Host-cell Response to Herpes Virus Infection in Central and Peripheral Nervous Tissue in Vitro

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SUMMARY

In an organotypic nerve cell culture system, all cells in both the central and the peripheral nervous system (CNS, PNS) components supported replication of herpes simplex virus types 1 and 2 (HSV 1, HSV 2). In HSV 1 infection, cellular response was particularly characterized by the formation of small syncytia (which involved neurons) and by the presence of bundles of interwoven fine filaments within the nuclei of infected cells. In HSV 2 infection, groups of parallel tubules characteristically formed in the nuclei of infected cells. All cells in the CNS or PNS succumbed to virus infection, some within 24 h (e.g. oligodendrocytes) and others after 48 h (e.g. neurons), with the exception of astrocytes. Although among the first cells to develop virus nucleocapsids in their nuclei, astrocytes became swollen and filled with increased numbers of bundles of glial filaments within 24 h after infection; by 48 h the actual number of astrocytes was increased by as much as three- to fourfold over the number in controls. The results suggest that astrocytes may have a unique mechanism which modifies virus infection and the cells not only survive, but can also become reactive.

INTRODUCTION

The characteristics of herpes simplex virus type 1 (HSV 1) or type 2 (HSV 2) infection in organotypic cultures of central nervous system (CNS) or peripheral nervous system (PNS) have been described in a number of studies (Feldman et al. 1968; Leestma et al. 1969; Dubois-Dalcq et al. 1972; Hill & Field, 1973; Fournier et al. 1977; Whetsell et al. 1977; Ziegler & Pozos, 1977). No studies, however, have attempted to analyse, characterize and compare the responses of the various cellular components within the cultures in the face of HSV infection. There remains a controversy about whether all cells are infected by herpes simplex virus particles (Feldman et al. 1968; Leestma et al. 1969; Whetsell et al. 1977; Ziegler & Pozos, 1977) or whether production of enveloped virus is restricted to neurons in the PNS tissue (Hill & Field, 1973) or restricted to astrocytes in the CNS tissue (Fournier et al. 1977). Similarly, it is not clear whether neurons can (Ziegler & Pozos, 1977) or cannot (Feldman et al. 1968; Leestma et al. 1969; Dubois-Dalcq et al. 1972) be involved in syncytial formation.

In two previous comparative studies of the effects of HSV 1 and HSV 2 in organotypic cultures of mouse spinal cord with attached dorsal root ganglia (Ecob-Johnston et al. 1978, 1979), attention was focused upon the effects of the virus infection on myelin. Those studies indicated that both HSV 1 and HSV 2 could cause a loss of myelin as a

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primary effect of virus replication in the nuclei of cells responsible for the synthesis and maintenance of central myelin (oligodendroglia) and peripheral myelin (Schwann cells). These cultures have been further evaluated morphologically to determine specific responses of the different cell types in the cultures (CNS and PNS) to herpes virus infection. The results of this ultrastructural morphological study are presented here.

**METHODS**

**Cultures and medium.** Spinal cord from 13 to 14 day embryonic mouse foetuses (Swiss-Webster) was cut into cross-sections one vertebral segment long such that a pair of dorsal root ganglia were still attached to each cross-section (Ecob-Johnston et al. 1978, 1979). Two cross-sections were placed on each collagen-coated coverslip (Masurovsky & Peterson, 1973) and mounted in a Maximow slide assembly (Bornstein, 1973). Culture medium contained 33% serum [human placental serum initially, replaced by horse serum before and after infection of the cultures (Ecob-Johnston et al. 1978)], 10% chick embryo extract and 50% Eagle's minimum essential medium (MEM) supplemented with 600 mg % glucose.

**Virus infection of cultures.** The Mp strain of HSV 1 (Hoggan & Roizman, 1959) and the MS strain of HSV2 (Gudnadotter et al. 1964), both previously passaged in HeLa cells, were used to infect the cultures (Ecob-Johnston et al. 1978, 1979). Mature, well-myelinated cultures after 26 to 31 days in vitro, were washed with Hanks' balanced salt solution. Then 0.04 ml virus inoculum (containing $1 \times 10^4$ TCID$_{50}$ in Eagle's MEM plus 10% foetal bovine serum) was adsorbed for 2 h at 37 °C. The inoculum was removed, the cultures re-fed and re-assembled and then incubated at 37 °C for the duration of the experiments. Control cultures were mock-infected with the diluent.

