Cell Tropism and Expression of Mouse Hepatitis Viruses (MHV) in Mouse Spinal Cord Cultures[1]

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Mouse hepatitis viruses (MHV) are coronaviruses which cause various infections in mice affecting lung, intestine, liver, and other organs as well as the central nervous system. The replication of three different MHV strains was studied in mouse dissociated spinal cord cultures containing differentiated neurons and nonneuronal cells (NN) (including astrocytes). Cell tropism and maturation of each virus strain was analyzed by immunolabeling methods using antisera to the virion or to purified membrane glycoproteins (E_1 and E_2) and by electron microscopy (EM). Wt-JHM, which causes acute encephalitis in mice, produces acute cytopathic changes in both neurons and NN cells. In neurons, virions mature in smooth ER cisternae closely associated to the Golgi apparatus. As judged by EM, fewer virions are produced by neurons than NN cells and neurons do not fuse or stain for E_2 as do NN cells. NN cells contain large inclusions made of nucleocapsid strands. A temperature-sensitive mutant of JHM, Ts8-JHM, which causes demyelination in mice, infects NN cells but not neurons. Infected NN cells synthesize E_1 and E_2, and contain large inclusions but few mature virions, even at permissive temperatures. These inclusions appear granular and rarely contain nucleocapsid strands in contrast to wt-JHM infection. NN cells infected with this mutant also display numerous membrane whorls. The *hepatotropic strain A59* lacks tropism for neurons and primarily infects NN cells, thus resembling ts8-JHM. Infected NN cells become loaded with intracytoplasmic virions which are secreted from the cells. E_1 can only be detected in the perinuclear area of these cells while E_2 rapidly spreads throughout the cytoplasm. The cytoplasm of A59 infected NN cells frequently contains large tubular structures often in the lumen of the RER. In conclusion, in primary CNS cultures consisting of neurons and NN cells: (1) wt-JHM replicates in both neurons and NN cells but has different effects on these cells; (2) Ts8-JHM exhibits no productive infection of neurons, and in NN cells appears to be defective in assembly and to stimulate membrane synthesis; (3) A59 also shows tropism restricted to NN cells which produce many viruses and display differential distribution of the two virion glycoproteins. Thus, in the absence of the immune system, the MHV strains assayed exhibit differences in viral tropism, cytopathic changes, and viral assembly in CNS cells, and these differences may account for the different disease patterns.

INTRODUCTION

Tropism and expression of virus in the nervous system result from complex interactions between viruses and differentiated neuronal and nonneuronal cells. To study these interactions we have used *in vitro* systems that preserve some of the specialized structure and function of nerve cells (Dubois-Dalcq et al., 1980; Faulkner et al., 1979; Hooghe-Peters et al., 1979; Rentier et al., 1981). This study investigates the replication of some coronavirus strains in CNS cultures of mice susceptible to these viruses. Several strains of mouse

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hepatitis virus (MHV) cause CNS disease in mice. The wild-type (wt)-JHM strain of MHV produces either a fatal acute encephalomyelitis (Knobler *et al.*, 1981b) or a demyelinating disease, depending upon the age of the animal at the time of inoculation and the virus passage level (Haspel *et al.*, 1978; Herndon *et al.*, 1975; Lampert *et al.*, 1973; Stohlman and Weiner, 1981; Weiner, 1973). In contrast, a chemically induced temperature-sensitive mutant of wt-JHM, ts8-JHM, rarely induces fatal encephalomyelitis in the same mouse strains but regularly leads to demyelination (Haspel *et al.*, 1978). *In vivo* electron microscopic and immunocytochemical studies have shown that neurons do not appear to support replication of ts8-JHM, while oligodendrocytes do (Knobler *et al.*, 1981a). Finally, a hepatotropic MHV strain, A59 (Sturman and Takemoto, 1972), can also produce chronic CNS disease in C_3H mice (K. V. Holmes and P. O. Leinikki, unpublished observations.)

A59 is presently the best characterized coronavirus strain: it has three virion-associated polypeptides which have been isolated and purified (Holmes and Behnke, 1982; Holmes *et al.*, 1981, 1982; Sturman and Holmes, 1977; Sturman *et al.*, 1980). The membrane glycoprotein E_1 appears to be involved in virus assembly, while the peplomeric glycoprotein E_2 is required for infectivity and virus-induced cell fusion. Monospecific antibodies have been raised against these proteins as well as to detergent-disrupted virions (Sturman *et al.*, 1980).

We have compared the tropism and maturation of two JHM virus strains with that of the A59 strain in CNS cells, using immunolabeling and electron microscopy. Our results suggest that specific properties of each virus strain and host factors present in differentiated nerve cells act in concert to control the features and outcome of MHV CNS infection at the target cell level.

MATERIALS AND METHODS

Cells. Cultures of dissociated CNS cells were obtained from spinal cord dissected from 12- to 13-day-old C57Bl/6 mouse embryos as described before (Faulkner *et al.*, 1979; Hooghe-Peters *et al.*, 1979). C57Bl/6 mice are permissive for the JHM strains. Cells were grown on 12-mm glass coverslips or 35-mm petri dishes coated with collagen and supplemented with medium as described (Faulkner *et al.*, 1979; Hooghe-Peters *et al.*, 1979). Fibroblast growth was inhibited by a 2-day antimitotic treatment with a mixture of FudR and uridine after 1 week *in vitro*. Cultures were used after 2 to 3 weeks of *in vitro* maturation when neurons and astrocytes had acquired their mature stage (Figs. 1a and b). Three cell types were identified in these cultures. The neurons represent 5 to 10% of the cells; they are either isolated or in clusters and show a characteristic morphology with long processes as seen by phase microscopy (Fig. 1a) (Faulkner *et al.*, 1979; Hooghe-Peters *et al.*, 1979). The nonneuronal (NN) cells are comprised of at least two cell types as judged on the basis of positive or negative immunoreactivity for glial fibrillary acidic protein (GFAP), a specific protein of astrocytes (Bignami *et al.*, 1980). GFAP+ cells represent 50 to 70% of the NN cells; these astrocytes have a pleiomorphic shape, contain radiating fibrils, and form groups under the neurons. GFAP− cells probably represent primary fibroblastic cells of CNS origin (meningeal or perivascular). Oligodendrocytes were not detected in these cultures.

