi, M.D., Institut für Neuropathologie, Freie Universität Berlin, Hindenburgdamm 30, D-1000 Berlin 45, FRG

Key words: Transsynaptic (transneuronal) virus spread, Axonal spread of virus, Herpes viruses, Spinal cord, Sensory systems, Neuroanatomy, Rhesus monkeys (macaques), Viral pathogenesis, Central autonomic pathways

Introduction

Herpesvirus simiae (B virus) is widely distributed among rhesus monkeys, causing vesicular inflammatory lesions on the mucous membranes of the oral cavity and, less frequently, on the skin (Ludwing et al., 1983; Palmer, 1987). B virus is inclined to latency in the trigeminal ganglia and its activation leads to recurrent oral herpetic lesions. In the few fatal cases examined pathologically liver lesions, and, in the brain stem, perivascular cuffing and microglial infiltration have been described (Keeble, 1960; Loomis et al., 1981). In contrast to monkeys, the rare human infection leads to a very severe, in about 80% of the cases fatal, encephalomyelitis (Palmer, 1987). Herpes simplex virus (HSV) type 1 and 2 is known to travel inside the axons (Goodpasture and Teague, 1923; Hill et al., 1972; Cook and Stevens, 1973; Bak et al., 1977; Kristensson et al., 1978; Georgsson et al., 1987). It has always been postulated, but never documented experimentally that B virus also propagates along axons.B virus infection of monkey bears many similarities to the HSV infection of humans. While the pathogenesis of HSV infection has been intensively studied experimentally, the pathogenesis of B virus infection remains little elucidated. In order to clarify the evolution of B virus infection, the events were studied histologically and immunohistochemically in a mouse model after various modes of peripheral inoculations. Topographically-directed virus isolation studies were not done because of the small size of the pertinent structures (ganglia, spinal cord segments) involved in virus spread in the mouse. Pathogenetic

considerations were based on the distribution of viral antigen in various phases of infection. It will be shown that the basic mechanisms of spread of HSV and B virus are very similar, with some differences, however, in their cellular tropisms.

Materials and methods

Virus strains

A strain of B virus was isolated from uninoculated monkey kidney cell cultures. The virus was identified by various types of spontaneously occurring cytophathic effects (Falke, 1961); it was typed as a B virus by Dr. K.E. Schneweis, Bonn, FRG, and could be differentiated by CF-tests from HSV type 1 and 2 (Falke, 1964).

Animals

NMRI mice were used and altogether 70 animals were infected. Twenty-nine mice were 10 days old, 14 mice 13 days old, 6 mice 21 days old and 21 mice 2 to 4 months old at the time of infection. All animals were infected peripherally. According to the site of virus inoculation, the animals were divided into four groups. *Group 1.* I.m. infection in one of the upper extremities: 36 mice. *Group 2.* I.m. infection in the right lower extremity: 5 mice. *Group 3.* S.c. infection in the upper half of the abdominal wall: 12 mice. *Group 4.* I.p. infection: 17 mice. For inoculation 0.2ml virus suspension was used with a titer of 10 PFU/ml. The animals were killed by Nembutal overdosage 3 to 9 days after infection.

Histological techniques

The brains were fixed in Bouin's solution, sliced in the frontal plane and embedded in paraffin. The inoculated limb, the spine, and, in about 70% of the animals, the entire trunk were also immersed in Bouin's fixative. After 48 hrs, the pieces were sliced, decalcified with 25% EDTA and embedded in paraffin. The trunks were sectioned transversally, the extremities longitudinally and the spines either transversally or longitudinally. 5 µm paraffin sections were stained with haematoxylin and eosin (H&E) and partly for myelin.

Antibodies and immunohistochemical techniques

For the demonstration of B virus antigen a B virus-specific and an HSV-specific polyclonal rabbit antibody (AB) were applied to the sections. Both sera came from hyperimmunized rabbits. The B virus-specific serum was absorbed with uninfected tissue. The HSV 1-specific serum (M8) was prepared under syngeneic conditions (Ludwig et al., 1983). Since gB and gD of HSV 1 and B virus carry common epitopes (Ludwig et al., 1983), both hyperimmune sera could be used to demonstrate B virus antigens. Thus, M8 AB was used to control and corroborate the results gained by the anti-B virus AB on adjacent sections. Several tissue sections were immunostained for myelin basic protein (MBP) and glial fibrillar acidic protein (GFAP). On a few sections double immunoenzymatic labelling of B virus antigen and GFAP was also performed. For the detection of AB binding sites the PAP technique, the avidin-biotin-peroxidase technique (ABC, Vector) and the APAAP technique (DAKOPATTS) were used.

Electron microscopy

A few elected brain areas were excised from the paraffin blocks, the paraffin was dissolved and the tissue fragments were embedded in Araldite. Thin sections were contrasted with uranyl acetate and lead citrate and viewed in a Zeiss EM 10 electron microscope.

Results

Clinical signs of the disease could be observed in the group of mice infected when 10 days old. Those infected in the hindlimb showed paresis of the right leg 4 days post infection (p.i.), and later paraparesis. Several mice, inoculated in one of the upper extremities, showed signs of paraparesis 5-6 days p.i. The group of mice inoculated when 13 and 21 days old and 2-4 months old, showed no apparent signs of disease.

Histology and immunohistology. Group 1. I.m. inoculation in *one of the upper extremities.* In the early phase, virus antigen together with some infiltration of mononuclear cells could be demonstrated only at the site of inoculation. Later, viral antigen was present in the nerve bundles of the upper extremity, in the brachial plexus and homolateral sympathetic ganglia. In the peripheral nerves viral antigen appeared in the Schwann cells, within the ganglia in the satellite cells and autonomic neurons. The arrival of infection to the spinal cord was hallmarked by the appearance of viral antigen in several homolateral spinal roots, spinal ganglia and in the ipsilateral posterior horn of the upper thoracic and lower cervical segments (Fig. 1). Simultaneously, virus antigen could already be detected in the ipsilateral dorsal column. Subsequently, it appeared with a characteristic pattern (Fig. 1) in further sectors of the spinal white matter. Positivity of the ipsilateral posterior funiculus was a constant feature throughout and remained strictly unilateral. Antigen was frequently present bilaterally at the border of the lateral and anterior funiculi in the ventral root entry zone. Furthermore, it appeared rather diffusely in the homolateral, but in a few cases also in the contralateral lateral funiculus. Apart from the homolateral posterior horn, positivity in the spinal grey matter was rather scanty; in the

