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Acute and Persistent Viral Infections of Differentiated Nerve Cells

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Within the nervous system the highly specialized structure and function of nerve cells renders the pathogenesis of viral infections amazingly complex. In vivo and in vitro studies reveal that viruses may display tropism for distinct types of cells such as neurons, myelin-forming cells, or astrocytes. In neurons, RNA viruses mature in the cell body and in dendrites close to synapses, from which they can spread to synaptic endings. Undefined host factors and stage of differentiation may favor defective viral assembly, which, in turn, results in persistent infections of neurons. In myelin-forming cells, lytic infection results in degeneration of myelin and, consequently, in altered conduction in those axons that are ensheathed by a myelin-forming cell. In addition, breakdown of myelin may induce an autoimmune response, which then leads to further demyelination. Autoimmune demyelination may also occur when glial cells other than myelin-forming cells are infected. Astrocytes are prone to persistent infection or viral transformation.

The highly specialized structure and function of nerve cells give a unique imprint to interactions between viruses and host cells in the nervous system. As reviewed recently by Johnson [1], the selective vulnerability of distinct nerve cells to viruses is based on two simple principles: that viruses can show tropism restricted to one type of cell; and that viruses can show various degrees of virulence in each type of cell. Since the nervous system (NS) contains several morphologically and functionally distinct types of cells, the pathological lesions in viral infections are amazingly diverse. From the interaction between virus and receptor to the entry, uncoating, transcription, translation, and assembly of the virus, any step may fail to occur. The virus can produce lysis of the nerve cell or spare the morphologic integrity of the cell while altering its functions by virus-specific factors. In some cases, viral genomes can become sequestered in the cell, a situation that can lead to transformation [1].

When a virus infects a differentiated nerve cell, some specific events occur that can be studied both in vivo and in vitro with combined morphologic and immunolabeling methods. In this brief review, we first describe the structure of the three major types of nerve cells, then summarize the methodology used to examine viral infections of these cells, and, finally, review a few situations

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in which a virus can affect the structure and function of differentiated nerve cells.

Structure of Nerve Cells

Three major types of differentiated cells are present in the parenchyma of the peripheral nervous system (PNS) and of the central nervous system (CNS): neurons, myelin-forming cells, and astrocytes. All three exchange trophic, mitotic, and differentiating influences (figure 1). Mature neurons are postmitotic cells; the other cells can be triggered to division and repair. Brain ependymal and endothelial cells, which have important functions in filtering nutritive substances from cerebrospinal fluid and blood, are not considered here.

Neurons

Neurons assure communication through synapses, where the release of neurotransmitters from synaptic vesicles triggers receptors on the specialized postsynaptic membrane (figure 1). There are at least 15–20 different neurotransmitters in the mammalian brain. Therefore, the function of a neuron depends not only on its location and specific connections but also on the transmitters it synthesizes [2]. The neuron has two types of processes: axon and dendrite (figure 1). The axon can be extremely long and contains a rigid cytoskeleton with neurotubules, neurofilaments, mitochondria, and smooth endoplasmic reticulum (ER) cross-linked by a microtrabecular network [3]. Axons do not contain rough ER. They terminate in a synaptic ending, a specialized region of contact with the dendrite of another neuron (or with a target cell). A unique bidirectional cytoplasmic transport system operates in the axon [4]. The anterograde flow appears to convey major skeletal elements down the axon at a slow speed (1-4 mm/day) and various other materials (such as calcium, proteins, and transmitters) associated with smooth ER at a fast speed (100–400 mm/day). The fast retrograde flow brings up the axon endogenous and exogenous materials, which may influence macromolecular synthesis in the neuron soma. Most neurons have several relatively short dendritic processes, which often branch. Dendrites contain organelles, including rough ER [5], and terminate at the postsynaptic membrane, which receives input from axons. Not much is known about cytoplasmic flow in dendrites.

Astrocytes

The brain contains 10 times more astroyctes than neurons; yet the function of astrocytes is still enigmatic. They have "end feet" closely apposed to basement membranes surrounding brain vessels. Astrocytes form gap junctions, which are involved in electrotonic coupling and metabolic cooperation [6]. Intermediate filaments of astrocytes contain glial fibrillary acidic protein (GFAP), which is used as a specific marker for astrocytes [7]. In astrocytic hypertrophy, intermediate filaments containing GFAP increase in number; this is a frequent astrocytic reaction to virtually any type of brain injury. Astrocyte processes surround the neuron soma, axon bundles, and synapses. Astrocytes may exchange with neurons trophic factors as well as hormonal signals. For instance, astrocytes have adrenergic beta receptors, and their response to the neurohormones' adrenergic beta amines might regulate the synthesis of neurotransmitters by neurons [8].

Myelin-Forming Cells

During development of the nervous system, these cells synthesize the myelin sheath that surrounds most CNS axons as well as PNS afferent and efferent fibers. The myelin sheath insulates the axon and, because the sheath is interrupted periodically at the nodes where the axon is exposed, saltatory conduction can take place [9]. The internodal myelin is made up of one spiralled membrane with 20–100 compact layers. This membrane is an extension of the plasma membrane of the myelin-forming cells [5].

There is a fundamental difference between myelin-forming cells of the CNS (oligodendrocytes) and those of the PNS (Schwann cells). One oligodendrocyte can send several processes (up to 20 or more) that myelinate internodes of different axons, whereas one Schwann cell forms only one myelin internode (figure 1) [5]. During myelination, cells can make 1×10^5 μm^2 of myelin membrane per day (quoted in [5]). Oligodendrocytes also produce vast quantities of plasma membrane. In the adult, myelin-forming cells nor-