Viruses. The A59 virus, a hepatotropic strain of mouse hepatitis virus, was used as described before (Sturman and Takemoto, 1972; Sturman and Holmes, 1977; Sturman *et al.*, 1980). The origin and propagation of the wt and ts8 strains of JHM have been published (Haspel *et al.*, 1978). The multiplicity of infection of 10 plaque-forming units (PFU)/cell was used with A59, whereas a multiplicity of infection of 0.3 to 1 PFU/cell was used with both JHM strains (the JHM stock viruses had much lower titers). Viral adsorption was carried out for 1 hr at 37° as previously described (Knobler *et al.*, 1981a). Cultures were incubated at 37°, although in a few experiments paired cultures were also incubated at 34°. The cultures were observed daily
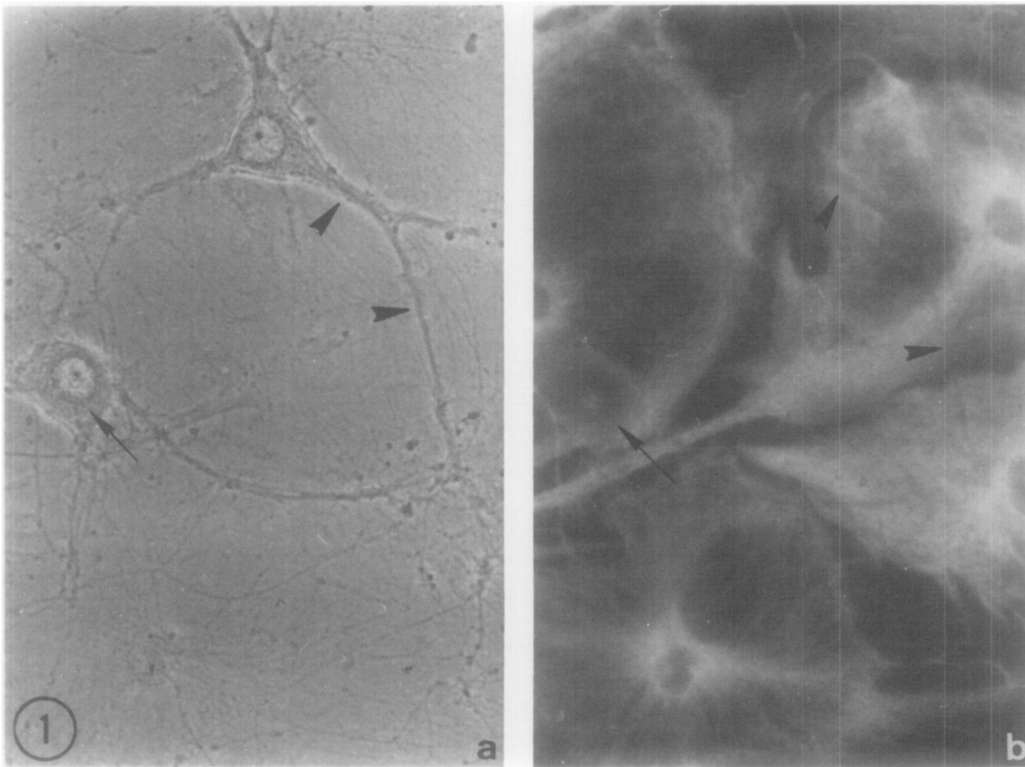
FIG. 1. The same field of dissociated CNS cultures of mouse is shown using phase (a) or immunofluorescence microscopy after glial fibrillary acidic protein (GFAP) staining (b). Two neurons with their characteristic processes are lying over flat astrocytes containing radiating fibers stained by antibody to GFAP. The location of the cell body of one is indicated by the arrow, while the processes of the second neuron are indicated by arrowheads. ×245.

with a Zeiss inverted phase scope. Viral infectivity of supernatant fluids was determined by plaque assay in L2 or 17CL 1 cells as described (Sturman and Takemoto, 1972).

Antiserum and immunolabeling. Four different rabbit antisera were used. Rabbit antiserum against GFAP was kindly provided by Dr. Rebecca Pruss; the rabbit had been injected with a suspension of human spinal cord. When reacted with cord proteins separated by agar gel electrophoresis, immune reactivity of this serum was found to be restricted to GFAP. Preparation and characterization of monospecific polyclonal rabbit antisera to the isolated E_1 and E_2 glycoproteins of A59 virion envelope has been previously described (Sturman *et al.*, 1980). Rabbit antisera directed against gradient-puri-

fied NP40-disrupted A59 virions were also prepared. This hyperimmune serum immunoprecipitated the three major structural polypeptides of both A59 and JHM from radiolabeled infected cells.

For fluorescent antibody (FA) staining, dissociated CNS cells grown on glass coverslips were fixed in a mixture of 5% acetic acid and 95% ethyl alcohol at −20° (for GFAP staining) or in acetone (for E_1 or E_2 staining) before being incubated for 1 hr in various dilutions of antisera. In some instances, living cells were incubated with antiviral sera at 4° or room temperature in order to detect only antigens on the cell surface. The second reagent used to detect bound antibodies was either protein A–fluorescein (Pharmacia, Piscataway, N. J.) or goat anti-rabbit IgG coupled to fluorescein. This conjugate was absorbed with

TABLE 1

EFFECTS OF THREE MHV STRAINS ON DISSOCIATED
CULTURES OF MOUSE SPINAL CORD

Virus strain	Type of cells infected[a]	Duration of infection[b] (days)	24 hr virus yield[c]
Wt-JHM	N and NN[d]	2 to 3	6×10^8
Ts8-JHM	NN	5 to 7	1×10^2
A59	NN	5 to 7	1×10^5

[a] Viral antigens were stained with antibody directed against detergent-disrupted A59 virions.

[b] This indicates the time at which 30% of the N and/or NN cells showed rounding or fusion.

[c] Yield of infectious virus released into the supernatant medium in plaque-forming units/ml at 37°.

[d] N, Neurons, NN, nonneuronal cells including astrocytes and fibroblasts.

liver powder or 17CL 1 cells. For immunoperoxidase labeling, staining was performed as described before (Knobler et al., 1981a). Briefly, the cells were fixed in 4% formaldehyde with 0.3 to 0.5% glutaraldehyde for 20 min, frozen and thawed in a sucrose-glycerol mixture in order to render the cell membrane permeable to antibody molecules. The cells were then allowed to react with antibody to the virion or to glycoprotein E_1 or E_2. Protein A–peroxidase was used as a second layer and cells were further incubated in the peroxide-diaminobenzidine substrate. Immediate observations by light microscopy were done on a Zeiss photomicroscope III using a water immersion phase contrast (40X) objective for immunoperoxidase or an oil immersion (40X) objective for fluorescence.

When double labeling with antisera to both GFAP and viral antigens was performed, glass coverslip cultures of CNS cells were fixed with acid–alcohol at −20°. After washing with serum containing medium, the coverslips were incubated successively in: (1) rabbit antiserum to GFAP, (2) goat anti-rabbit IgG coupled to rhodamine (Miles Laboratory, Elkhart, Ind.) (3) polyvalent mouse antiserum to MHV (Microbiological Associates, Bethesda, Md.), and (4) goat anti-mouse IgG coupled to fluorescein (Progressive Laboratories, distributed by Roboz Institution Company,

Washington, D. C.). Preparations were successively examined with rhodamine and fluorescein filters.

Electron microscopy. As described earlier (Faulkner et al., 1979), glass coverslips covered with cells were used for scanning electron microscopy (SEM), whereas 35-mm petri dishes were used for transmission electron microscopy (TEM) studies. Cells were usually fixed with Karnovsky fixative consisting of a mixture of 1.25% glutaraldehyde and 1% formaldehyde in 0.06 M cacodylate buffer at pH 7.2. For SEM studies, cells were then postfixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 2 hr, and dehydrated in methanol, critical point dried in CO_2 in a Balzer apparatus, coated under high vacuum with gold–palladium alloy, and observed in an ETEC Autoscan at 20 kV and a tilt angle of 45°. For TEM studies, monolayers in petri dishes that had been labeled by the immunoperoxidase method (as described above) or fixed *in situ* with the Karnovsky fixative, were postfixed with osmium tetroxide 1% in cacodylate buffer for 30 min, stained with uranyl acetate at pH 5, progressively dehydrated in ethanol, and embedded in Epon 812. The technique for circling and drilling out immunolabeled cells or syncytia from the embedded monolayer has been published in detail (Knobler et al., 1981a). Sections were cut with a diamond knife and examined in Philips 201 or Philips 400-T electron microscopes. Thin sections were usually counterstained with uranyl acetate and lead salts, and examined at 80 kV.