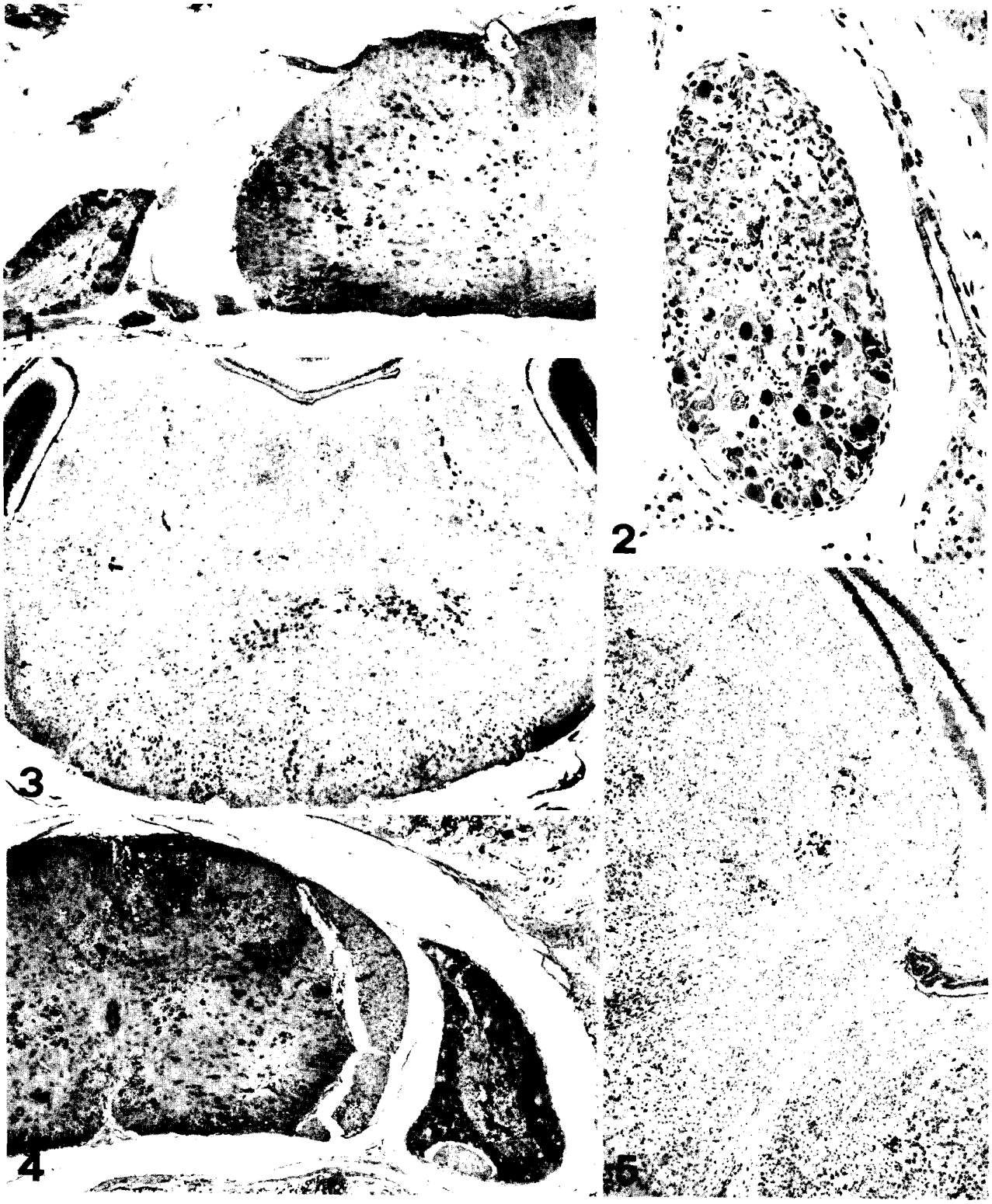


Fig. 1. Cervical spinal cord representing the entry segment; i.m. inoculation into the left forelimb. Virus antigen is present in neurons of the sensory ganglion, in the posterior root, in scattered neurons and glial cells of the spinal grey matter, in glial cells of the lateral and anterior funiculi and in several cells of the ipsilateral posterior funiculus. Immunostaining with a HSV-specific polyclonal AB (M8). PAP technique. × 61

Fig. 2. Thoracic sympathetic ganglion with B virus antigen-containing neurons and satellite cells; i.m. inoculation into the right forelimb. Immunostaining with the polyclonal M8 AB. APAAP technique. × 200

Fig. 3. Bilateral, asymmetric presence of immunoreactive cells in the pontine tegmentum following i.m. inoculation of virus into the right upper extremity. Immunostaining with a polyclonal AB against B virus. ABC technique. × 31

Fig. 4. Lumbar spinal cord. B virus antigen-containing cells in the sensory ganglion (arrows), dorsal root entry zone, posterior horn, lateral and anterior funiculi; i.m. inoculation into the right forelimb. Immunostaining with the HSV specific M8 AB. APAAP technique. × 103

Fig. 5. Groups of immunoreactive cells in the thalamus after i.m. inoculation of virus into the right upper extremity. Immunostaining with the M8 AB. APAAP technique, × 40.5

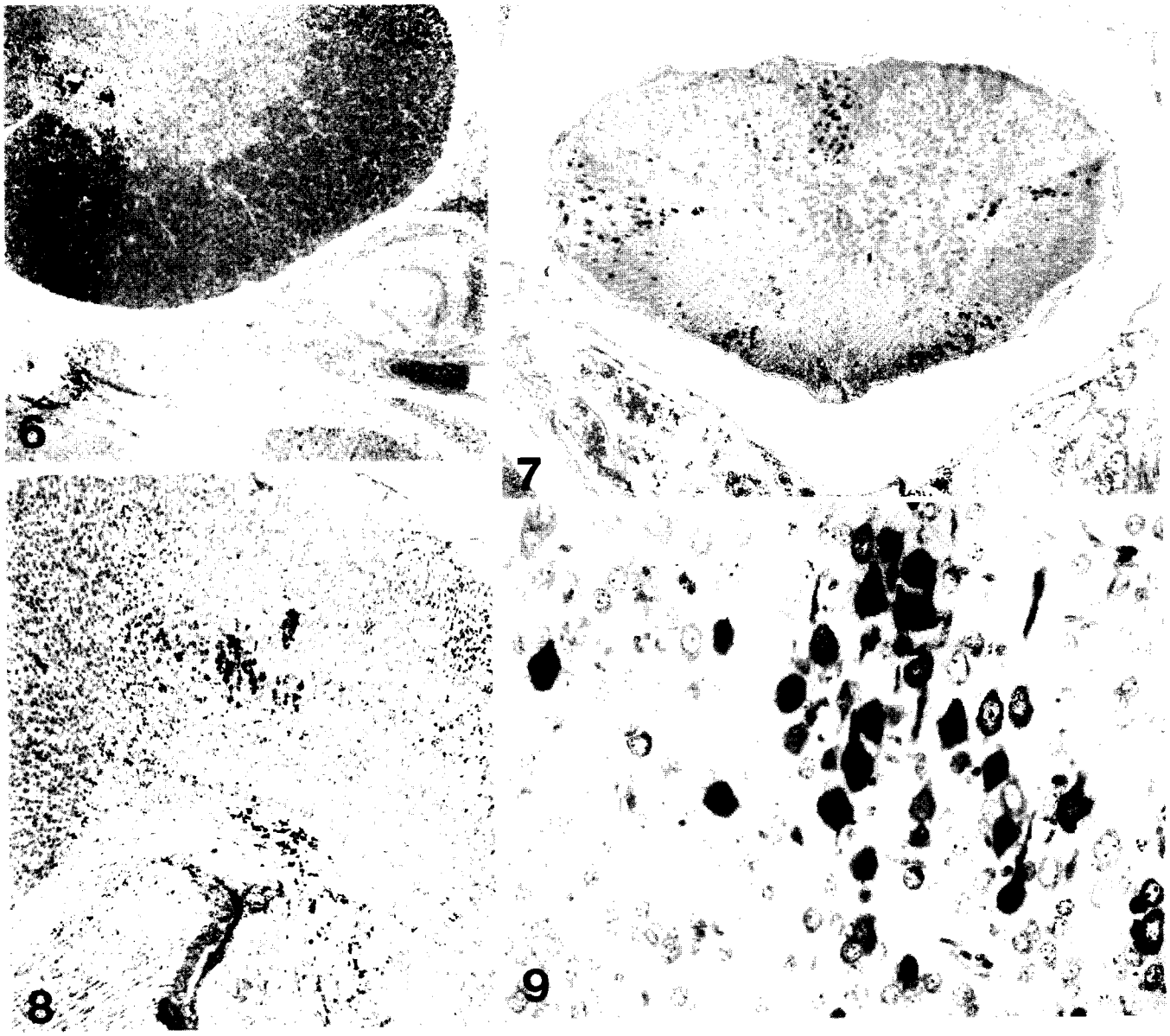


Fig. 6. Small groups of leukocytes positive for viral antigen in the thymus (solid arrow) and in a thoracic lymph node (empty arrow); i.m. inoculation into the right upper extremity. Immunostaining with a polyclonal B virus-specific AB. APAAP technique. × 58