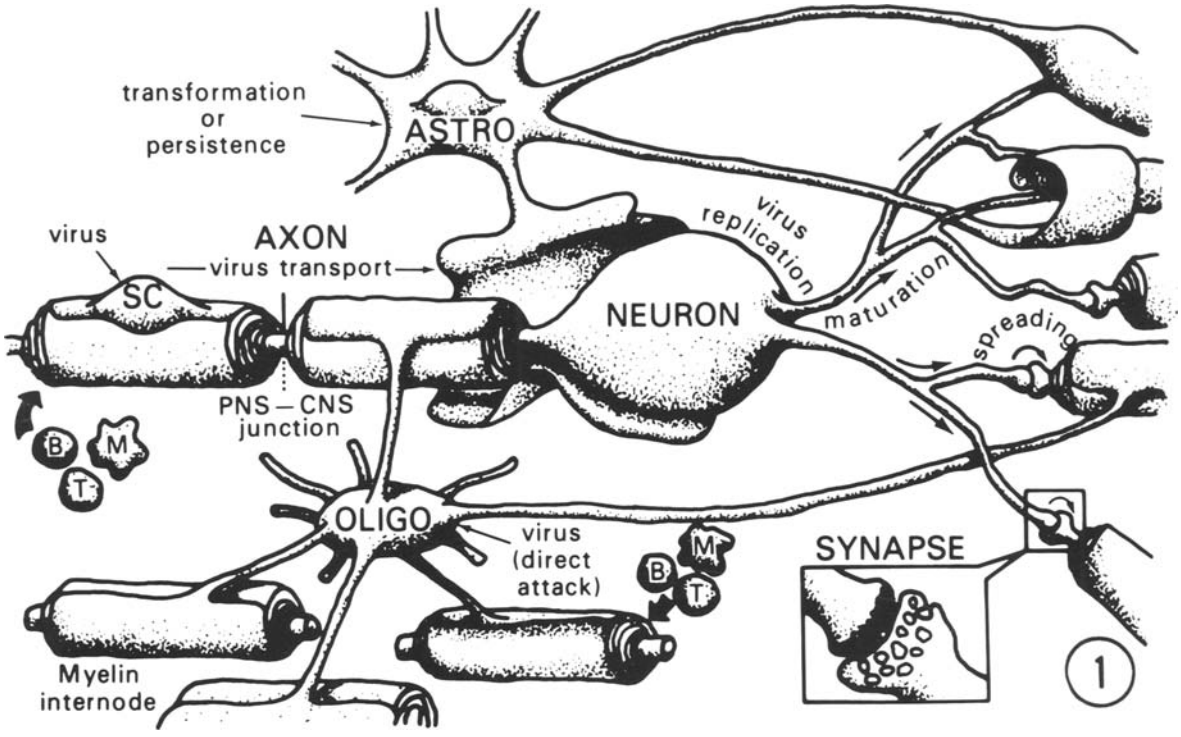


Figure 1. A schematic representation of interactions between viruses and the different types of cells of the nervous tissue parenchyma. "Astro" stands for astrocyte, "oligo" for oligodendrocyte, and SC for Schwann cells. The words associated with arrows in the intercellular space usually refer to viral events occurring in each specific type of cell. Viruses can be transported to the neuronal soma by retrograde axonal flow from the peripheral nervous system (PNS) to the central nervous system (CNS). Replication of viruses and maturation of enveloped RNA viruses can occur in the neuronal soma and along the dendrites as well as close to the synaptic junction. Some viruses can spread from the postsynaptic area to the presynaptic ending. Myelin-forming cells can be directly attacked by viruses, but in some cases sensitized cells, macrophages (M), and B- and T-lymphocytes enhance myelin destruction. Astrocytes are prone to viral persistence and transformation.

mally do not proliferate unless some nerve or brain damage occurs. Regeneration consists of cell migration and mitosis, followed by axon contact and remyelination. It occurs much more readily in PNS than in CNS; Schwann cells can invade the CNS and remyelinate its axons, as seen in some multiple sclerosis cases [10]. Several mitogens for Schwann cells are known [11, 12], whereas mitotic factors for oligodendrocytes have not yet been identified.

Methodology

Direct Examination of Infected Tissue from the Nervous System

In animals (and sometimes in humans), the virus-induced alteration of the nerve cells within the natural organization of the nervous system can be studied directly, so that viral expression can be correlated with disease. Viral antigens can be localized immunocytochemically by light and electron microscopy in two ways. In the pre-embedding staining technique, animals are perfused through the heart with fixatives that preserve viral antigenicity. Nonembedded nervous tissue fragments are cut with a vibratome, and the resulting sections are incubated in antibodies and conjugates, as schematized in figure 2 [13]. Immunolabeled sections are then flat-embedded in plastic for electron microscopic studies. In the postembedding staining technique [14, 15], done on paraffin or epoxy sections, a 1μ section of plastic-embedded nervous tissue is cut and the plastic is etched with sodium ethoxide before incubation with antibodies and conjugates. Such a

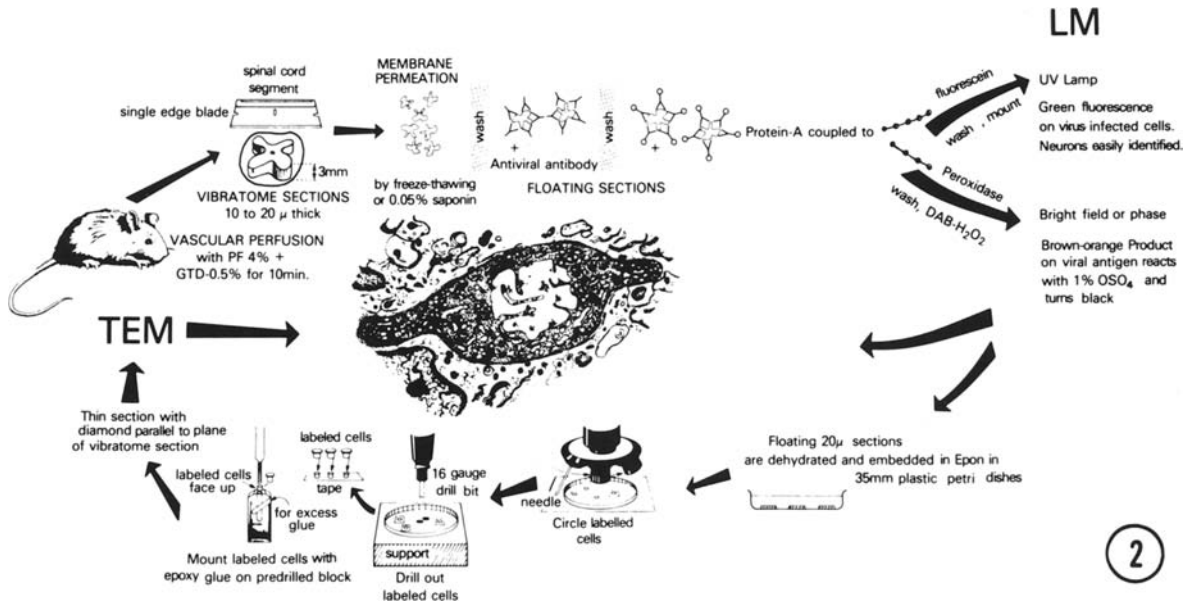


Figure 2. Flow chart of the procedures used to label viral antigen in samples of nervous tissue (in this case, mouse spinal cord) before embedding and sectioning for electron microscopy. This preembedding incubation technique allows good correlation between the light and the transmission electron microscopic localizations of viral antigen because single cells (labeled by the immunoperoxidase technique) can be drilled out of the plastic block and studied by electron microscopy [13] (see also figure 11). DAB-H_2O_2 = diamino-benzidine-peroxide mixture; OSO_4 = osmium tetroxide; GTD = glutaraldehyde; PF = paraformaldehyde.

stained section allows detailed localization of stained antigen in the light microscope and staining for viral- and cell-specific antigens on serial sections. In addition, the ultrastructure of the same cell can be studied on thin sections adjacent to the stained 1μ section.

Cultures of Differentiated Nerve Cells for Infection with Viruses

In vitro systems can allow analysis of different aspects of infections of nerve cells, such as the role of the host cell and its degree of differentiation, the mechanism of viral spreading, and the induction of viral persistence by antiviral antibody or defective interfering viral particles. Explant cultures from the CNS or PNS of rodents [16] partly preserve the three-dimensional organization of the brain and allow myelination. In these cultures, immunolabeling and embedding in situ with scoring of individual cells, as shown in figure 2, are difficult. Similarly, scanning electron microscopy (SEM) is unrevealing because these cultures are often covered with a conjunctive layer.

Cultures of dissociated nerve cells allow good differentiation of neurons and astrocytes but rarely contain myelin [17, 18, 19]. The method for culturing human fetal nerve cells has been developed recently (figure 3) [20]. Because the surfaces of these dissociated nerve cells are directly in contact with the medium, one can easily control viral input, allow interaction with antiviral antibodies, perform surface immunolabeling and SEM studies, and embed in situ and score infected cells (identified in phase microscopy) for thin section and transmission electron microscopy (TEM) studies [21, 22, 23]. In addition, the behavior and motility of living, dissociated nerve cells can be followed for several days with video-intensification microscopy (VIM) [24]. In phase microscopy, VIM reveals changes in shape, movement, migration, mitosis, and fusion. VIM also allows the use of fluorescent probes [25] on living cells before, during, and after viral infection. These probes could consist of the virus itself, virus genome injected into a large neuron, antiviral antibodies, or protein precursors, among others.