RESULTS

I. Virus Maturation of wt-JHM in Cultures of Dissociated Nerve Cells

When CNS cultures were inoculated with wt-JHM, cytopathic changes were first detected at 18 to 24 hr and resulted in the destruction of the monolayer in 2 or 3 days. At 18 hr postinoculation (PI), 30% of the neurons contained viral antigens (as detected by antiserum to the virions) and progressively rounded up. No fusion of neurons was noted. In contrast, NN cells started to fuse and express viral
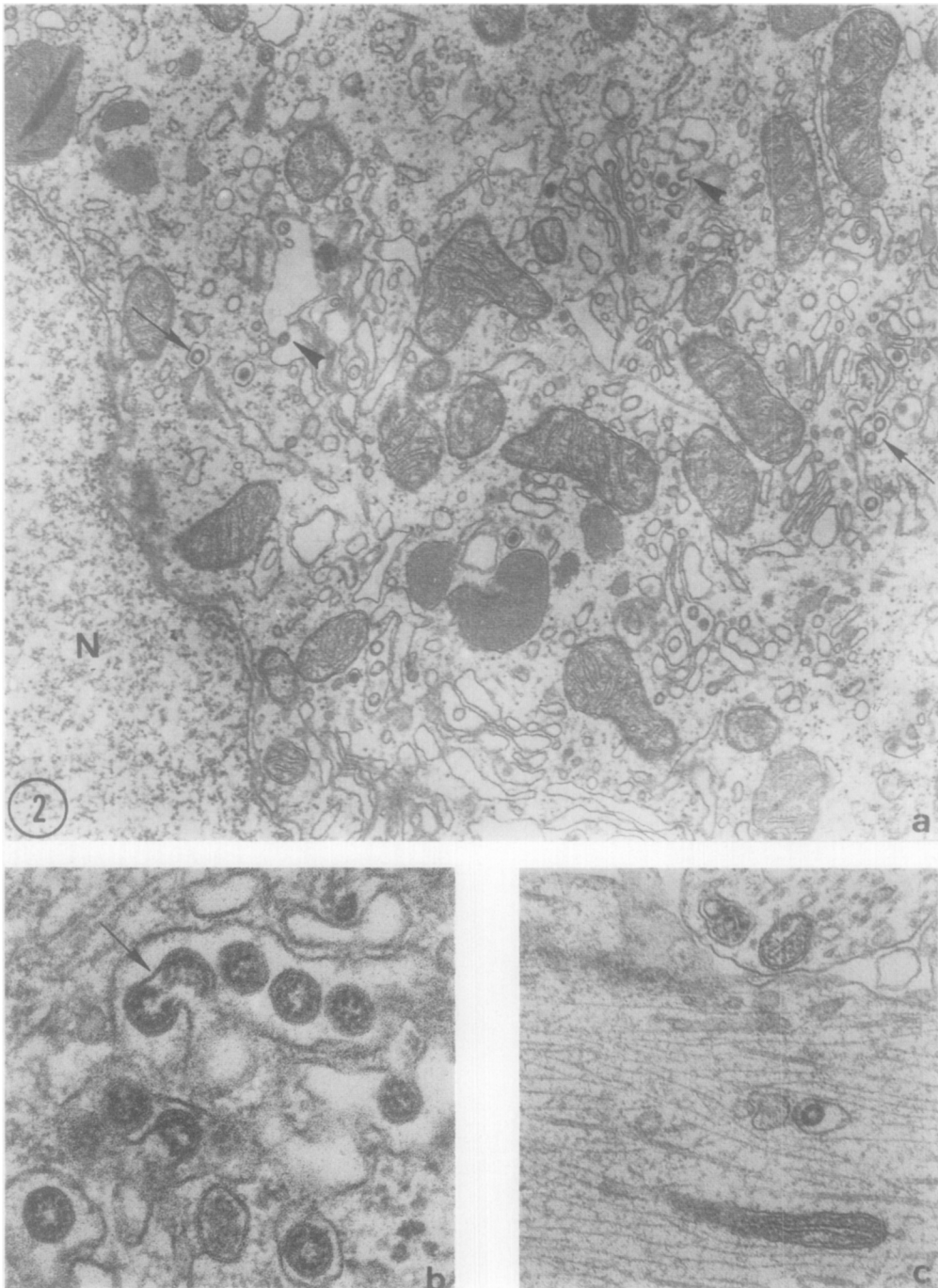
FIG. 2. Ultrastructural aspects of wt-JHM virus maturation in neurons infected for 18 hr. (a) Virus matures in smooth membrane cisterns closely associated with Golgi system (budding sites at arrowheads; free virus at arrows). Indented nucleus (N) of neuron is seen on the left. (b) During budding in the Golgi-related cisterns, the nucleocapsid, an electron-dense helix, is closely apposed to the modified membrane (arrow points to double viral bud). (c) A vacuole containing a single virion is seen within a dendrite containing many filaments and tubules. (a) ×20,000; (b) ×86,000; (c) ×48,000.
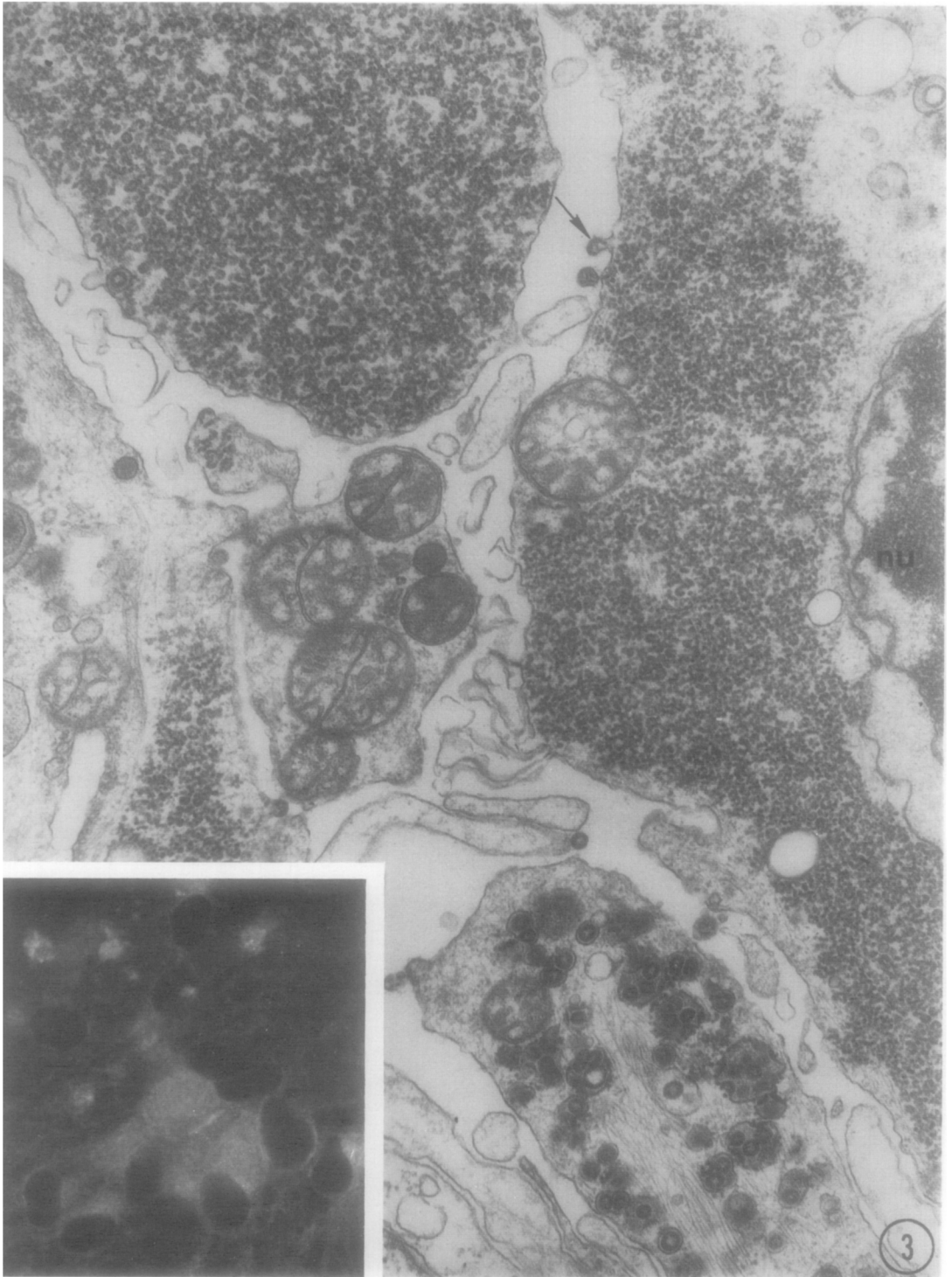