**Electron microscopy.** At 12, 24, 36 and 48 h post inoculation (p.i.) a control culture and cultures infected with HSV type 1 and type 2 were fixed for electron microscopy by immersion in cold 2% glutaraldehyde for 1 h, postfixed in 1% OsO$_4$ for 1 h in the cold, dehydrated and embedded in epon. Ultrathin sections of all cultures were stained with uranyl acetate and lead acetate and examined with an Hitachi 12A electron microscope.

**Cell counts.** All the nuclei in one section were identified; their cell types, the presence and location of enveloped and unenveloped virus, any nuclear changes (including reduplication and thickening of the nuclear membrane and the presence of unusual structures) or cytoplasmic changes, as well as the formation of syncytia, were recorded. At each time point, at least five different sections from comparable areas of replicate cultures were observed and 150 to 250 cells were studied.

**RESULTS**

**Electron microscopic identification of cell types in uninfected cultures**

The different cell types were identified on the basis of specific criteria. About 5% of the cells did not fulfil the criteria for a particular cell type and were therefore excluded from the counts.

In the CNS the cells were divided into neurons, oligodendroglia and astrocytes. The neuronal population included cells with large amounts of cytoplasm as well as others with sparse cytoplasm, but in both cases the cytoplasm contained abundant mitochondria, Golgi apparatus and rough endoplasmic reticulum. Neuronal nuclei displayed evenly-dispersed pale-staining nuclear chromatin and a single nucleolus. Oligodendroglia had small nuclei which stained darkly and their cytoplasm contained only a few organelles and some microtubules. Astrocytes were typically characterized by the presence of bundles of glial filaments with few, if any, microtubules in the abundant cytoplasm. The large nucleus contained pale-staining fine chromatin granules which were unevenly dispersed.

In the PNS there were neurons, fibroblasts, satellite cells and Schwann cells. Neurons were
Table 1. The proportion* of each cell type that contained intranuclear virus nucleocapsids at different times post infection (h p.i.) with either herpes simplex virus type 1 (HSV 1) or type 2 (HSV 2)

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Time p.i. (h)</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV 1-infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron</td>
<td>0 (0/82)</td>
<td>66 (33/50)</td>
<td>82 (40/49)</td>
<td>82 (55/67)</td>
<td></td>
</tr>
<tr>
<td>Oligodendroglion</td>
<td>2 (2/89)</td>
<td>57 (34/60)</td>
<td>67 (34/51)</td>
<td>84 (32/38)</td>
<td></td>
</tr>
<tr>
<td>Astrocyte</td>
<td>17 (6/36)</td>
<td>90 (102/113)</td>
<td>88 (114/129)</td>
<td>97 (145/149)</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>4 (8/207)</td>
<td>76 (169/223)</td>
<td>82 (188/229)</td>
<td>91 (232/254)</td>
<td></td>
</tr>
<tr>
<td>HSV 2-infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron</td>
<td>2 (2/85)</td>
<td>66 (43/65)</td>
<td>100 (56/56)</td>
<td>94 (62/66)</td>
<td></td>
</tr>
<tr>
<td>Oligodendroglion</td>
<td>5 (4/76)</td>
<td>46 (25/54)</td>
<td>100 (34/34)</td>
<td>100 (8/8)</td>
<td></td>
</tr>
<tr>
<td>Astrocyte</td>
<td>4 (2/50)</td>
<td>68 (37/54)</td>
<td>100 (40/40)</td>
<td>100 (65/65)</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>4 (8/211)</td>
<td>62 (105/173)</td>
<td>100 (130/130)</td>
<td>97 (135/139)</td>
<td></td>
</tr>
<tr>
<td>Peripheral nervous system</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV 1-infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron</td>
<td>0 (0/64)</td>
<td>58 (41/71)</td>
<td>80 (66/83)</td>
<td>74 (86/116)</td>
<td></td>
</tr>
<tr>
<td>Satellite</td>
<td>0 (0/45)</td>
<td>22 (8/37)</td>
<td>32 (20/62)</td>
<td>18 (23/130)</td>
<td></td>
</tr>
<tr>
<td>Schwann</td>
<td>13 (11/83)</td>
<td>15 (10/67)</td>
<td>36 (43/119)</td>
<td>31 (86/277)</td>
<td></td>
</tr>
<tr>
<td>Fibroblast</td>
<td>57 (20/35)</td>
<td>89 (8/9)</td>
<td>69 (11/16)</td>
<td>73 (19/26)</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>14 (31/227)</td>
<td>36 (67/184)</td>
<td>50 (140/280)</td>
<td>39 (214/549)</td>
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<tr>
<td>HSV 2-infected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron</td>
<td>0 (0/58)</td>
<td>47 (24/51)</td>
<td>85 (35/41)</td>
<td>97 (32/33)</td>
<td></td>
</tr>
<tr>
<td>Satellite</td>
<td>0 (0/35)</td>
<td>44 (8/18)</td>
<td>60 (18/30)</td>
<td>62 (28/45)</td>
<td></td>
</tr>
<tr>
<td>Schwann</td>
<td>10 (10/98)</td>
<td>10 (7/73)</td>
<td>73 (68/80)</td>
<td>87 (94/108)</td>
<td></td>
</tr>
<tr>
<td>Fibroblast</td>
<td>49 (19/39)</td>
<td>66 (25/38)</td>
<td>88 (14/16)</td>
<td>77 (7/9)</td>
<td></td>
</tr>
<tr>
<td>Total cells</td>
<td>13 (29/230)</td>
<td>36 (64/180)</td>
<td>75 (125/167)</td>
<td>82 (161/195)</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as a percentage of the total number of nuclei of each cell type that were present in the sections. That is,