Limitations of these primary cultures of dissociated nerve cells are their small quantity and

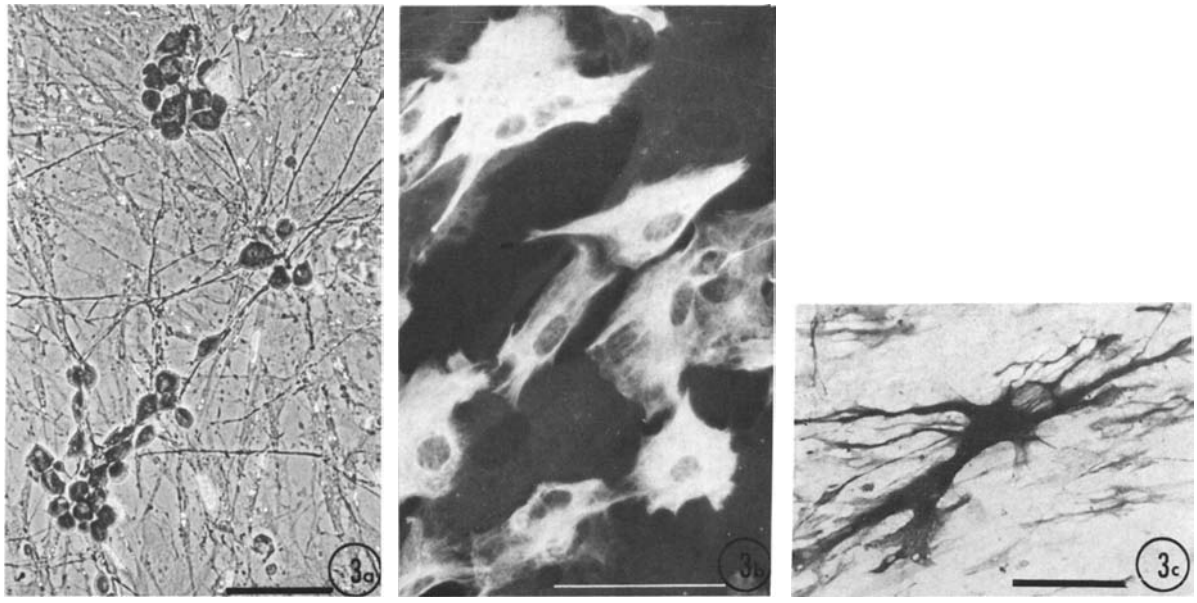


Figure 3. Cultures of dissociated human nerve cells. Figure 3a shows cultures of human fetal dorsal root ganglia. After 10 days of growth in a medium containing mouse nerve growth factor, neurons and their long neuritic extensions stain for neuron-specific enolase, as detected by immunoperoxidase labeling. In figures 3b and 3c, dissociated human spinal cord was cultured for three weeks before the immunostaining of glial fibrillary acidic protein. Figure 3b shows a cluster of astrocytes stained with a rhodamine conjugate, whereas figure 3c shows a more differentiated astrocyte (outside the clusters) stained with peroxidase-labeled protein A. Bar = 100 μm.

heterogeneity. However, viral proteins can be analyzed in such systems. Methods of immunoprecipitation have considerably increased sensitivity and accuracy in the identification of viral proteins, even when small amounts of cellular material are available [26, 27]. In addition, large amounts of pure populations of Schwann cells and astroyctes can be obtained by immune-mediated killing of fibroblasts and mitogenic stimulation in the rat [24, 28]. Biochemical analysis can be more easily performed and combined with sequential immunolabeling and morphologic studies on CNS aggregate cultures from the brains of fetal rats [29, 30]. These aggregates form microspheres 200–500 μm in diameter; their organization and differentiation mimic those of the brain. The cells within these aggregates develop from undifferentiated neuroepithelial cells to a population of morphologically mature neurons, astrocytes, and oligodendrocytes [29]. Synaptic contacts and myelinated axons appear as the cells differentiate. The replication of influenza viruses in nerve cells at various stages of differentiation was studied recently using this system [31], which may provide a powerful tool for neurovirologic investigations.

Viral Infections of Differentiated Nerve Cells

The Neurons

Maturation and propagation of viruses. We assume that viruses are picked up and uncoated at the nerve terminal and that their nucleic acid is transported by retrograde flow to the neuron soma [32]. Viruses such as rabies and herpes simplex (HSV) can replicate outside the nervous system (in a muscle or cornea, for instance) and then centripetally move along the nerves up to the neuron soma (of CNS neurons in rabies, PNS neurons in HSV) [32–35]. Controversy continues concerning the exact site through which HSV spreads along the nerves: inside the nerve fibers or by cell-to-cell transmission in the supporting Schwann cells [36, 37]. Once the virus has started to replicate and accumulate progeny in the neuron soma, mature virions are seen occasionally in axons. A few HSV virions were detected close to the

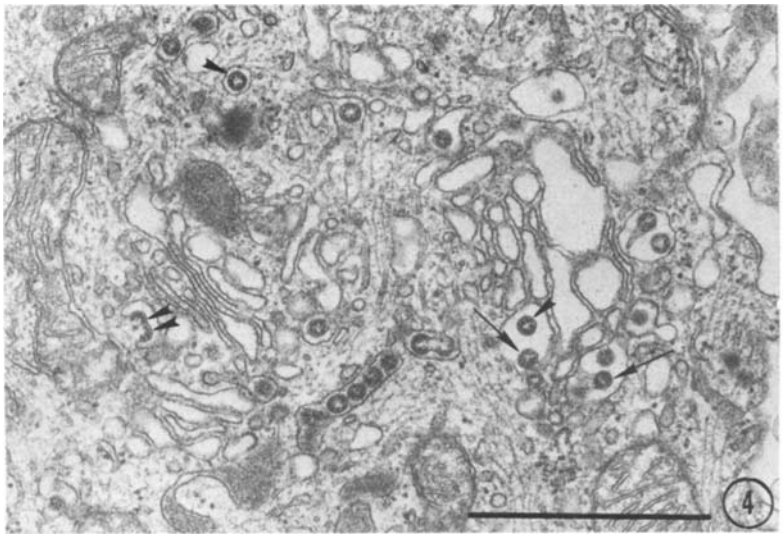


Figure 4. Maturation of a coronavirus (JHM strain of mouse hepatitis virus) in cultures of mouse spinal cord. The virus matures in smooth membrane cisternae closely associated with the Golgi apparatus. Note the budding sites at arrows and the free viruses at arrowheads. Twin budding sites are also seen (double arrowhead). Bar = 100 nm.

sensory neuron soma [38], while rabies virions have been observed at nodes of Ranvier of peripheral fibers [39]. Sites of the assembly and maturation of viruses are rarely recognized in axons, but mature virions can travel in axonal smooth vesicles, probably with the anterograde flow. Intracellular inoculation of viral nucleocapsids into a single cultured neuron might elucidate this point.

Once in the neuron soma, the virus uses the cell machinery to replicate, and different maturation sites are observed for different viruses. For example, the wild-type JHM neurotropic strain of mouse hepatitis virus, a coronavirus, often matures in Golgi-related cisternae [39a] (figure 4), while rhabdoviruses, such as vesicular stomatitis virus (VSV) [22] and rabies [40], and paramyxoviruses, such as measles, produce viral components mostly near the plasma membrane [26] (figures 5 and 6). In these latter cases, viral budding from the neuron plasma membrane often occurs along the entire neuron soma and dendrites (figure 5) [41]. Such viral maturation sites are strongly labeled with specific antiviral antibodies (figure 5, lower inset; figure 6). Finally, reovirus type 3 accumulates clusters of virions in dendrites where particles are closely associated with neurotubules [42].