FIG. 3. Wt-JHM maturation in NN cells infected for 2 days: nucleocapsid inclusions are very large and occupy the space between the nucleus (NU) and the plasma membrane. Rarely virus buds directly from the plasma membrane (arrow) but usually virions mature by budding into intracytoplasmic vacuoles as seen in the lower part of the figure. ×35,000. Inset shows E_2 localization in the perinuclear area of large syncytium of NN cells at 24 hr PI with wt-JHM. ×310.
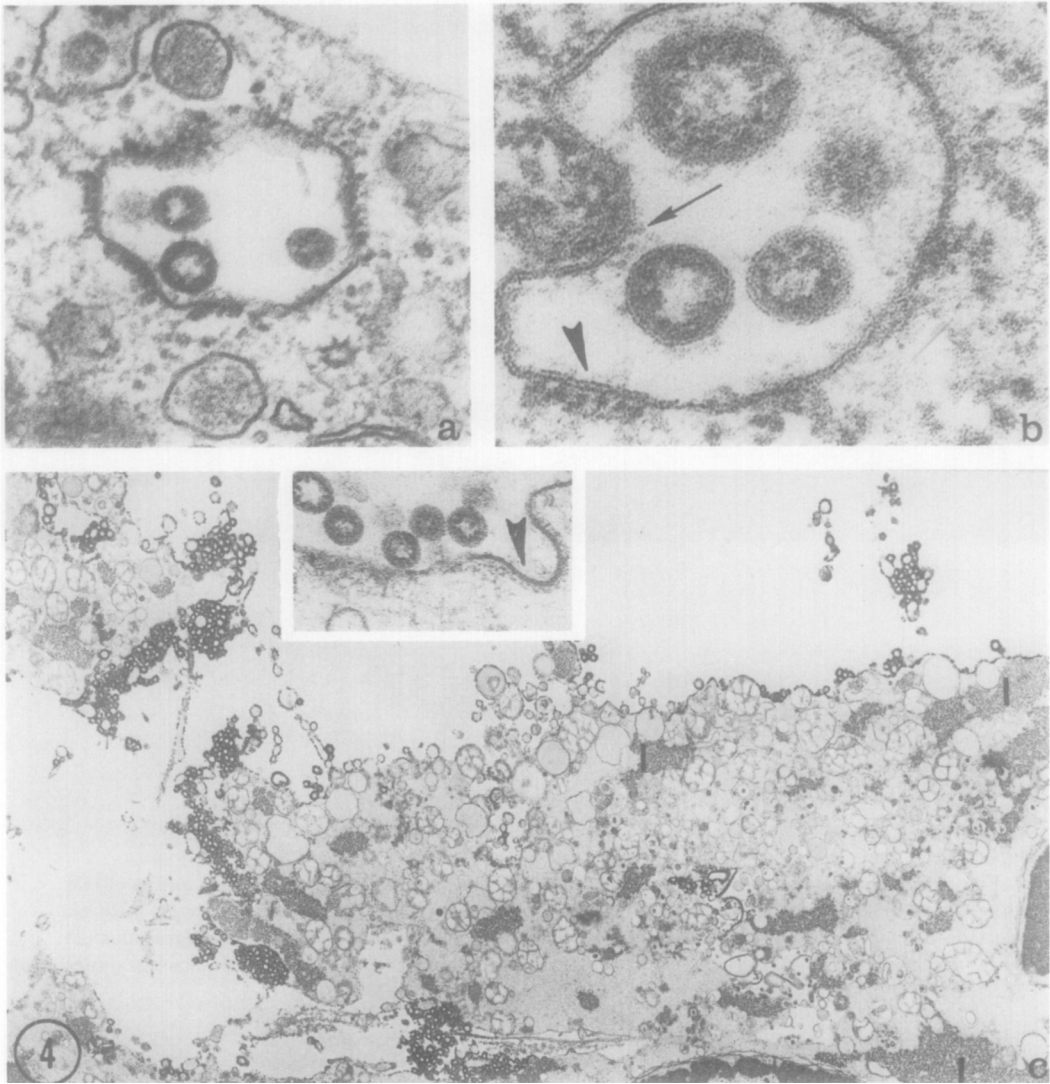
FIG. 4. In wt-JHM-infected NN cells, early budding stages of the virus are detected along the vacuole membrane in which other viruses have already matured (a and b). In (a), loosely folded strands of nucleocapsid are closely apposed to four different areas of this ER cistern membrane. In (b), at arrowhead, six cross sections through the nucleocapsid strand are seen along 80 nm of membrane. Spacing between the crosscuts is 6 nm. During budding, the virion membrane acquires definite spikes (arrow). (c) Numerous virions, specifically labeled by immunoperoxidase, are clustered together or released along the surface of a giant cell which contains several RNP inclusions (I). Inset: Released virion has coiled RNP strand localized under the viral envelope. These extracellular virions are sometimes close to coated pits (arrowhead). (a) ×85,500; (b) ×180,500; (c) ×7200; inset, ×57,000.

proteins between 16 and 18 hr. Titers of released virus were always lower than titers of CNS cells infected with A59 strain (Table 1).