Fig. 7. Upper thoracic spinal cord; i.m. virus inoculation into the right hindlimb. Immunoreactive cells in the ipsilateral posterior and bilateral lateral and anterior funiculi. The grey matter is free from virus antigen. Immunostaining with the M8 AB. ABC technique. × 77.5

Fig. 8. Group of immunoreactive neurons in the motor cortex and several immunoreactive glial cells in the subcortical white matter and internal capsule; i.m. virus inoculation into the right hindlimb. Immunostaining with the HSV-specific polyclonal M8 AB. APAAP technique. × 48

Fig. 9. Closer view of the immunoreactive cortical neurons shown in Fig. 8. Virus antigen is present in the nuclei, perikarya and dendrites. Immunostaining with the M8 AB. APAAP technique. × 335

ventral horns it was rare; if present, it was bilateral and mainly restricted to small neurons. Big ventral horn motoneurons were only exceptionally infected. In the levels above the entry segments of the virus the grey matter was free of antigen-containing cells, positivity was restricted to the funiculi. In the spinal segments, caudal to the entry zone, viral antigen could hardly be detected. The ganglia of the sympathetic chain and their connecting branches always displayed a strong unilateral positivity for viral antigen around the entry zone (Fig. 2). Scattered positive autonomic ganglion cells and positive autonomic nerve segments could be found in the ipsilateral lower thoracic, and, exceptionally, in the contralateral upper thoracic levels

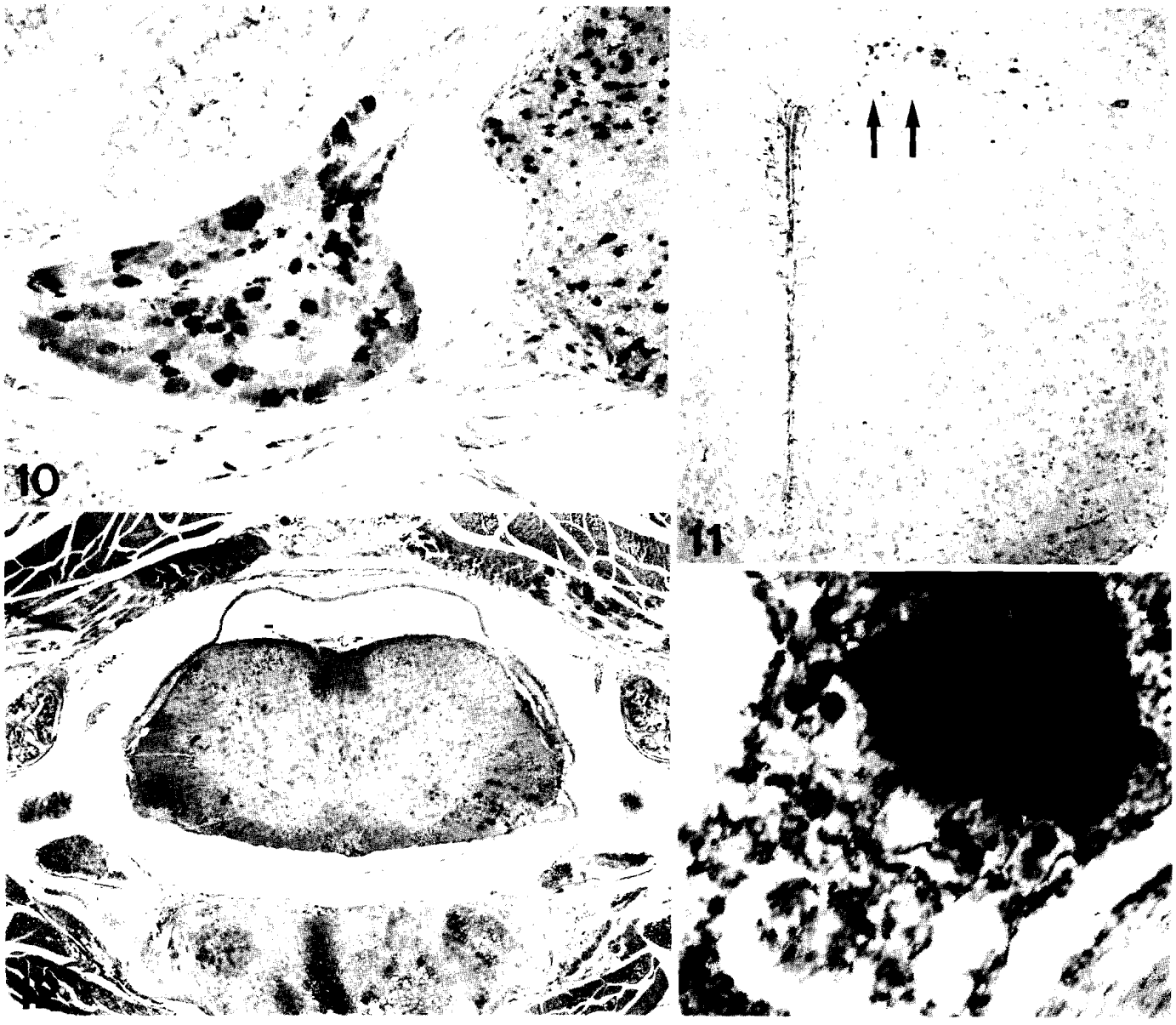


Fig. 10. Upper thoracic spinal segment. B virus antigen-bearing neurons and satellite cells in the spinal ganglion and immunoreactive glial cells in the lateral and anterior funiculi. Subcutaneous inoculation of B virus. Immunostaining with the M8 AB. ABC technique. × 145

Fig. 11. B virus antigen-containing cells in the N. paraventricularis hypothalami (arrows); i.p. inoculation of B virus. Immunostaining with the M8 AB. The slender astrocytes perpendicular to the wall of the IIIrd ventricle are stained with anti-GFAP AB. Double immunoenzymatic labelling, APAAP and ABC technique. × 77.5

Fig. 12. Cervical spinal cord. B virus antigen-containing glial cells bilaterally at the border of the anterior and lateral funiculi; i.p. inoculation of virus. Immunostaining with the M8 AB. ABC technique. × 63

Fig. 13. Intranuclear and cytoplasmic B virus particles in the brain stem. × 42,000

of the sympathetic chain. In the dorsal horn, small and medium-sized neurons were lodging viral antigen in both the nucleus and cytoplasm, and occasionally only in the nucleus. Around the neurons numerous glial cells were also positive for B virus antigen. In the funiculi, both astrocytes and oligodendrocytes harboured viral antigen. On H&E preparations numerous Cowdry type A inclusions were present in the nuclei. An inflammatory infiltration of mononuclear cells always followed the appearance of viral antigen, though with a considerable delay. The inflammatory infiltrates were rather mild in the spinal and autonomic ganglia, very intense in the dorsal root entry zone, again weaker in the white matter of the entry segments, and hardly discernible in the spinal tracts above the entry segments. Spread of the virus to

Tables 1-4. Explanations in the text. v: ventral root, d: dorsal root, sp: spinal ganglion, sy: sympathetic ganglion.