Three different enveloped RNA viruses, which have a predilection for neurons and cause encephalitis in adult BALB/c mice, can replicate and mature in dendrites close to synapses. In mice infected with a measles neurotropic strain (HNT), some groups of neurons in the brain are selectively infected [43]. Viral antigen accumulates in cortical dendrites, often close to the postsynaptic specialized membrane (figure 7a) [43]. No inflammatory cells or intense lysis of neurons are found in infected areas; yet the affected animals develop convulsions and spastic paralysis. Thus, the virus might have a direct effect on the process of neuron communication at synapses. Similarly, the coronavirus JHM matures inside vacuoles of postsynaptic endings in the spinal cord (figure 7b) [13], and the rhabdovirus R_1-VSV (a revertant of group 1 *ts* mutant of VSV) matures on the side of postsynaptic density in anterior horn cells [44]. Rhabdoviruses sometimes bud directly into a coated pit on the side of the presynaptic ending, as seen in cultured neurons infected with VSV and maintained in medium containing antiviral antiserum (figures 7c and 7d) [23]. In this situation, the virus escapes neutralization by antibody and spreads from postsynaptic to presynaptic terminals. Thus, while electrical and chemical communication between neurons is mostly oriented from pre- to postsynaptic endings, some enveloped RNA viruses can spread "transsynaptically" in the opposite direction and enter through coated pits on the presynaptic terminals. Interestingly, coated pits and vesicles are involved in the recycling of synaptic vesicle membranes [45] and in uptake of trophic factors [46].

Persistence of viruses. Multiple mechanisms

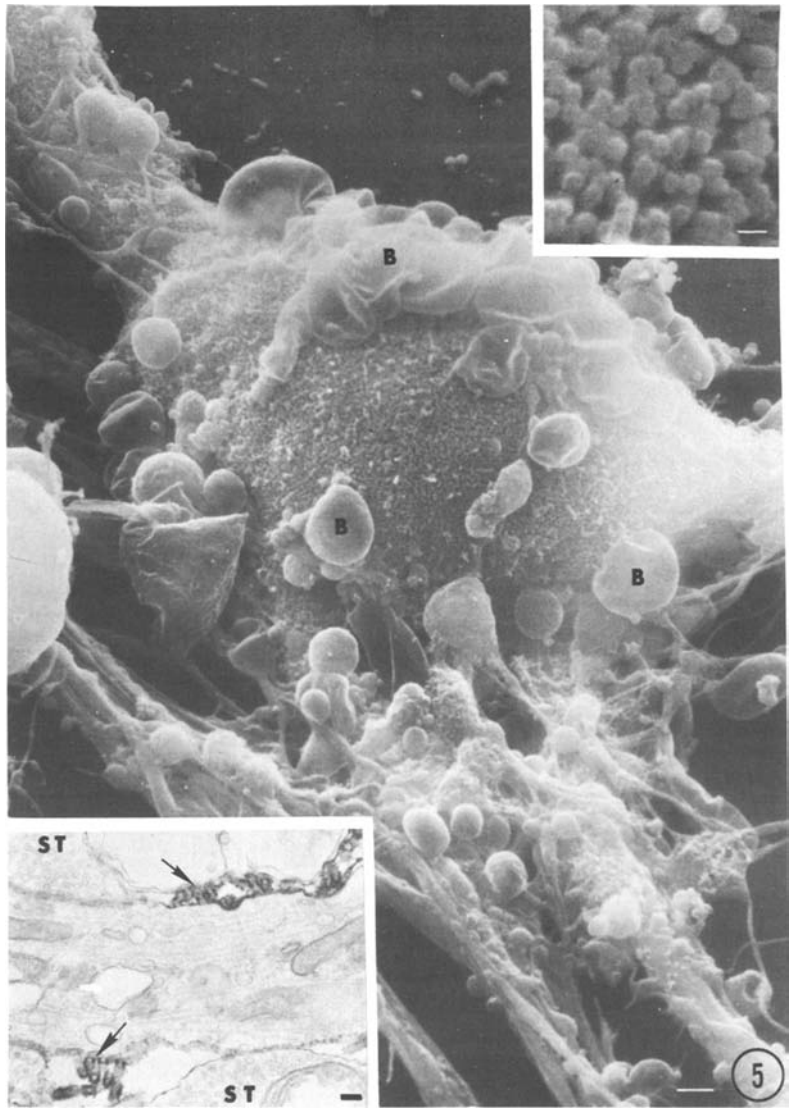


Figure 5. Maturation of a rhabdovirus, vesicular stomatitis virus (VSV), at a mouse neuron membrane in vitro. Scanning electron microscopy demonstrates numerous viruses budding from the entire neuronal surface and along a dendritic process (upper left corner) 24 hr postinfection. There are numerous membrane blebs (B) on the neuron, which shows rounding and retraction. The upper inset shows a detail of the viruses budding from the neuron surface at higher power. The lower inset is a thin section through an infected dendrite to which synaptic terminals (ST) are apposed. Virions and budding particles (arrows) are covered with electron-dense peroxidase product after immunolabeling for vesicular stomatitis virus antigens. Bar = 1 μm; bars in insets = 100 nm.

allow viruses to persist in nerve cells [47]; latency of HSV in sensory ganglia is one of the most intriguing [48]. Viral persistence in differentiated neurons can be induced in vitro, and such systems should allow analysis of how viruses alter specific neuronal markers or functions. Chronic infection of CNS neurons was readily obtained with a sheep retrovirus (visna) inoculated into cultures of mouse spinal cord [21]. This virus produces slow, progressive neuronal fusion with little lysis. Viral-specific proteins are expressed in the virtual absence of viral assembly. These giant neurons are stained by neuron-specific enolase antiserum (figure 8) and maintain synaptic contacts on their processes [21]. These multinucleated neurons represent an attractive tool for electrophysiologic analysis of virus-induced neuronal dysfunction. Alteration of the parameters of action potential has been observed in the rat dorsal root ganglia acutely infected with HSV type 1 [49], but the study of possible dysfunction of chronically infected sensory neurons awaits the development of a model in vitro for HSV latency.

The establishment of viral persistence in CNS neurons often depends on the degree of neuronal differentiation. For instance, the addition of defective interfering particles or antiviral antibodies to wild-type virus results more readily in