Virus expression and maturation of wt-JHM was examined successively in infected neurons and NN cells. Infected neurons showed diffuse immunofluorescent staining for E_1 from 18 to 48 hr but no staining with anti-E_2 antiserum. At 18 hr
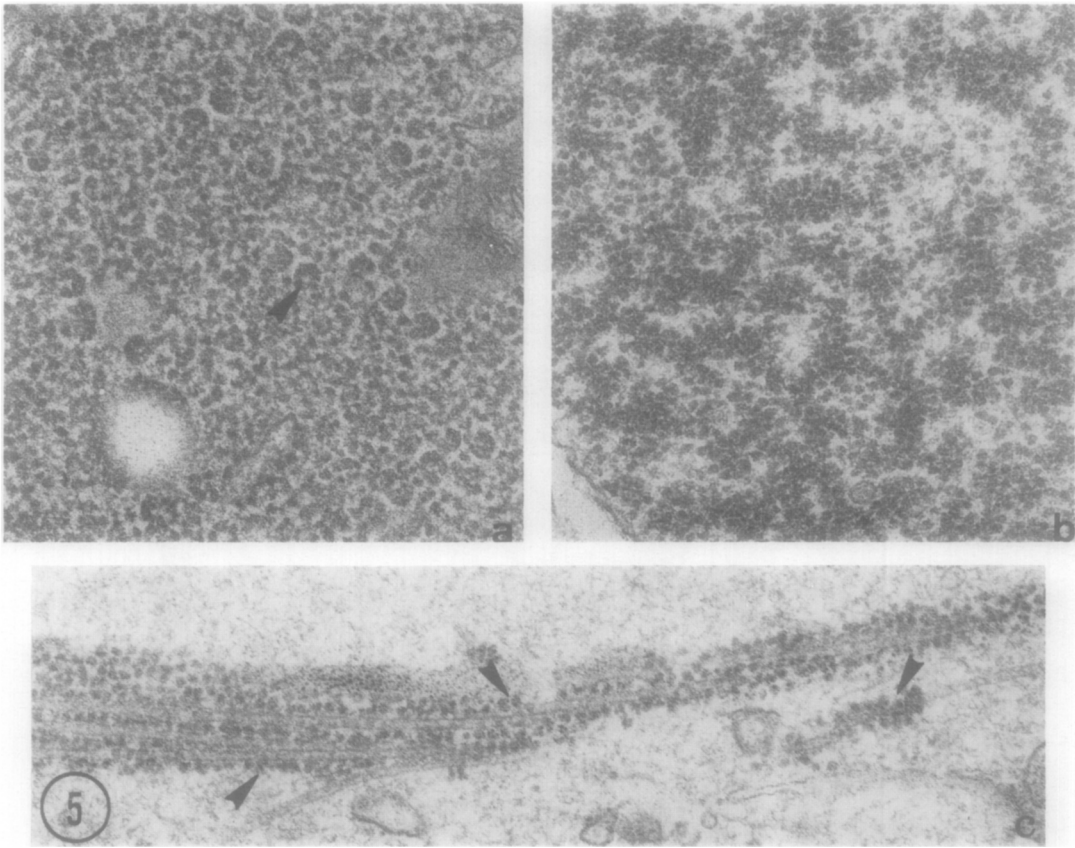
FIG. 5. Comparison between viral inclusions in NN cells infected with wt-JHM (a) and ts8-JHM (b). The wt-JHM inclusion contains loosely coiled strands (8 nm in diameter) or cores with an electron-lucent center (arrowheads in a). The ts8-JHM inclusions are made of dense granules (20 to 30 nm in diameter) (b). Figure 5c shows 18-nm tubules surrounded by small dense granules (arrowheads) in ts8-JHM infections. (a) ×68,200; (b) ×51,000; (c) ×81,000.

PI, the organization of the cytoplasm of infected neurons was not altered since they had an extensive rough ER system and a normal distribution of other organelles. Budding and free virions with electron-lucent centers were detected in cisternae of smooth membranes closely associated with the Golgi apparatus (Figs. 2a, b). Virus was also found occasionally in small vacuoles within dendrites (Fig. 2c) as seen in infected mouse spinal cord in vivo (Knobler et al., 1981a). At 48 hr PI, the cytoplasm of infected neurons showed disorganization of RER membranes and vacuolization. Scattered budding virions were seen in association with intracytoplasmic membranes as described before

(Knobler et al., 1981a). Fewer cytoplasmic vacuoles containing virions were detected in thin sections of infected neurons than in giant cells formed by NN cells (illustrated in Figs. 3 and 4). Similarly, clusters of extracellular virions were rarely found around infected neurons, whereas they were numerous around NN cells (Fig. 4c). This correlates with previous in vivo observations on infected neurons of the spinal cord (Knobler et al., 1981a).