Table 1. Pattern of B virus propagation following i.m. inoculation in the upper limb.

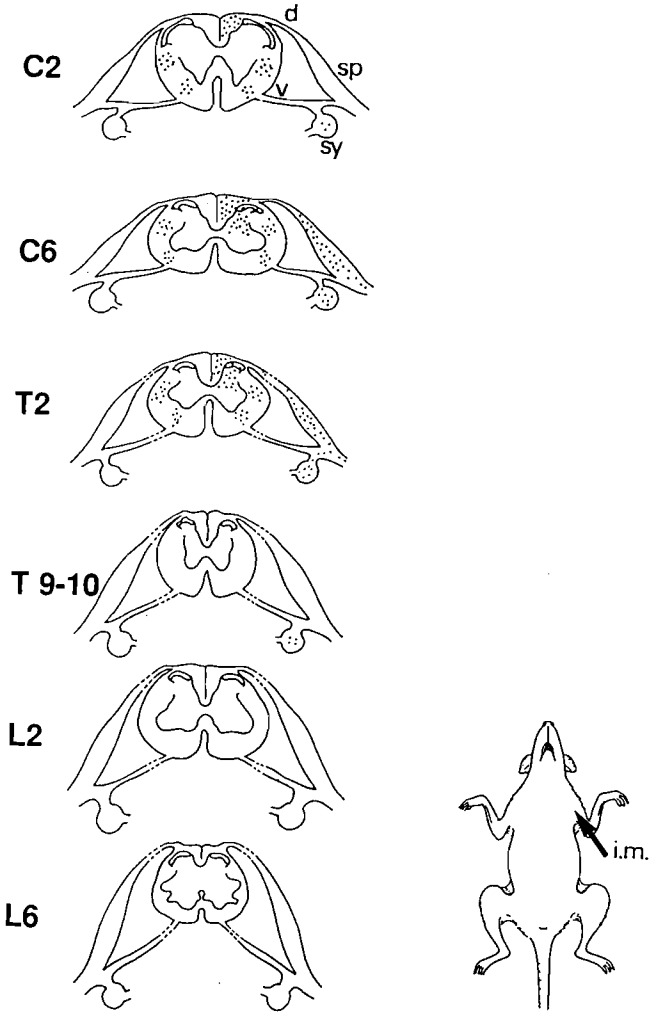
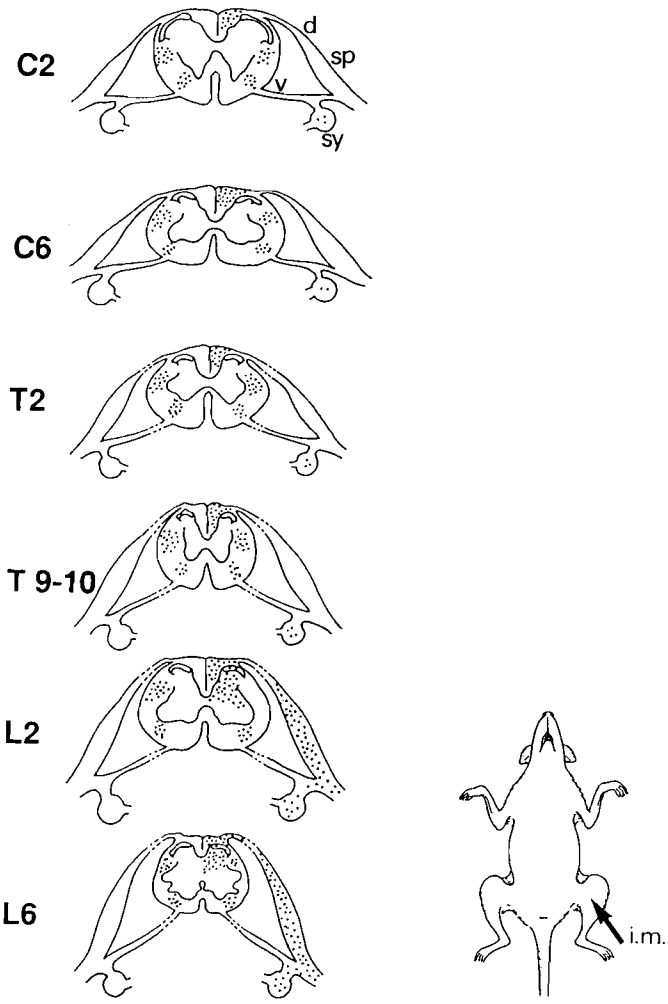


Table 2. Pattern of B virus propagation following i.m. inoculation in the lower limb.



the brain stem and the cerebrum could be observed only in mice killed after a longer survival following infection. In the brain stem, besides long projecting tracts multiple sites exhibited viral antigen (Fig. 3). Positivity was usually bilateral, but expressed more strongly on the contralateral side. The infection reached the cerebrum in only four mice of this group. In the first animal, positive neurons were found in the contralateral thalamus (Fig. 5), in the second, bilaterally in the thalamus, in the third bilaterally in the hypothalamus, while in the fourth, the spread of virus could be traced along the contralateral internal capsule up to the motor cortex.

This group also includes 16 animals between 2 and 4 months of age. In their central and peripheral nervous system no B virus antigen could be demonstrated, though occasionally focal inflammatory infiltrates were found in the spinal cord. These animals most probably survived the acute infection, eliminated the virus, but some residual inflammatory signs still remained.

Another subgroup of 6 mice, inoculated when 13 days old, was either completely negative for, or exhibited only minimal amounts of viral antigen at the inoculation site. These animals most probably underwent an abortive infection.

Group 2. I.m. inoculation into the *right lower extremity.* The distribution of the viral antigen showed a pattern similar to Group 1. At the lumbosacral levels unilateral, plurisegmental positivity of the dorsal roots, spinal ganglia and a few sympathetic ganglia could be documented (Fig. 4). The dorsal root entry zone and the posterior horn ipsilaterally abounded in immunoreactive cells. The

Table 3. Pattern of B virus propagation following s.c. inoculation into the abdominal wall.