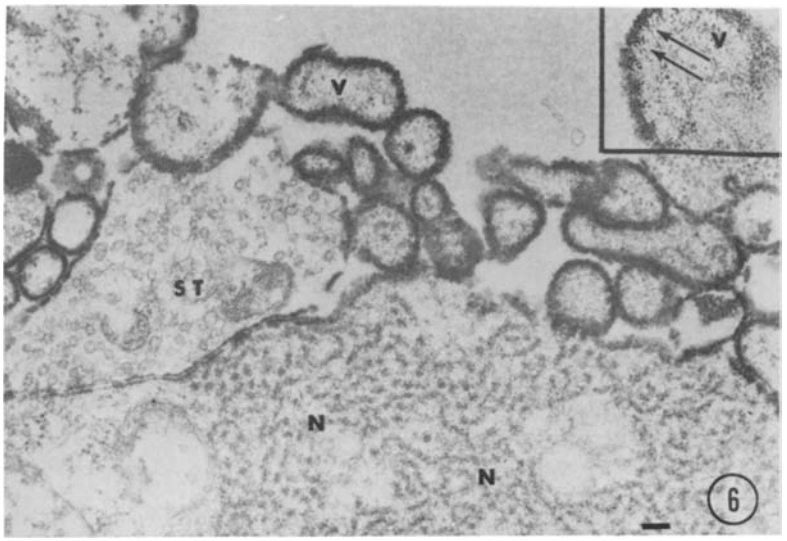
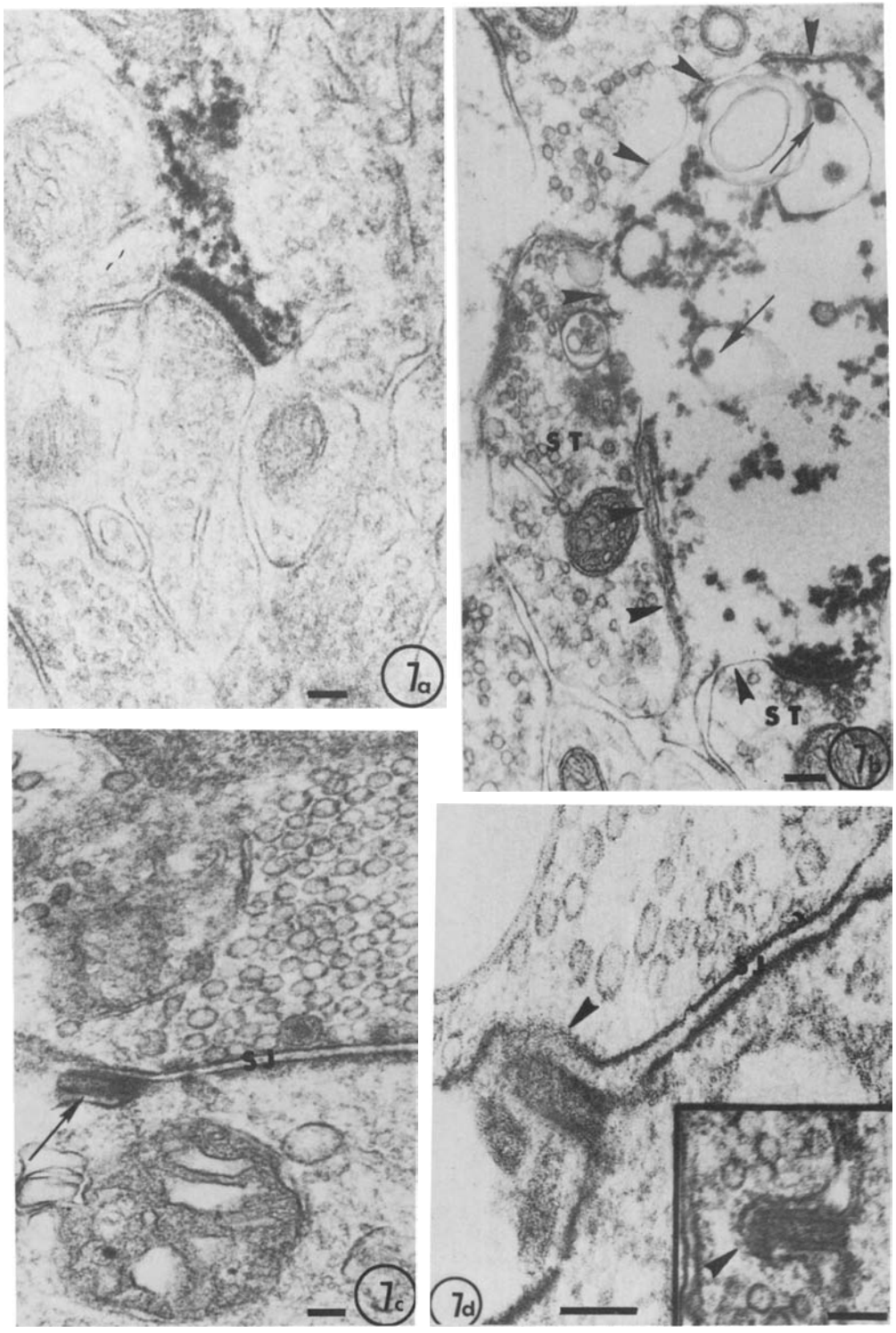


Figure 6. Transmission electron micrograph of a cultured mouse neuron persistently infected with wild-type measles virus at seven days postinfection. The cell surface and virions are covered with electron-dense immunoperoxidase staining for measles antigen. Note the presence of a synaptic terminal (ST) on the left. Numerous viral nucleocapsids (N) accumulate in the neuron cytoplasm right under the membrane, but these nucleocapsids are not aligned under the plasma membrane or incorporated into the viral buds. Compare the virus (V) produced by the neuron with the virus produced by the monkey kidney cells and shown in the inset; nucleocapsids are seen only in the latter (double arrow). Bar = 100 nm.

persistent VSV infection in mature mouse neurons than in undifferentiated cells [22, 23]. Cultures of mouse neurons can be chronically infected with wild-type measles virus, provided infection occurs at a precise stage of neuronal maturation in vitro [26]. These neurons develop intricate dendritic networks and interneuronal contacts (figure 9). However, they contain large inclusions of viral nucleocapsids, and their plasma membranes are covered by viral surface antigens. Infected neurons fail to incorporate nucleocapsids into viral buds (figure 6); hence, no infectious virus is produced. Measles virus polypeptides have been analyzed by polyacrylamide gel electrophoresis after immunoprecipitation of cell lysates by hyperimmune antibody to measles virus or monoclonal antibody to virion hemagglutinin (HA). All viral polypeptides are present, including M protein, which is known to play a role in viral asembly, but HA from infected neurons migrates faster than HA from productively infected Vero cells. This modification could be due to a change in glycosylation, which is an important step in virus maturation, or to an alteration of the nonglycosylated intramembrane portion of the HA protein. The modification impairs neither the binding of the glycoprotein to polyclonal or monoclonal anti-HA antibodies nor the hemadsorbing property of the glycoprotein. However, the altered HA apparently cannot interact normally with internal components of the virion. This abnormality could explain the failure of infected neurons to redistribute antigen-antibody complexes on their plasma membranes [50]. Cocultivation of infected neurons with Vero cells readily results in production of infectious virus and in restoration of the normal electrophoretic mobility of the HA protein. This indicates the major role of the host cell in the establishment of this persistent infection of neurons with measles virus.

Figure 7. Visualization of viral components and viral maturation in dendrites close to synapses. In figure 7a, the cerebral cortex of a BALB/c mouse infected with a neurotropic strain of measles shows accumulation of viral-specific label in postsynaptic areas after indirect immunoperoxidase labeling for measles antigen. Note that the rest of the neuropile is devoid of electron-dense label [43]. In figure 7b, the spinal cord of a BALB/c mouse infected with a coronavirus (JHM strain of mouse hepatitis virus) contains mature virions within dendrites close to synaptic contacts. Black label is seen on virions at arrows, and clusters of viral antigen are scattered inside the postsynaptic area of this dendrite (delimited by arrowheads). Synaptic terminals (ST) around the infected dendrite are devoid of viral-specific label [13]. In figures 7c and 7d, cultures of mouse neurons have been infected with vesicular stomatitis virus and treated with antiviral antibodies for two days. In figure 7c, a virus is budding from a dendrite perpendicular to the plasmalemma surface (arrow) on the side of the synaptic junction (SJ). In figure 7d, a virus on the postsynaptic side is directly budding into a coated pit on the lateral side of the synaptic junction (SJ). Coated pits and vesicles are characterized by a fuzzy material on the cytoplasmic side of their membranes (arrowheads) [23]. Bar = 100 nm.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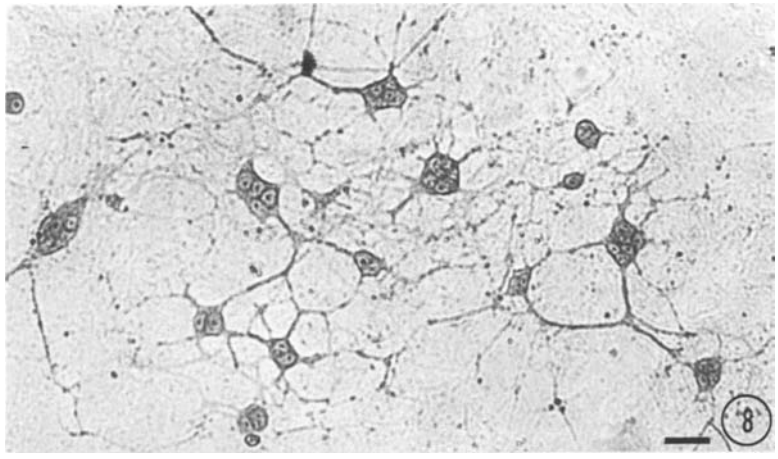


Figure 8. Effect of visna virus (a retrovirus) on cultured mouse neurons inoculated at four weeks of maturation in vitro. Multinucleated neurons progressively formed by fusion during the next three weeks. These giant neurons and their processes are stained here for neuron-specific enolase by the immunoperoxidase method. Bar = 100 μm.