Infected NN cells contained both E_1 and E_2 glycoproteins at 16 hr PI. These proteins were predominantly localized in the perinuclear area of giant cells (Fig. 3, inset). Fusion between GFAP+ and GFAP− was also observed. EM examination of
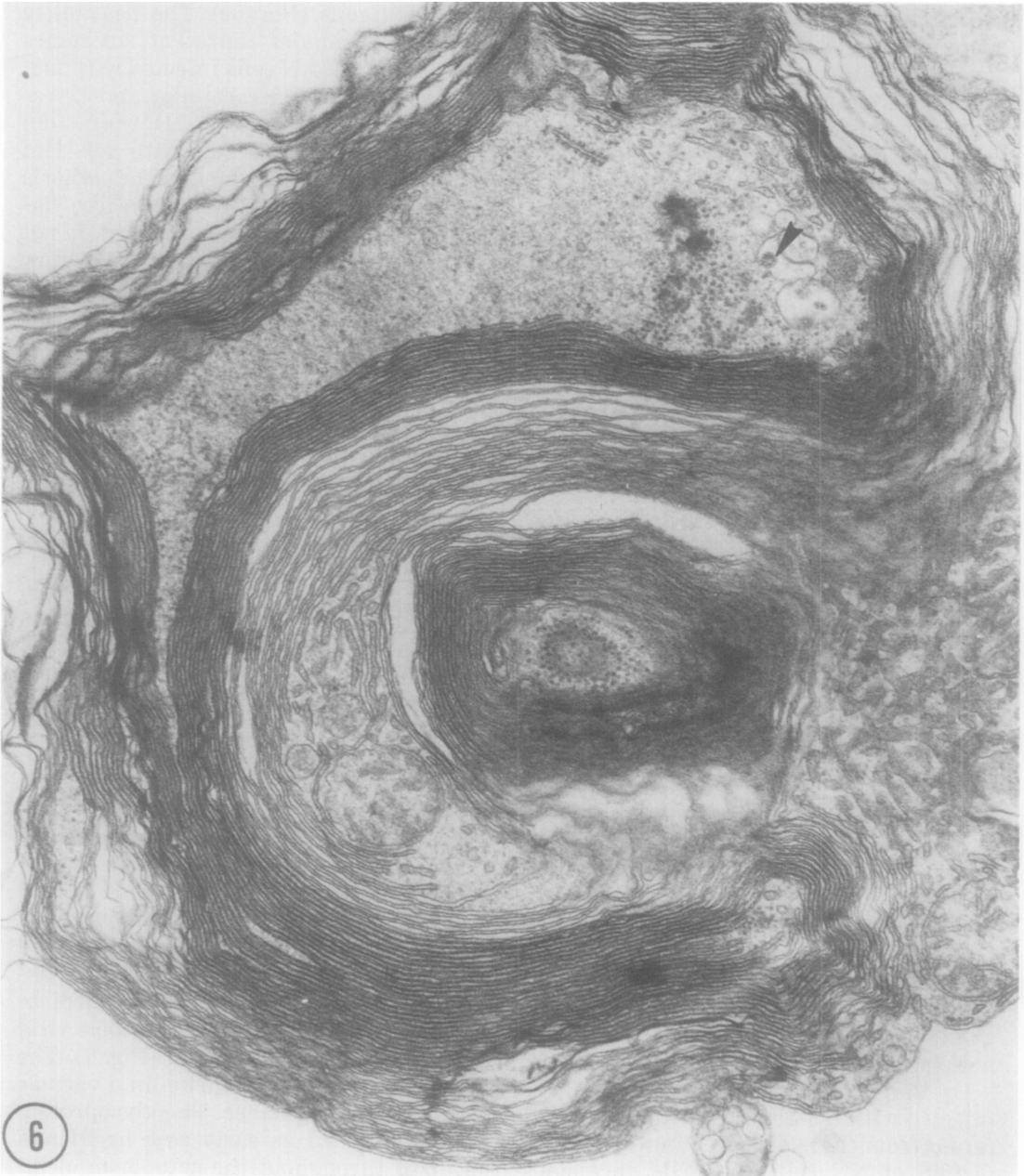
FIG. 6. Membrane whorls in NN cells infected with ts8-JHM. The myelin figure surrounds part of the cytoplasm which contains accumulation of dense granules and a virus budding into a vacuole (arrowhead). Myelin whorl is made of 40 pairs of membranes with an interspace of 12 to 20 nm. ×39,000.

these fused NN cells revealed nucleocapsid strands accumulating in large inclusions and numerous virions budding in vacuoles (Fig. 3). Before budding occurred, loosely folded nucleocapsid strands approximately 8 nm in diameter were seen in close apposition to the ER membrane (Figs. 4a and b). During budding, the nu-

cleocapsid was closely apposed to the virion membrane of the bud which had acquired definite spikes (Fig. 4b). Virions were budding from RER as well as smooth ER. Using antiserum against purified A59 virions, MHV antigens were detected in all membranes directly associated with virion formation and in the virion surface (data not shown).

Numerous free virions were released by giant cells and formed clusters firmly attached to infected cell surface and heavily labeled after immunoperoxidase staining (Fig. 4c). Free virions in the extracellular space resembled those seen in vacuoles and were 80 to 100 nm in diameter (Fig. 4c, inset).

II. Virus Maturation of ts8-JHM in Cultures of Dissociated Nerve Cells

Neurons showed no cytopathic changes or viral antigens after inoculation with this strain. However, scattered foci of NN cells slowly developed fusion over 3 to 5 days and contained viral antigen. Both GFAP+ and GFAP− cells were infected as demonstrated by double labeling for viral- and cell-specific antigens. The glycoproteins, E_2 and E_1, were detected in the perinuclear area of giant cells at 24 hr and stayed in these regions throughout infection. Slow progression of infection led to disintegration of the monolayer after 5 to 7 days. NN cells infected with ts8-JHM released at least 10 times less virus than cells infected with wt-JHM (Table 1), even at the permissive temperature (34°) (data not shown).

By EM, inclusions present in NN cells infected with ts8-JHM were markedly different from those observed in wt-JHM infection of NN cells. Wt-JHM inclusions contained nucleocapsid strands 8 nm in diameter as well as crescent-shaped core-like structures (37–40 nm in diameter), usually with an electronlucent center (Fig. 5a). Ts8-JHM inclusions contained numerous dense granules (20 to 30 nm in diameter). Strands of nucleocapsid could not be clearly identified in these inclusions (Fig. 5b). In addition, rows of dense granules (18 nm in diameter) were seen aligned along cytoplasmic tubules of ts8-JHM-infected NN cells (Fig. 5c). The possibility that these granules represent ribosomes is not excluded. NN cells infected with ts8-JHM showed less intracellular and extracellular virions than in wt-JHM infection (Fig. 4c). NN cells infected with ts8-JHM displayed numerous membrane whorls sometimes organized in myelin-like figures (Fig. 6). However, our cultures do not normally contain oligodendrocytes or show myelination. These membrane whorls surrounded cytoplasmic compartments containing ER, dense granules, and, occasionally, budding virions (Fig. 6). Some whorls were made of 40 pairs of membranes with an interspace of 12 to 20 nm. Immunoperoxidase labeling of viral antigens did not reveal staining of membrane whorls.