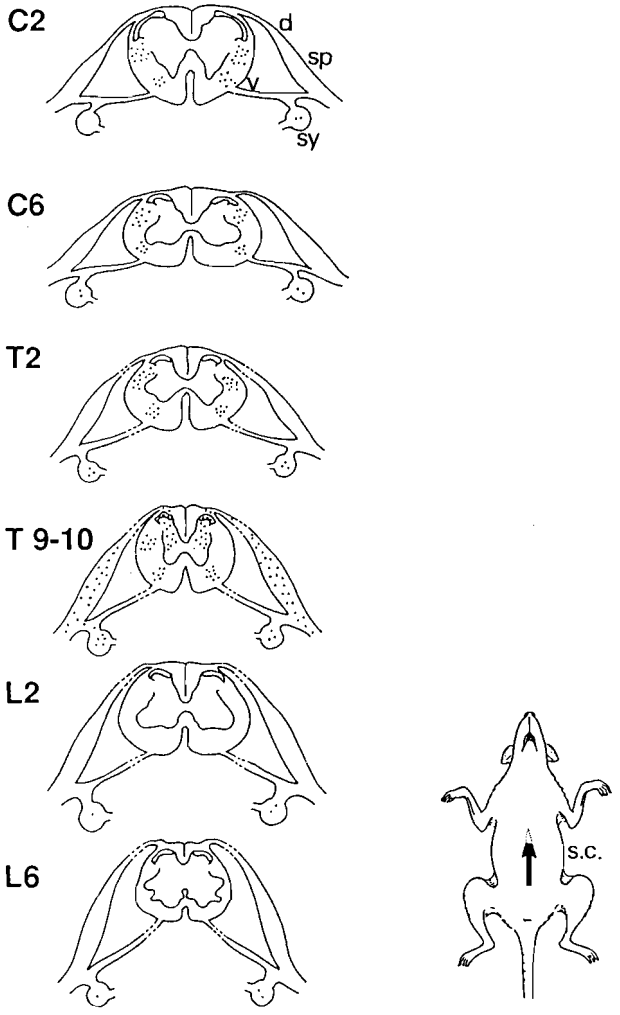
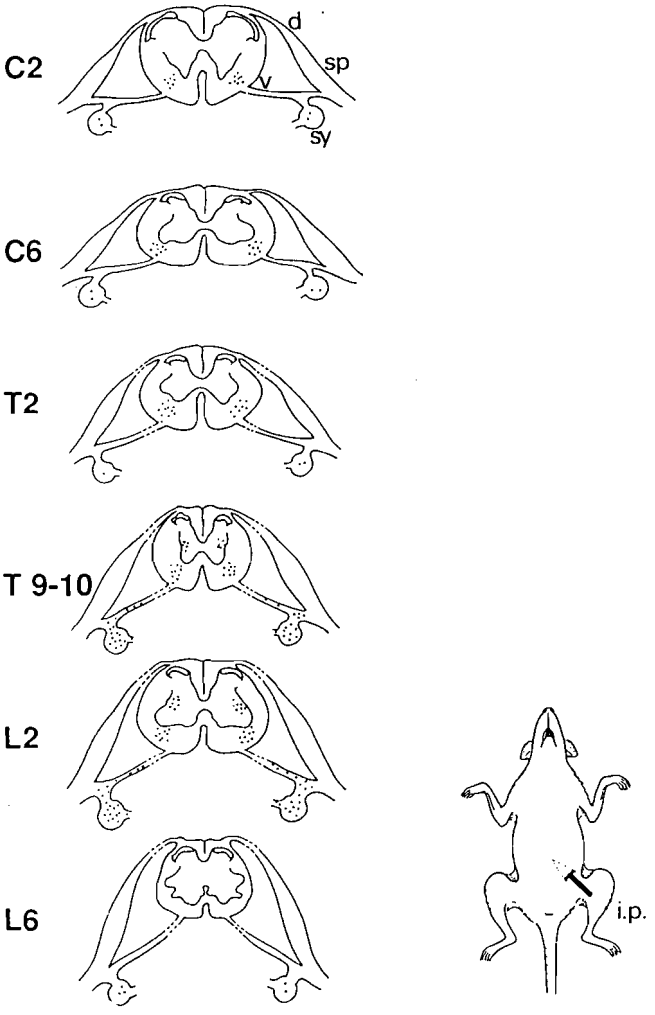


Table 4. Pattern of B virus propagation following i.p. inoculation.



ipsilateral positivity of the dorsal column was most conspicuous. The lateral funiculi and the border between the lateral and anterior funiculi were bilaterally rich in antigen-positive cells. The scanty involvement of the ventral roots and ventral horn motoneurons was most impressive here also (Fig. 4). In the thoracic and cervical segments the funiculi exhibited the same distribution of B virus antigen-bearing cells as in the lumbar segments, while the grey matter was free of antigen (Fig. 7). In the cerebrum the most significant finding was the positivity of the glial cells in the contralateral internal capsule and/or positivity of a few pyramidal neurons in the motor cortex in 3 of 5 animals (Figs. 8, 9). In a single mouse, B virus-bearing neurons were found unilaterally in the N. paraventricularis of the hypothalamus.

Group 3. S.c. inoculation *into the abdominal wall.* Only 3 of 12 animals were found to harbour virus antigen in the spinal cord. In the spinal ganglia (Fig. 10), many ganglion cells contained virus antigen bilaterally in the middle thoracic entry segments. In two animals, spread of infection was symmetrical in the lateral and anterior funiculi. The posterior funiculi remained conspicuously negative. In the third animal, however, the posterior funiculi became even bilaterally involved, most probably due to the infection of the bilateral abdominal muscles together with their sensory terminals.

Group 4. I.p. inoculation. Of 17 mice 7 exhibited marked involvement of the spinal cord. As a rule, virus antigen appeared bilaterally in several sympathetic ganglia and autonomic nerves, and afterwards multisegmentally and bilaterally in the lower thoracic levels of the cord. In typical cases, the dorsal roots, spinal ganglia and posterior funiculi were negative for B virus antigen. In contrast, the borderline between lateral and anterior funiculi was always symmetrically strongly positive (Fig. 12). The lateral funiculus showed bilateral positivity for B virus antigen. In the grey matter the lateral horns harboured

Table 5. Involvement of various neuronal systems depending on the mode of virus inoculation.

	i.m.	s.c.	i.p.
Peripheral motoneuron	-	-	-
Central motoneuron	+	-	-
Proprioceptive system	+	-	-
Spinothalamic system	+	+	-
Autonomic system	+	+	+

many immunoreactive cells. A few cases contained, in addition, a couple of immunoreactive cells in one of the spinal ganglia, dorsal roots and, unilaterally, in the posterior funiculus. The involvement of the latter structures can be explained by the concomitant unilateral infection of the abdominal or paravertebral muscles in the course of i.p. inoculation.

A peculiar feature of this mode of infection was the preference of B virus for propagating along the chain of sympathetic ganglia, reaching even the superior cervical ganglia. In two animals the ganglion of the vagus nerve at the base of the skull was also infected bilaterally. In 3 of 7 animals B virus-positive cells were seen at several sites in the brain stem. In one mouse, positive cells were found unilaterally in the N. paraventricularis of the hypothalamus (Fig. 11).

Histological changes in organs other than the nervous system were scarce or absent. In about 10% of the mice, interstitial pneumonia could be assessed. A few leukocytes of the infiltrates were positive for B virus antigen. Such cells could occasionally be seen in lymph nodes and the thymus (Fig. 6). No histological damage could be seen in the liver.

Electron microscopy

Particles with cores and empty capsids were repeatedly found in glial and neuronal nuclei and perikarya. The appearance of the particles was in accordance with the morphology of herpes viruses (Fig. 13).