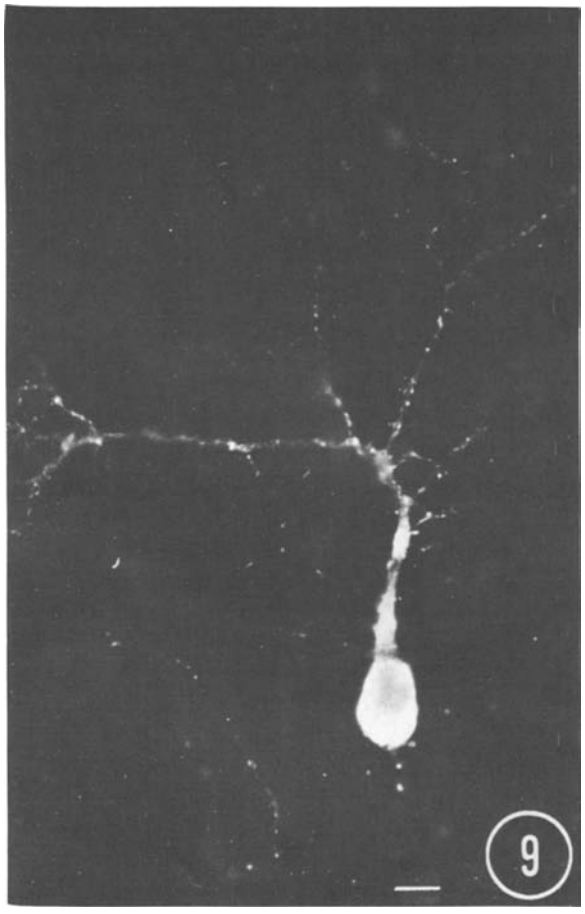


Figure 9. Cultured mouse neuron chronically infected with Edmonston measles virus for three weeks. In this case, the culture was inoculated with the virus after only 10 days of maturation in vitro; immunofluorescence staining for viral antigen was performed three weeks later. This neuron contains measles viral antigen throughout its cytoplasm and dendritic network. Bar = 10 μm.

The Nonneuronal Cells (Myelin-Forming Cells and Astrocytes)

Myelin-forming cells can be directly attacked by lytic viruses, such as JC human papova virus isolated from patients with progressive multifocal leucoencephalopathy (PML) and the JHM strains of coronavirus in mice. Viral destruction of one oligodendrocyte can result in focal demyelination and alteration in the conduction velocities of several axons. In addition, such viruses may interact variably with the myelin membrane itself. In PML, hundreds of particles can accumulate in the nucleus and cytoplasm of one oligodendrocyte [51]. Virions can be easily concentrated and their DNA purified and cloned into a suitable vector directly from the brain without passage in culture [52]. PML virions have occasionally been seen at the intraperiodic line inside the myelin sheath (figure 10) [53], and the infection induces a decrease in immunocytochemical staining of a glycoprotein associated with CNS myelin (MAG) [14]. A temperature-sensitive (*ts*) mutant of JHM mouse coronavirus has tropism restricted to nonneuronal cells, and viral antigen is found in the oligodendrocyte cytoplasm and processes in animals showing demyelination (figure 11) [13]. However, viral antigens have not yet been identified in vivo in the myelin sheath. At semipermissive temperature in vitro, this mutant triggers the formation of membrane whorls by nonneuronal cells and appears defective in assembly [39a].

There are several virus-induced demyelinating diseases in animals in which the attack on myelin appears to be "a bystander effect" (reviewed in [54]). Destruction of myelin-forming cells by a

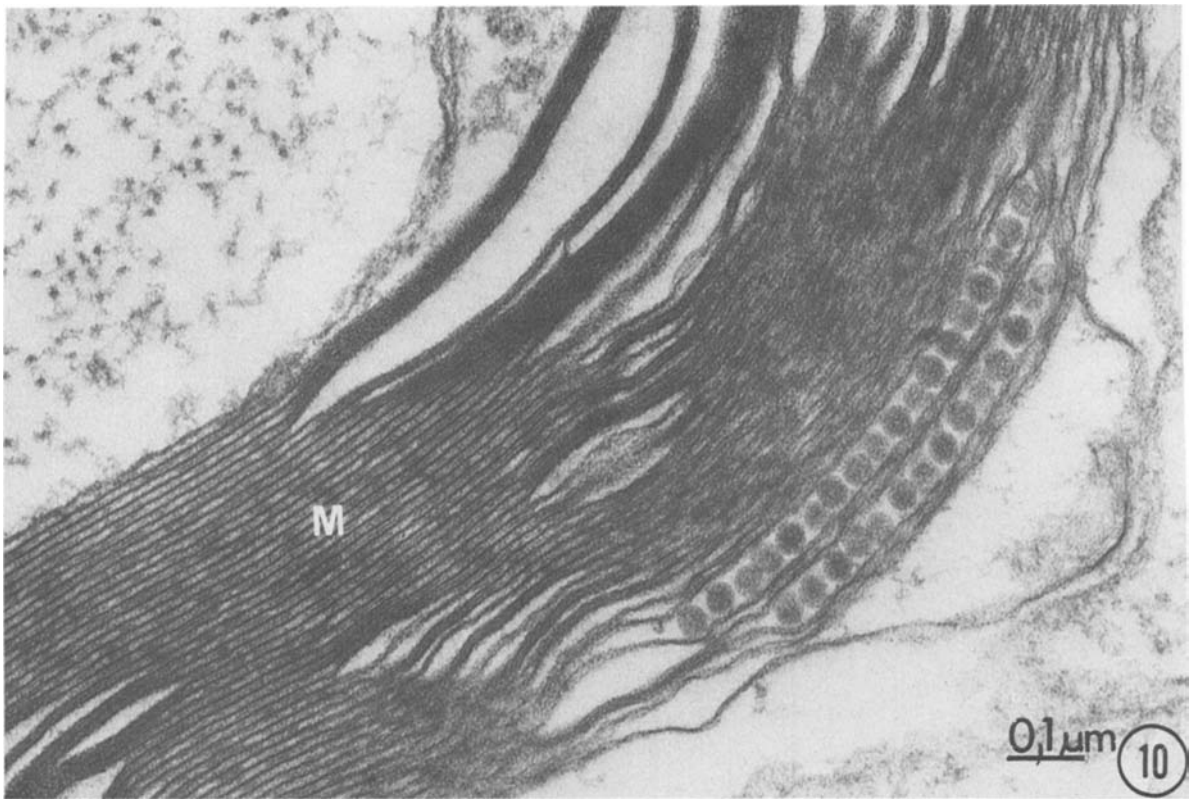


Figure 10. JC papova virions are seen in between the loosened lamellae of myelin sheath (M) in the human brain of a patient with progressive multifocal leucoencephalopathy [53]. Courtesy of Dr. M. Mazlo.