III. Virus Maturation of A59 in Cultures of Dissociated Nerve Cells

As with ts8-JHM, no infected neurons were found in CNS cultures inoculated with A59 and the infection appeared restricted to NN cells. Mild fusion of NN cells was observed and infection lasted for 5 to 10 days. Infected GFAP+ cells progressively retracted their fibrillar processes. A59-infected cultures produced 10 times more infectious virus than those infected with wt-JHM (Table 1) even though wt-JHM replicated in both neurons and NN cells. Infected astrocytes were wrapped around healthy, apparently uninfected neurons as seen by EM (Fig. 7). These astrocytes were very rich in intermediate filaments and contained numerous viral particles in their ER system (Fig. 7). The inset of Fig. 7 shows a virion in a vacuole close to the cell surface. The glycoprotein E_2 was detected as early as 8 hr PI and localized throughout the cytoplasm of infected NN cells, while E_1, first detected at 16 hr, remained predominately perinuclear (Figs. 8a and b). Such differential localization of these proteins has been observed in infected 17CL 1 fibroblasts (E. W. Doller et al., submitted for publication). Interestingly, virus maturation in perinuclear cisternae was frequently noted by EM (Fig. 8c). A59-infected NN cells also contained numerous large tubular structures of 25 to 30 nm in diameter, often in
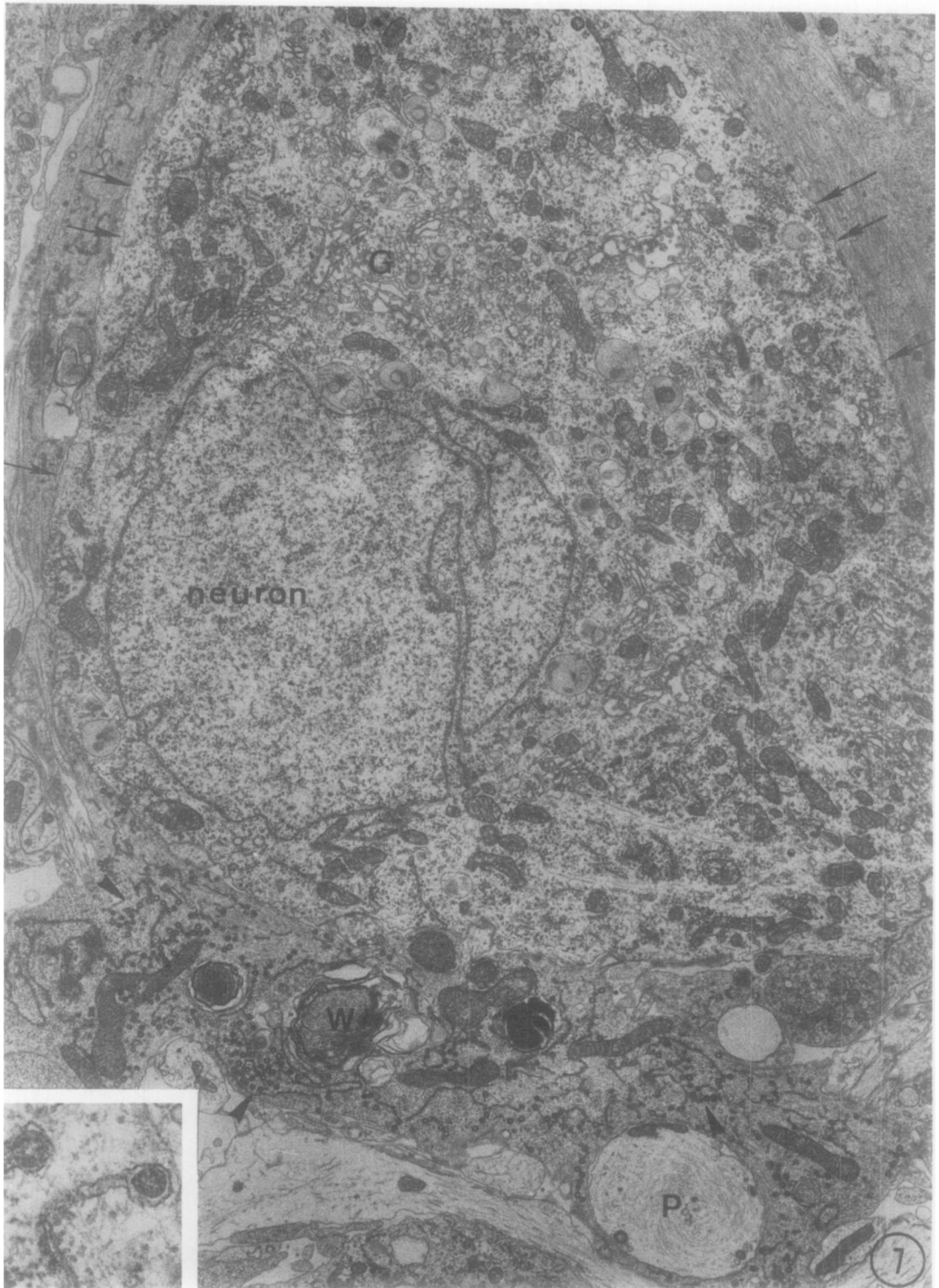
FIG. 7. In A59 infection, a healthy neuron with extensive Golgi apparatus (G), indented nucleus, and well-developed ER system is surrounded by astrocyte (arrows point to limiting membrane between the two cells). This astrocyte contains many filaments and numerous virions maturing in the endoplasmic reticulum (arrowheads). A detail on one virion in a vacuole close to cell surface is shown in inset. Infected astrocyte contains some membrane whorls at W and surrounds a neuron process (P). ×7700; inset, ×54,000.
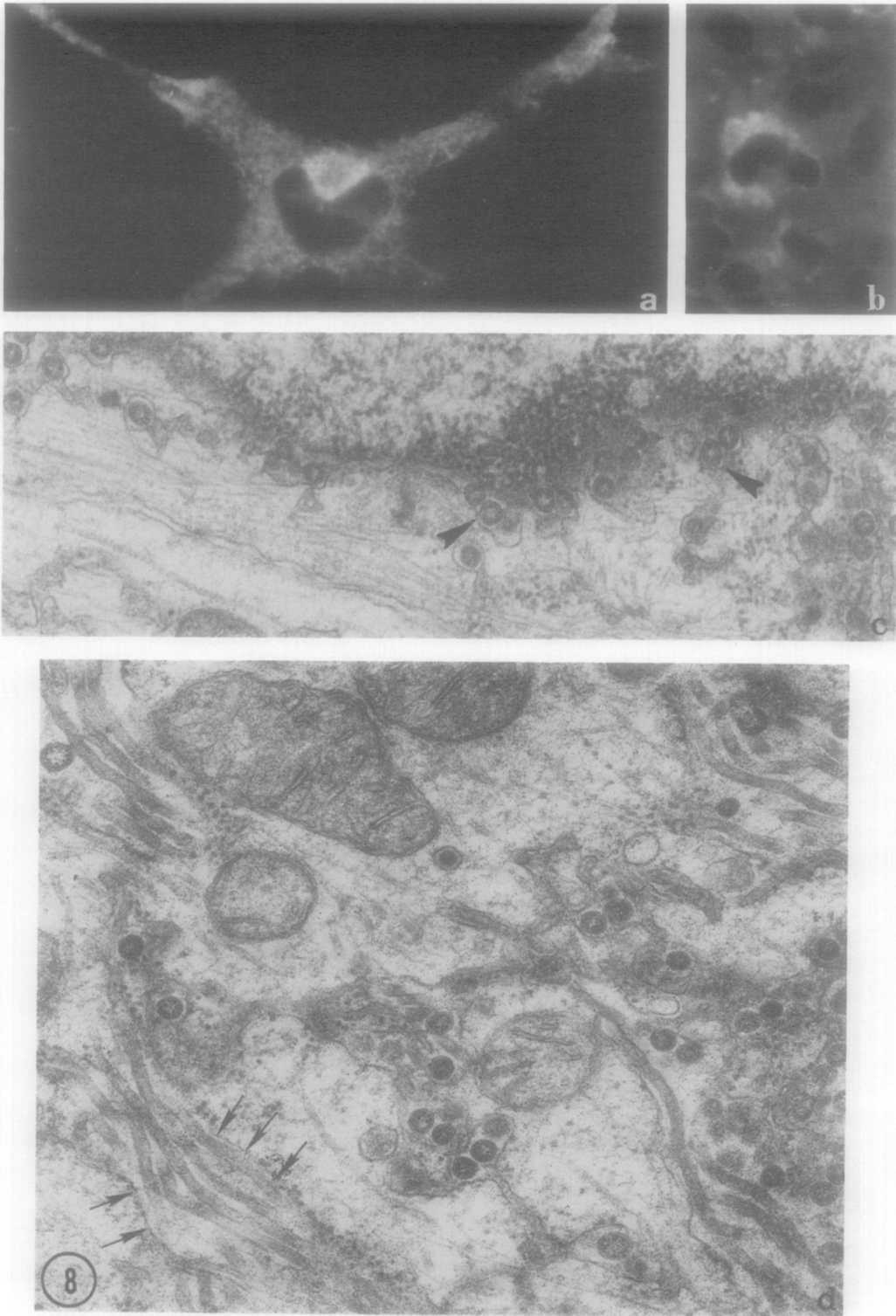
FIG. 8. A59 infection of NN cells. (a) At 8 hr PI, the peplomeric glycoprotein E_2 is detected in scattered NN cells. Immunofluorescent staining is diffuse and spreads into the processes, although some condensation is seen in the perinuclear area. (b) At 16 hr PI, the membrane glycoprotein E_1 is detected only in the perinuclear area. (c) At 2 days PI, numerous particles are seen in the

the lumen of the RER (Fig. 8d). These tubular structures had a higher density than cellular microtubules. A59-infected NN cells also showed some membrane whorls but these were smaller than those observed in ts8-JHM infection of NN cells (Fig. 7).