Discussion

While the epidemiology and serology of simian and human B virus infections has been extensively studied (Ludwig et al., 1983; Palmer, 1987), little has been done to elucidate their pathogenesis. In the generalized disease, several organs are involved, but the signs of damage to the central nervous system (CNS) govern the symptomatology. Our knowledge about the mode of CNS infection is lacking. The recovery of virus from the trigeminal and other sensory ganglia of rhesus monkeys with latent infection (Boulter, 1975; Vizoso, 1975; Zwartorouv and Boulter, 1984) and the reactivation of mucocutaneous lesions in these animals indicate that B virus, like HSV, can travel along axis cylinders.

In pathogenetic studies of viruses using the neural pathway for their spread, the comparative study of spread following various modes of inoculations is a very convenient technique, as has also been done with HSV type 1 and 2 (Hill et al., 1972; Cook and Stevens, 1973; Renis et al., 1976; Anderson and Field, 1983; Georgsson et al., 1987; Ugolini et al., 1989). In the present study, B virus was inoculated i.m. into the upper or lower extremity, s.c. to the abdominal wall and into the peritoneal cavity. After each mode of inoculation a characteristic pattern of spread emerged. Analysis of these patterns indicates that B virus spreads axonally and transsynaptically. Spread of a virus can best be tracked by immunohistochemical demonstration of viral antigens. Inoculation of virus into a distal axonal segment is followed by retrograde axonal transport of the agent. This phase, however, cannot be detected immunohistochemically since the amount of ascending virus is just sufficient to confer infection. Subsequently, when virus is replicated in the perikaryon, part of the virus progeny starts a centrifugal (orthograde) migration along the axon. In this phase, axonal presence of the virus can already be shown with immunohistochemical or electron microscopic techniques (Gosztonyi, 1979). The amount of herpes viruses, however, even in this phase is not sufficient for their immunohistochemical demonstration. Nevertheless, all the herpes viruses have the particular feature that a part of them leaves the axon while wandering in an orthograde direction, resulting in a limited infection of the neighbouring oligodendrocytes, astrocytes or Schwann cells (Lascano and Berria, 1980; Martin and Dolivo, 1983; Stroop et al., 1984). In nonmyelinated fibres, this shedding of virus can continue along the entire length of the axon; in myelinated fibres it only occurs at the nodes of Ranvier. The infected sheath cells and astrocytes express viral antigen, so that the spread of herpes viruses can be followed. In the present study, in the case of i.m. inoculation in the fore- or hindlimb (Groups 1 and 2), the virus was taken up by axons of sensory neurons innervating muscle spindles, tendon organs and by receptors of deep cutaneous sensibility. Accordingly, the viruses spread along the ipsilateral dorsal column and, bilaterally, in the anterolateral funiculus (Tables 1, 2). To reach the latter systems, the viruses must pass a synapse and infect second order sensory neurons in their segments of entry. The ascending axons of the latter carry the virus further by orthograde axonal transport. The positivity for viral antigen in the anterolateral funiculi was bilateral and more or less symmetrical. This can be explained by the fact that the main central projection,

the spinothalamic system, contrary to the «classical» concept, is both contra- and ipsilateral in the rat (Granum, 1986; Kemplay and Webster, 1986) and, most probably, also in the mouse. Another important projection, the spino-reticular pathway, also has bilateral connections to the brain stem. A further important route for the centripetal spread of virus from the muscle to the spinal cord constituted the postganglionic and preganglionic sympathetic neurons. The positivity of the sympathetic ganglia, of the rami communicantes, and, frequently, of the intermediolateral column in the spinal grey matter document this mode of spread. The rich autonomic plexuses offer an extraspinal pathway of virus spread (Table 2).

In spite of i.m. inoculation, the virus did not enter the terminal axonal segments of motoneurons, as indicated by the overwhelming negativity of their perikarya in the entry segments. The exceptional positivity of a few small ventral horn neurons can be explained by the spread of infection through reflex collaterals of primary sensory neurons ipsi- but also contralaterally. The s.c. inoculation of virus into the abdominal wall (Group 3) resulted in a bilateral entry of virus through multiple sensory roots into the spinal cord and symmetrical ascending spread in the anterolateral funiculi (Table 3). Except for animals in which the abdominal muscles had also become infected, the dorsal columns were spared, i.e. virus spread was restricted to the spinothalamic and spinoreticular systems. Postganglionic and preganglionic sympathetic neurons were also infected, obviously through their branches innervating cutaneous blood vessels and exocrine glands.

Following i.p. inoculation virus spread was mainly restricted to the autonomic nerves, ganglia and their bilateral central connections (Table 4). The few cases in which infected Schwann cells could be shown in the ventral roots, signalling the entry of postganglionic fibres, belonged to this group. The bilateral positivity of the intermediolateral column, the site of perikarya of preganglionic autonomic neurons, was most prominent. An involvement of these neurons has also been described after i.p. infection of HSV type 1 (Irie et al., 1989). In the white matter, the symmetrical positivity of the transition between lateral and anterior funiculi, present also in the other groups, was most conspicuous in these mice with i.p. inoculation (Table 4). This area, besides spinothalamic projections, may accommodate a central autonomic pathway. Apart from these "pure" i.p. animals there were a few others, in which an additional infection of subcutaneous and/or muscle tissue resulted in the involvement of proprioceptive neurons and of those with spinothalamic projections.

As can be seen, at the spinal level the proprioceptive, superficial sensory, autonomic and the motor neuronal systems exhibit individual, specific patterns of involvement depending on the mode of inoculation (Table 5). A very similar behaviour of these neuronal types has been revealed in a excellent analytical study on the infection of the trigeminal nerve and ganglion with *Herpesvirus suis* (Martin and Dolivo, 1983). The detectability of virus antigen at multiple sites in the brain stem can be explained by the abundance of spinal connections to these segments of the neuraxis and by direct spread along the vagus nerves in the i.p. group, just as in the case of HSV type 1 infection (Irie et al., 1989). In contrast, virus antigen-bearing cells have never been seen in the cerebellum. In the cerebrum, virus infection was rare and restricted to a few areas. Infection of the thalamus was observed in two animals and was bilateral of the thalamus was observed in two animals and was bilateral in one, unilateral in the other. Virus reached the thalamus by the aid of the orthograde axonal transport in spinothalamic neurons, using their bilateral projections (Granum, 1986; Kemplay and Webster, 1986). Spread of the virus to the contralateral motor cortex through the internal capsule was the result of the retrograde axonal transport. Infection of the pyramidal neurons of the motor cortex was present only in the i.m. groups, where the inoculation was unilateral. The fact that the peripheral motoneurons were hardly ever infected does not rule out the involvement of central motoneurons. Virus could reach the terminals of the latter through the reflex collaterals of proprioceptive neurons. Interestingly, the spinal segments of the pyramidal tracts, located in the rat and mouse in the ventral parts of the dorsal columns, were not positive in these cases, in contrast to the positivity of this tract within the internal capsule. Evidently, the "visible" phase of axonal spread started only after the produciton of virus progeny in the perikarya of pyramidal neurons in the motor cortex. The orthograde spread of newly produced virus could not yet reach the spinal segements of the tracts. HSV will also be carried to the motor cortex in a similar manner by retrograde transport after footpad inoculation (Lascano and Berria, 1980) or after inoculation of the contralateral ulnar, median or sciatic nerves in rats (Ugolini et al., 1989).