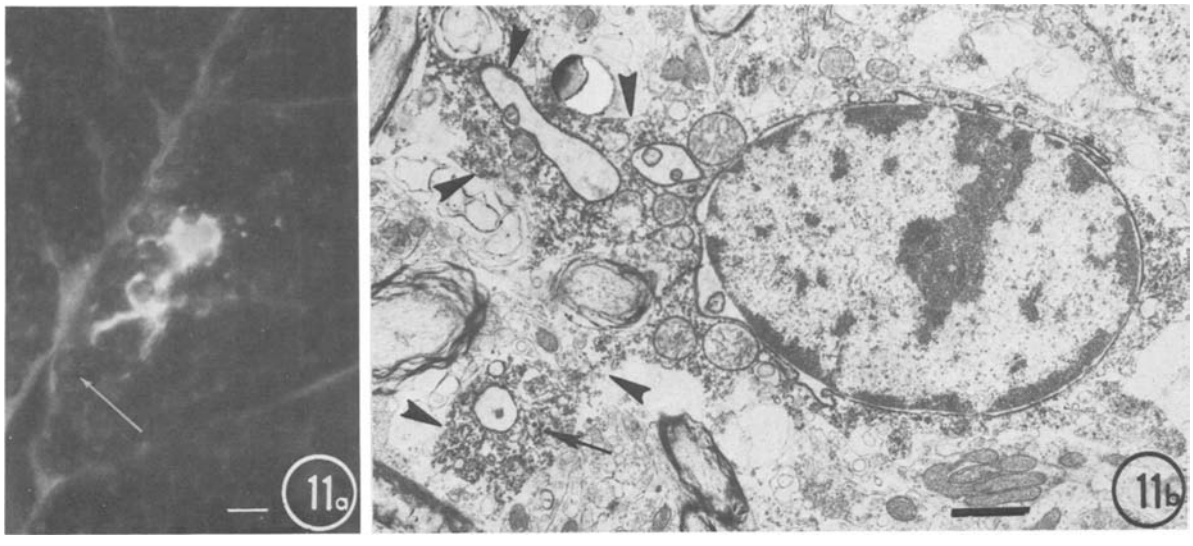


Figure 11. Spinal cord white matter of a mouse infected with *ts*8 mutant of JHM strain of mouse hepatitis virus, a coronavirus. Vibratome sections were cut and immunolabeled following the technique illustrated in figure 2. Viral antigen is diffuse in oligodendrocytes and its processes. In figure 11a, which shows immunofluorescence labeling, the arrow points to myelin sheath in contact with the processes of this infected oligodendrocyte. In figure 11b, after immunoperoxidase labeling, electron microscopy reveals a virion at the arrow; arrowheads point to two processes of oligodendrocyte containing electron-dense label for viral antigen [13]. Bar in 11a = 10 µm, in 11b = 1 µm.

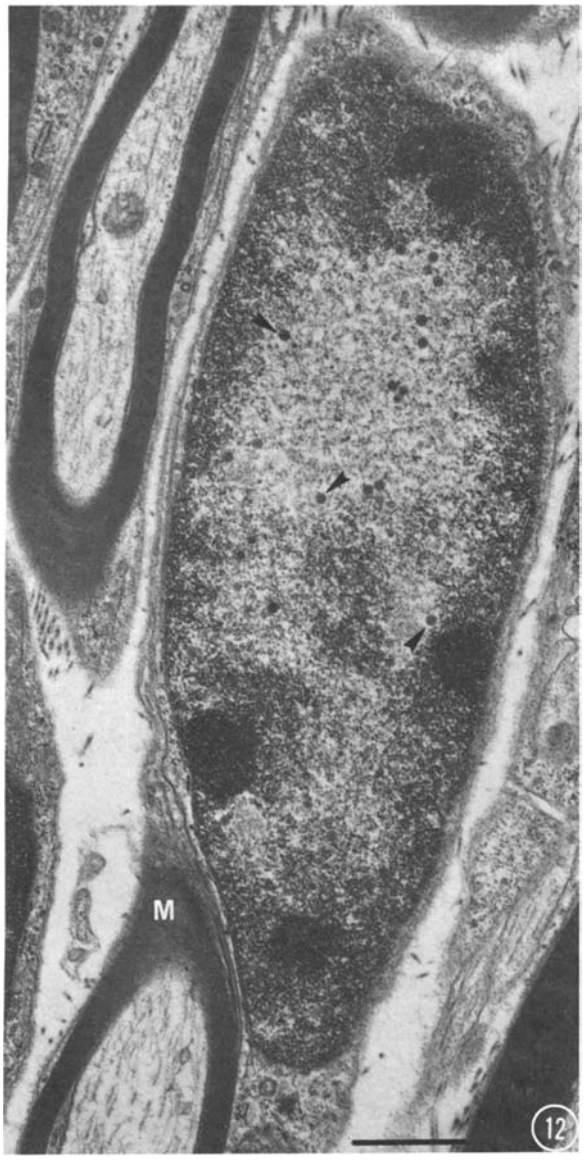
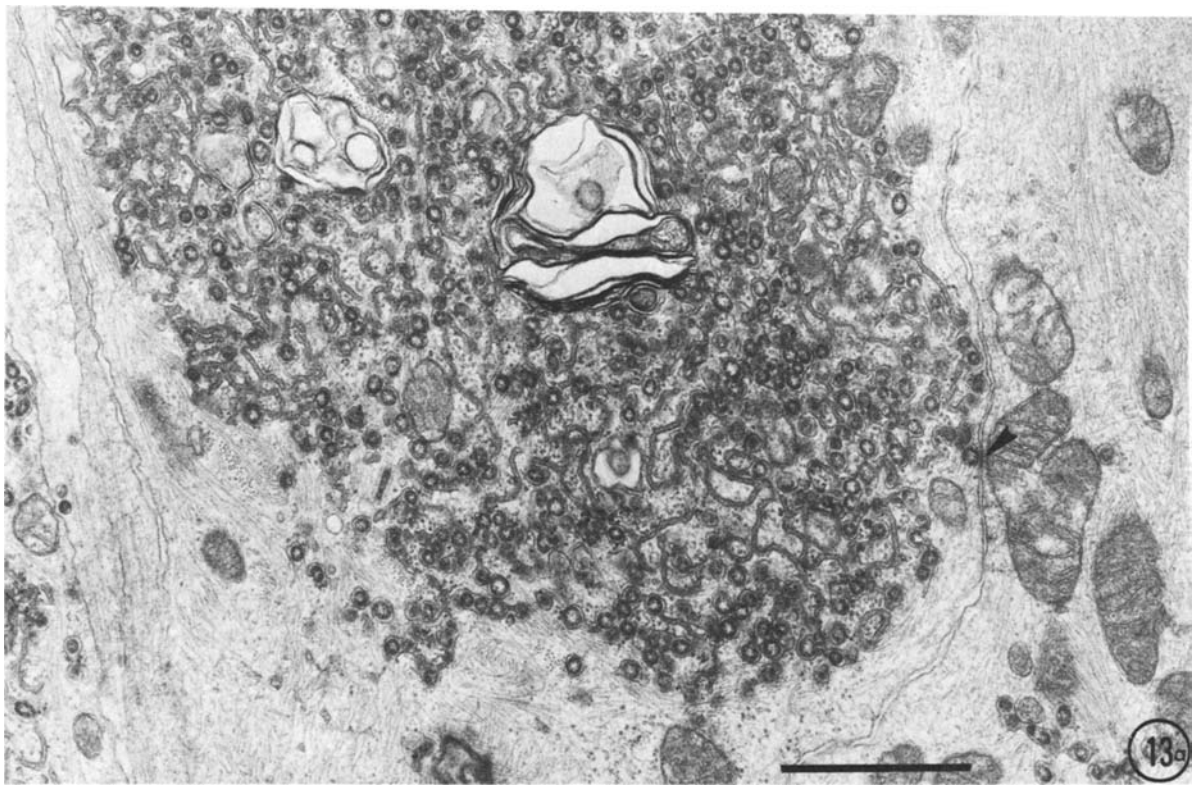