DISCUSSION

The present study describes how three different MHV strains interact *in vitro* with cultured neurons and NN cells, including astrocytes, isolated from mouse spinal cords. Previous studies have demonstrated that persistent infection with the JHM strain could be obtained in cloned cell lines of glial and neuronal origin (Lucas *et al.*, 1977, 1978; Stohlman and Weiner, 1978). Here, we have used primary cultures of differentiated nerve cells, an *in vitro* system that seems closer to the *in vivo* situation. Our observations are summarized in Table 2.

Wt-JHM is the only strain able to replicate in mature neurons as well as NN cells and this correlates well with *in vivo* observations (Knobler *et al.*, 1981a). Wt-JHM never produces fusion of neurons as do other viruses (Hooghe-Peters *et al.*, 1979), but rather induces neuronal rounding and lysis with little virus release. Simultaneously, extensive fusion and virus production occur in the nearby NN cells. Thus wt-JHM has very different effects in these two CNS cell types. In addition, the peplomeric glycoprotein E_2 was not detected in infected neurons but was observed in NN cells. Thus, JHM virions produced in neurons may either lack E_2 or have an altered E_2 no longer reacting with antisera used. Virions lacking peplomers and E_2 are formed in A59-infected 17CL 1 cells treated with tunicamycin (Holmes *et al.*, 1981, 1982). Thus it is possible for buds lacking E_2 to form but such buds are known to be noninfectious (Holmes *et al.*, 1981, 1982). The possible differences in E_2 glycoprotein synthesis between neurons

and NN cells infected with wt-JHM should be further studied provided that purified neuron cultures could be obtained (Knobler *et al.*, 1982).

The other two MHV strains, ts8-JHM and A59, infect only NN cells. Similarly, in weanling mice, ts8-JHM does not appear to replicate in neurons (Knobler *et al.*, 1981a). One possible explanation for this restricted tropism is that ts8-JHM and A59 may lack some structural aspects of the surface glycoprotein E_2 necessary for its interaction with virus receptors on neurons. Alternately, the virus may have infected the neurons but intracellular factors of an unknown nature may have blocked its replication. In a study of restricted tropism of VSV ts mutants, it was suggested that "secondary" virulence genes may control viral replication in cells through adequate or inadequate interactions of virus specific polymerase complexes with some specific host cell factor (Preble *et al.*, 1980).

The process of virus maturation in NN cells infected with wt-JHM and A59 is closely similar to that observed in fibroblast cell lines and liver cell cultures (David-Ferreira and Manaker, 1965) but more stages are captured in primary CNS cells. TEM analysis of these stages reveals that a close interaction between a folded nucleocapsid strand and a virus-modified ER membrane is occurring during the budding process, as is the case for other enveloped RNA viruses. There is a close association of the virus maturation sites with the Golgi system. Virus contained in vacuoles is "secreted" since these vacuoles eventually fuse with the plasma membrane (Holmes and Behnke, 1982). This release process is also observed with other enveloped viruses undergoing intracellular maturation such as herpes simplex (Rodriguez and Dubois-Dalcq, 1978).

A59 infected CNS cultures produce more virus than wt-JHM infected ones, even though A59 infects only NN cells in a slow, progressive way. In addition, differential

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perinuclear cistern (arrowheads). (d) After 3 to 5 days, numerous large tubular structures (25 to 30 nm in diameter) are seen within ER cisternae which sometimes also contain virions. Arrows point to membranes limiting the cisternae with tubular structures. (a) ×485; (b) ×310; (c) ×36,000; (d) ×53,000.

TABLE 2

VIRUS EXPRESSION OF THREE MHV STRAINS IN CNS NEURONS AND NONNEURONAL CELLS

	Replication[a]	Fusion	E_2	Lysis	Yield of infectious virus	Cytoplasmic inclusions	Tubular structures in RER	Membrane whorls in cytoplasm
Neuronal cells								
Wt-JHM	+	−	−	+	+	−	−	−
Ts8-JHM	−							
A59	−							
Nonneuronal cells								
WT-JHM	+	+++	+	+++	++	++[b]	−	−
Ts8-JHM	+	++	+	++	+	+++[c]	+	++
A59	+	+	+	+	+++	+[b]	++	+

[a] As assayed by viral antigen containing cells.
[b] Nucleocapsid strands.
[c] Granular.

migration of E_1 and E_2 was observed in A59 infected NN cells (as in 17CL 1 fibroblasts, E. W. Doller et al., submitted for publication) but not in wt-JHM-infected NN cells. In A59 infections, the membrane glycoprotein E_1 appears to remain in the Golgi area while the peplomeric glycoprotein E_2 migrates rapidly to the plasma membrane (Figs. 8a and b). In contrast, the E_2 synthesized in NN cells by the JHM strains did not disperse rapidly but remained near the cell center (Fig. 3, inset). Possibly the signals for migration of the E_2 glycoprotein are different for the JHM and A59 strains of MHV. These differences may account, at least in part, for the different yields of virus from cells infected with wt-JHM or A59.

Ts8-JHM-infected NN cells showed viral inclusions different from those observed in wt-JHM infection. These alterations suggest a defect in the ribonucleoprotein, resulting in defective assembly of ts8-JHM occurring even at permissive temperatures. Such an alteration was not observed in fibroblastoid cell lines but only in CNS cells (Dubois-Dalcq et al., unpublished observations). Thus the host cell appears to influence expression and maturation of this virus. In addition, the cytopathic changes produced by ts8-JHM in NN cells were slowly progressive and resulted in the accumulation of abnormal whorls of cytoplasmic membranes. Such accumulation was not observed in fibroblastoid cell lines. In vivo, oligodendrocytes infected with JHM strains appear to

lay down more myelin lamellae (Powell and Lampert, 1975). Thus membrane proliferation induced by MHV infection may be a specific event occurring in differentiated glial cells.

Analysis of RNA and protein synthesis of ts8-JHM might elucidate the molecular basis of the ability of this mutant to produce chronic demyelination in vivo. There is no evidence yet that the host immune response plays a critical role in the induction of virus persistence in vivo since immunosuppression does not alter the course of the disease (Weiner, 1973). Rather, our in vitro study demonstrates differences in viral tropism and cytopathic changes between neurons and NN cells which may be correlated with differences in disease patterns in vivo. Our study also points to the advantages of using cultures of specialized nerve cells to elucidate virus–nerve cell interactions.

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