Another example for the retrograde axonal transport of B virus is its spread to the hypotalamus. In two animals the paraventircular nucleus was positive unilaterally, in one, the laterodorsal hypothalamic neurons wre immunoreactive bilaterally. A direct descending connection to spinal preganglionic sympathetic neurons originates from both the paraventricular nucleus (Swanson and Kuypers, 1980; Tucker and Saper, 1985; Hosoya and Kohno, 1987) and the dorsal lateral hypothalamic area (Haring and Davis, 1983). These three examples demonstrate the feature of axonal virus spread, viz., it can span over great distances in the central and peripheral nervous system (Kristensson et al. 1978). Interestingly, virus can spread the fastest along direct mononeuronal pathways. Polysynaptic connections could not yield virus when central terminations of motor, sensory and autonomic neurons with monosynaptic connections were already infected.

The events of B virus infection in the mouse nervous system can best be explained in terms of

axonal and transsynaptic (transneuronal) virus spread. The spread of B virus is strictly bound to neuronal systems, it ensues according to the natural anatomical connections of the neurons infected first at the periphery. The strict confinement of virus spread to definite neuronal systems is the most important proof for the axonal transport and synaptic transfer of B virus. The «lateral» spread of virus to sheath cells and astrocytes is limited in space; the main pathogenetic event is the infection of the neurons. The time relationships also advocate the axonal spread; a spread along a row of sheath cells (Narang and Codd, 1978) would need multiple replication cycles and result in a much slower spread. The strict correlation of distribution of viral antigen with neuronal systems excludes a hematogenous spread of virus from the peripheral inoculation site to the CNS. Studies of the spread of HSV type 1 and 2 after peripheral inoculation have led to the same conclusion (Lascano and Berria, 1980; Georgsson et al., 1987).

Why are synapses particularly suitable for virus transfer from one neuron to another? The main reason is that with the exception of naked axons the synapses are almost the only spots where neurons are in intimate contact with each other. The perikarya and dendrites are covered with astrocytic processes, satellite oligodendroglia, and a great number of axons are ensheathed by oligodendroglia and/or Schwann cells. Furthermore, synaptic areas are metabolically very active, as they are the sites of frequent membrane transformations and various endocytotic mechanisms. Since impulse transmission is mediated by neurotransmitters, synaptic sites are rich in specific receptors for binding and recycling these mediator molecules. The synaptosomal fraction of brain tissue of rodents showed a high binding capacity to HSV, indicating a high density of specific receptors for this virus in these structures (Vahlne et al., 1978). A certain electivity in the involvement of neuronal systems is a further peculiar feature of B virus spread. At the periphery, this trend is manifested by the preferential involvement of sensory (both superficial and deep) and autonomic (sympathetic) systems and the sparing of the peripheral motoneuron (Table 5). This concept, however, contradicts Kristensson's observation (1970), who found the motoneurons of the spinal cord directly affected after i.m. inoculation of HSV in suckling mice. In another study α-motoneurons were found to be infected and gamma-motoneurons spared after inoculation of *Herpesvirus suis* into the masseter muscle of rats (Martin and Dolivo, 1983).

In spite of the rich and direct connections of the spinal cord to the cerebellum, in our experiments the virus did not spread to this part of the brain. Direct inoculation of HSV type 1 to the cerebellum of mice also exhibited a very low affinity to this formation in contrast to hippocampal inoculations (McFarland and Hotchin, 1987). A similar electivity towards certain neuronal systems has been described in experiments with stereotaxic inoculation of HSV into the neostriatum (Bak et al., 1977).

One factor to explain an elective involvement of neuronal systems is the kind of receptors distributed on their cell membranes. The specific distribution of neuronal systems working with specific neurotransmitters has led to a tentative explanation of the elective vulnerability of CNS areas towards various viral infections by the topographically different distributions of neurotransmitter receptor sites (Gosztonyi and Ludwig, 1984; Gosztonyi, 1985). Rabies virus shows a high affinity towards acetylcholine receptors (Lentz et al., 1982), and Borna virus towards aspartate and glutamate receptors (Gosztonyi and Ludwig, 1984; Gosztonyi, 1985; Ludwig et al., 1988).

The patterns of virus spread may depend on many factors, such as age, viral strains, infectious units and immunological responsiveness. However, in order to study the mechanisms of spread, a certain optimum of these factors must be achieved to establish a suitable experimental model. Animals of different ages were infected in this experimental series to find an optimal constellation for the study of propagation patterns. The 10-day-old age group proved to be the most suitable for the study. The older age groups, due to abortive infections and recovery following CNS infections, yielded very little conclusive evidence. In the 10-day-old group the choice of virus strain and of the infectivity units were optimal and the immune reaction did not seriously interfere with virus spread. Thus, further experiments using variables of these parameters did not seem necessary.

To assess the influence of virus strains we correlated our results with pathogenetic studies done with other types of herpes viruses (Renis et al., 1976; Kristensson et al., 1978; Lascano and Berria, 1980; Anderson and Field, 1983; Georgsson et al., 1987; Ugolini et al., 1989). Such comparisons indicate that the basic mechanisms in the neural spread of these agents are very similar. Minor differences in the spreading patterns are most probably due to different intensities of affinities toward specific surface receptors (Vahlne et al., 1978) that may result in slightly different distribution patterns or in a different sequence of involvement of neuronal systems.

The fact that B virus propagates intraaxonally has important implications as to the natural infection of macaques and laboratory infections of humans. Following a bite, in the non-immune individual the B virus binds quickly to neural elements and thereafter it travels axonally and transsynaptically in the nervous system. Once the virus is inside a neuronal chain, the immune system has very little influence on the outcome of the infection. The situation is very similar to that seen in infections with rabies virus, which also propagates axonally and transsynaptically (Gosztonyi, 1986). In many cases of natural infection, post-exposure anti-rabies vaccination cannot prevent the fatal outcome of the disease (Wunner, 1987; Charlton,

1988). However, somewhat more immune control can be experienced in B virus infection than in rabies: mortality of human cases is «only» about 80% (Palmer, 1987), and in our experimental series several older animals also recovered from CNS infection. Macaques with persistent trigeminal infection and periodic oropharyngeal herpetic manifestations possess a partial immunity so that a fatal encephalitis (just as human herpes simplex encephalitis) is a most infrequent event. In addition, B virus is indigenous in rhesus monkeys, like HSV in man. Well adapted viruses, as a rule, do not kill their hosts.

Acknowledgements. Thanks are due to Mrs. Renate Ehrnsperger and Ms. Michaela Schulze for their skillful technical assistance. This research was supported by the Deutsc Forschungsgemeinschaft; Grant No. Go 426/3-1.

References

Anderson J.R. and Field H.J. (1983). The distribution of herpes simplex Type 1 antigen in mouse central nervous system after different routes of inoculation. J. Neurol. Sci. 60, 181-195.

Bak I.J., Markham C.H., Cook M.L. and Stevens J.G. (1977). Intraaxonal transport of herpes simplex virus in the rat central nervous system. Brain Res. 136, 415-429.

Boulter E.A. (1975). The isolation of monkey B virus (Herpesvirus simiae) from the trigeminal ganglia of a healthy seropositive rhesus monkey. J. Biol. Stand. 3, 279-285.

Charlton K.M. (1988). The pathogenesis of rabies. In: Rabies. Campbell J.B. and Charlton K.M. (eds). Kluwer Academic Publishers. Boston. pp 101-150.

Cook M.L. and Stevens J.G. (1973). Pathogenesis of herpetic neuritis and ganglionitis in mice: Evidence for intraaxonal transport of infection. Infect. Immun. 7, 272-288.