\[
\left( \frac{\text{Number nuclei with nucleocapsids}}{\text{Number nuclei}} \right) \times 100 \% \text{ for each cell type.}
\]

large cells with abundant cytoplasm full of mitochondria, Golgi apparatus, rough endoplasmic reticulum and ribosomal rosettes. Neural nuclei were round with a single nucleolus and the nucleoplasm contained evenly-dispersed pale-staining chromatin granules. Neurons were surrounded by a satellite cell sheath. The satellite cell nuclei were small and round or triangular in shape with a densely staining nucleoplasm and a thin peripheral band of chromatin. Schwann cell nuclei also stained heavily and had a peripheral band of chromatin, an appearance identical to that of the satellite cell nuclei. The Schwann cells and satellite cells both exhibited a basal lamina. Nuclei of fibroblasts could appear as large as those of neurons, but were oval in shape and contained several nucleoli. The cytoplasm of fibroblasts contained few organelles and lacked Nissl substance.

Intranuclear nucleocapsids and subsequent cellular loss

Intranuclear nucleocapsids were seen in cultures 12 h after infection with either virus. At later times (36 and 48 h p.i.) nucleocapsids formed crystalline arrays, a phenomenon more characteristic of type 1- than type 2-infected cells. The infections progressed quickly from the most superficial layers of the culture to the deeper cells and by 48 h p.i., a high proportion of cells were showing advanced c.p.e. Identification of cells after this time became progressively more unreliable and so times after 48 h p.i. were not evaluated. The proportion of cells infected with respect to time after infection is summarized in Table 1.