Figure 12. Trigeminal entry root of a Swiss mouse inoculated with herpes simplex virus type 1 in the cornea. The nucleus of a myelinating Schwann cell contains several herpesvirus capsids (arrowheads). However, no virions are seen in the cytoplasm or the extracellular space, and the myelin (M) is intact. Bar = 1 μm. Courtesy of Dr. Jeanette Townsend.

virus may indeed trigger macrophages to clear away the myelin debris, and various types of immune cells can invade the lesions. Using monoclonal antibodies, it is now possible to identify which subpopulations of lymphocytes are active in these lesions of the nervous system [55]. Virus-induced demyelination can often be attenuated in immunosuppressed or nude mice [56, 57]. Thus, immune cells may become sensitized to components of the myelin sheath. Perhaps the unique nature of myelin enables it to elicit an autoimmune disease. In addition, some viruses appear to have a predilection for glial cells other than myelin-forming cells (astrocytes, satellite cells of neurons) [58, 59]; yet, antimyelin antibodies can be detected and demyelination occurs.

Following are outlined some of the morphologic events in these virus-induced demyelinating diseases. Canine distemper virus is a dog paramyxovirus that matures from the plasma membrane and is antigenically related to measles virus. Virus is often seen in astrocytes and sometimes in microglial cells, which also contain myelin debris. Chronic demyelination is the most striking lesion, and antimyelin antibodies have been demonstrated [60]. The DA strain of murine Theiler virus, a picornavirus that does not mature at the plasma membrane, produces in SJL/J mice extensive demyelination in the spinal cord white matter where mononuclear cells "invade" the myelin sheath [61, 62]. Yet no clear degeneration of oligodendrocytes is observed in the vicinity of the lesion. Immunosuppression abolishes the demyelinating phase but not the early cytolytic infection of neurons [56]. The possibility that the DA virus is sometimes associated with the oligodendrocyte or myelin membrane is not excluded. In hamster cells, DA virions mature between two double-membrane layers rather than from paracrystalline structures; these virions remain associated with the membrane after cell lysis [63].

An interesting model that shows differential effects of one virus on the myelin-forming cells of the PNS and the CNS is the demyelinating lesion of the CNS produced by HSV_1 in the trigeminal nerve after corneal inoculation in mice [57, 59]. Here again, the astrocytes close to the nerve entry in the CNS are the first to produce mature virions. Oligodendrocytes are infected later and myelin is fragmented. This fragmentation may trigger mononuclear cells to ingest myelin debris and present myelin antigens to lymphocytes [59]. This hypothesis is supported by the fact that nude mice infected with HSV_1 show minimal demyelination. On the PNS side of the trigeminal nerve, demyelination never occurs and the infection appears abortive in myelinating Schwann cells: only nuclear viral capsids are found, and no degenera-



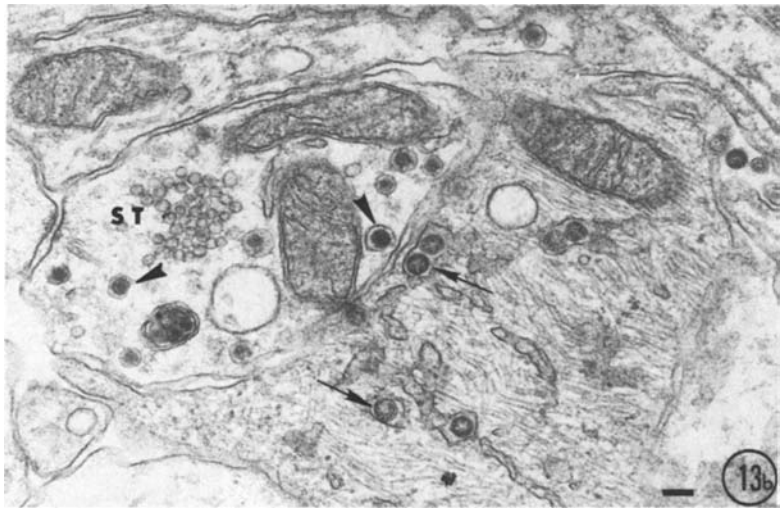


Figure 13. Maturation of mouse hepatitis virus A59, a coronavirus, in astrocytes cultured from mouse spinal cord. In figure 13a, a large inclusion is made of coronaviruses maturing in the endoplasmic reticulum. The inclusion is surrounded by numerous intermediate filaments typical of these cultured astrocytes. One virus is very close to the cell surface (arrowhead). In figure 13b, a process of an infected astrocyte with virions in the endoplasmic reticulum (arrows) is surrounding a synaptic terminal (ST) containing a cluster of synaptic vesicles and dense core vesicles (arrowheads). No viruses are seen in the neurons present in the same culture. Bar in 13a = 1 μm, in 13b = 100 nm.

tion of myelin related to infected cells is seen (figure 12). This observation is in contrast to the demyelination in the PNS that occurs in Marek's disease of the chicken caused by a herpes virus (MDV).

Marek's disease has been proposed as a model for Landry-Guillain Barré (LGB) syndrome in man [58]. Epstein-Barr virus (EBV) is most commonly associated with this human disease. EBV, as MDV, belongs to the herpes viridae family and resides in lymphocytes (reviewed in [58]). In Marek's disease, viruses cannot be detected in nerves showing demyelination in vivo. However, when nerves and ganglia are explanted, viruses are

detected by electron microscopy after a few days in culture, but only in nonmyelinating and satellite Schwann cells [58]. Thus, at least some Schwann cells may harbor a latent genome in vivo. In addition, immunoglobulins of the IgG type are present on the myelin sheath; specific cell-mediated and humoral immune response to chicken peripheral nerve myelin can be demonstrated early in the disease.

The development of sensitive probes for the intracellular detection of viral genome has recently allowed direct identification of genome in two other demyelinating diseases of the central nervous system by in situ hybridization [64, 65]. Visna viral genome is present in many brain cells of infected sheep, whereas viral proteins are rarely expressed. In a recent report [65], measles virus genome (but not measles virus proteins) was detected in demyelinating lesions of one out of four cases of multiple sclerosis. How viral genome can be triggered to express viral proteins and perhaps induce demyelination remains to be elucidated.

Astrocytes are rarely the sole target of neurotropic viruses. Two different coronavirus strains, A59 and the *ts*8 mutant of JHM strain of mouse hepatitis virus, have tropism restricted to cultured astrocytes, which slowly fuse without losing glial fibrillary acidic protein [39a]. Infected astrocytes contain large inclusions of viruses in ER and are wrapped around healthy neurons and their synaptic endings (figure 13). In PML, papova JC virions can induce both productive and abortive infection in astrocytes that show marked increase in filaments [66]. JC causes various types of tumors in the CNS of the hamster, but only glioblastomas in the squirrel and owl monkeys 15–25 months postinoculation [67]. Thus "in PML, the principle that different cells have different susceptibilities to one virus and that viruses can have varying effects on host cells is demonstrated within the same organ" [1]. JC antigen [66] and JC DNA have been demonstrated in these tumors (Nancy Miller, personal communication). Whether JC can produce glioblastoma in humans is presently being investigated; epidemiologic studies have shown that about 70% of normal adults have antibodies against JC.

In conclusion, viral persistence and specific tropism in the nervous system seem to result from a complex interaction between virus and differentiated nerve cells. Experimental systems that preserve the specialized structure and function of nerve cells are essential to the understanding of these interactions.

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