Falke D. (1961). Isolation of two variants with different cytopathic properties from a strain of herpes B virus. Virology 14, 492-495.

Falke D. (1964). Über die serologischen Beziehungen zwischen B- und Herpes simplex virus in der Komplementbindungsreaktion. Z. Hyg. Infektionskrkh. 150, 185-195.

Georgsson G., Martin J.R., Stoner G.L. and Webster H. deF. (1987). Virus spread and initial pathological changes in the nervous system in genital herpes simplex virus type 2 infection in mice. A correlative immunohistochemical, light and electron microscopic study. Acta Neuropathol. 72, 377-388.

Goodpasture E.W. and Teague O. (1923). The transmission of the virus of herpes febrilis along sensory nerves with resulting unilateral lesions in the central nervous system in the rabbit. Proc. Soc. Exp. Biol. Med. 20, 545-547.

Gosztonyi G. (1979). Possible mechanisms of spread of fixed rabies virus along neural pathways. In: Proceedings of the 4th Munich Symposium on «Mechanisms of Viral Pathogenesis and Virulence». June 6-7, 1979. Bachmann P.A. (ed). Munich. pp 323-345.

Gosztonyi G. and Ludwig H. (1984). Neurotransmitter receptors and viral neurotropism. Neuropsychiatr. Clin. 3, 107-114.

Gosztonyi G. (1985). Uber die elektive Vulnerabilität (spezielle Neurotropie) bei Virusinfektionen des Nervensystems. In: Drittes Neuropathologisches Symposion im Bezirkskrankenhaus Haar. Frydl V. (ed). Haar bei München. pp 69-85.

Gosztonyi G. (1986). Verbreitung von Viren entlang Neuronennetzen durch transsynaptische Passage - Ein Beitrag zur Pathogenese der Tollwut. Tierärtztl. Prax. 14, 199-204.

Granum S.L. (1986). The spinothalamic system of the rat. I. Locations of cells of origin. J. Comp. Neurol. 247, 159-180.

Haring J.H. and Davis J.H. (1983). Acetylcholinesterase neurons in the lateral hypothalamus project to the spinal cord. Brain Res. 268, 275-283.

Hill T.J., Field H.J. and Roome A.P.C. (1972). Intra-axonal location of herpes simplex virus particles. J. Gen. Virol. 15, 235-255.

Hosoya Y., Ito R. and Kohno K. (1987). The topographical organization of neurons in the dorsal hypothalamic area that project to the spinal cord or to the nucleus *raphé pallidus* in the rat. Exp. Brain Res. 66, 500-506.

Irie H., Harada Y., Yoshihashi H., Kimura T., Kojima M., Kataoka M., Saito M., Sugawara Y. and Mori W. (1989). Spread of herpes simplex virus type-1 (Miyama +GC strain) to the central nervous system after intraperitoneal inoculation: the role of the myenteric plexus of the gut. Arch. Virol. 105, 247-257.

Keeble S.A. (1960). B virus infection in monkeys. Ann. N.Y. Acad. Sci. 85, 960-969.

Kemplay S.K. and Webster K.E. (1986). A qualitative and quantitative analysis of the distributions of cells in the spinal cord and spinomedullary junction projecting to the thalamus of the rat. Neuroscience 17, 769-789.

Kristensson K. (1970). Morphological studies of the neural spread of herpes simplex virus to the central nervous system. Acta Neuropathol. 16, 54-63.

Kristensson K., Vahlne A., Persson L.A. and Lycke E. (1978). Neural spread of herpes simplex virus types 1 and 2 in mice after corneal or subcutaneous (footpad) inoculation. J. Neurol. Sci. 35, 331-340.

Lascano E.F. and Berria M.I. (1980). Histological study of the progression of Herpes simplex virus in mice. Arch. Virol. 64, 67-79.

Lentz T.L., Burrage T.G., Smith A.L., Crick J. and Tignor G.H. (1982). Is the acetylcholine receptor a rabies virus receptor? Science 215, 182-184.

Loomis M.R., O'Neill T., Bush M. and Montali R.J. (1981). Fatal herpesvirus infection in patas monkeys and a black and white colobus monkey. J. Amr. Vet. Med. Assoc. 179, 1236-1239.

Ludwig H., Pauli G., Gelderblom H., Darai G., Koch H.-G., Flügel R.M., Norrild B. and Daniel M.D. (1983). B virus *(Herpesvirus simiae).* In: The Herpesviruses. Vol. 2. Roizman B. (ed). Plenum Press. New York. pp 385-428.

Ludwig H., Bode L. and Gosztonyi G. (1989). Borna Disease: A persistent virus infection in the central nervous system. Prog. Med. Virol. 35, 107-151.

Martin X. and Dolivo M. (1983). Neuronal and transneuronal tracing in the trigeminal system of the rat using the *Herpes*

virus suis. Brain Res. 273, 253-276.

McFarland D.J. and Hotchin J. (1987). Contrasting patterns of virus spread and neuropathology following microinjection of herpes simplex virus into the hippocampus or cerebellum of mice. J. Neurol. Sci. 79, 255-265.

Narang H.K. and Codd A.A. (1978). The pathogenesis and pathway into the central nervous system after intraocular infection of herpes simplex virus type I in rabbits. J. Neuropathol. Appl. Neurobiol. 4, 137-150.

Palmer A.E. (1987). B virus, *Herpesvirus simiae*: Historical perspective. J. Med. Primatol. 16, 99-130.

Renis H.E., Eidson E.E., Mathews J. and Gray J.E. (1976). Pathogenesis of herpes simplex virus types 1 and 2 in mice after various routes of inoculation. Infect. Immun. 14, 571-578.

Stroop W.G., Rock D.L. and Fraser N. (1984). Localization of herpes simplex virus in the trigeminal and olfactory systems of the mouse central nervous system during acute and latent infections by *in situ* hybridization. Lab. Invest. 51, 27-38.

Swanson L.W. and Kuypers H.G.J.M. (1980). The paraventricular nucleus of the hypothalamus: Cytoarchitectonic subdivisions and organization of projections to the pituitary, dorsal vagal complex, and spinal cord as demonstrated by retrograde fluorescence double-labelling methods. J. Comp. Neurol. 194, 555-570.

Tucker D.C. and Saper C.B. (1985). Specificity of spinal projections from hypothalamic and brainstem areas which innervate sympathetic preganglionic neurons. Brain Res. 360, 159-164.

Ugolini G., Kuypers H.G.J.M. and Strick P.L. (1989). Transneuronal transfer of herpes virus from peripheral nerves to cortex and brainstem. Science 243, 89-91.

Vahlne A., Nyström B., Sandberg M., Hamberger A. and Lycke E. (1978). Attachment of herpes simplex virus to neurons and glial cells. J. Gen. Virol. 40, 359-371.

Vizoso A.D. (1975). Recovery of Herpesvirus simiae (B virus) from both primary and latent infections in rhesus monkeys. Br. J. Exp. Pathol. 56, 485-488.

Wunner W.H. (1987). Rabies viruses - Pathogenesis and immunity. In: The Rhabdoviruses. Wagner R.R. (ed). Plenum Press. New York. pp 361-426.

Zwartorouv H.T. and Boulter E.A. (1984). Excretion of B virus in monkeys and evidence of genital infection. Lab. Anim. 18, 65-70.

Accepted June 20, 1991