In the CNS component neurons, oligodendrocytes and astrocytes were equally susceptible
to infection with either HSV 1 or HSV 2 and an increasing number of cells contained nucleo-
capsids with increasing time after infection. The type 2 virus appeared to infect more cells
more rapidly in the CNS than did the type 1 virus. In HSV 2-infected cultures, 100% of the
cells contained intranuclear nucleocapsids by 36 h p.i., but in HSV 1-infected cultures, even
by 48 h p.i., only about 90% of the cells contained virus nucleocapsids in their nuclei. In
cultures fixed at 24 h after infection with either HSV 1 or HSV 2, there appeared to be about
half as many neurons as in control sibling cultures. By 36 h p.i., there was no further reduc-
tion of neurons; however, by 48 h p.i., there were only about 20 to 25% as many neurons
present as in the control cultures. Numbers of oligodendroglia were progressively decreased
at different time points in the course of infection. By 48 h p.i., about 15% of the nuclei in
HSV 1-infected cultures and approx. 6% of the nuclei in HSV 2-infected cultures could be
identified as oligodendrocytes whereas, in controls, about 35% of the nuclei were oligo-
dendroglial. Astrocytes, in HSV 1-infected cultures, were among the first cells in which
virus nucleocapsids were seen in nuclei (at 12 h p.i.), but virus infection did not appear to
result in cell death or a subsequent decrease in cell numbers. In fact, as early as 24 h p.i.,
there was an increased number of astrocytes and 48 h p.i. the number of cells identified as
astrocytes in HSV 1-infected cultures was three- to fourfold higher than in control cultures.
At that time, 97% of astrocytic nuclei in infected cultures contained intranuclear virus
nucleocapsids. The cytoplasm of these astrocytes appeared swollen and was packed with
bundles of long glial filaments. In cultures infected with HSV 2, 100% of astrocytic nuclei
were infected by 36 h p.i. A proliferation of astrocytes was not obvious in these cultures.
On the other hand, there did not appear to be a decrease in astrocyte numbers during the
experiment, although cells were swollen and full of bundles of glial filaments.

In the PNS portion, HSV 2 infection spread more rapidly than HSV 1, but the cellular
involvement with either virus was less than in the CNS. Even by 48 h p.i., in HSV 2-infected
cultures, only 82% of the PNS cells contained virus nucleocapsids, whereas in the CNS
fragment, 100% of the cells were infected by 36 h p.i. Virus nucleocapsids were found with
equal frequency in the nuclei of neurons and fibroblasts, infected for 24 h or more with
either type of herpes virus. Although fibroblasts appeared to be reduced in number by 36 h
p.i., most of the neurons were not lost from the cultures until after 48 h p.i. Nuclei of satellite
cells and Schwann cells contained virus nucleocapsids less often than did the nuclei of
neurons and fibroblasts in HSV 1-infected cultures, but in HSV 2-infected cultures nuclei of
all cell types contained nucleocapsids with equal frequency. It was possible, especially in
HSV 1-infected cultures, to find neurons with intranuclear nucleocapsids and cytoplasmic
enveloped virus which were surrounded by apparently uninfected satellite cells.

Changes in the nuclei of infected cells
There was reduplication and thickening of the nuclear membrane in all cell types infected by
both viruses, but it was seen more frequently in HSV 2-infected cultures. There was also
clumping of chromatin in neurons in both the CNS and PNS portions of cultures and a
distinct margination of chromatin in oligodendrocytes and astrocytes infected with either
virus. Astrocytic nuclei, especially those infected with HSV 1 for 36 or 48 h, also appeared to
form large 'buds' into the cytoplasm (Fig. 1a). These buds often contained groups of dense-
cored virus nucleocapsids.

Intranuclear membranous whorls (Fig. 2a) were found in nuclei late (after 48 h) in
infection. They were more common in the CNS than in the PNS portions, but were rarely
seen in oligodendroglia which did not often survive this late in the infection. Membranous
'tubules' and lengths of fused membranes (Fig. 2e), which appeared like rods, were also
occasionally seen in cells infected with either virus.

Certain nuclear inclusions were characteristic of the virus type used to infect the cultures.
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Fig. 1. Electron micrographs of cultures infected with HSV 1 for 36 h depicting astrocytes which have fused to form syncytia. (a) Buds of nucleoplasm, some containing dense-cored virus nucleocapsids (arrows), protrude from the infected nucleus which itself contains clumps of virus nucleocapsids (arrowheads). (b) Unenveloped dense-cored virus nucleocapsids (nc) interspersed among small vesicles, lie in the cytoplasm. Enveloped nucleocapsids can be seen in the nucleus on the right and between the inner and outer nuclear membranes of the nucleus on the left. Bundles of fine filaments are present in the nucleus on the right (arrows).
Fig. 2. Electron micrographs of virus-induced structures in the nuclei of cells infected with HSV 1 (a, c, d, e) or HSV 2 (b). (a) Whorls of membranes which may or may not be associated with virus nucleocapsids occur within nuclei of cells infected with HSV 1; electron-dense round structures (arrow) are usually associated with nucleocapsids. Similar inclusions are also seen in HSV 2-infected nuclei. (b) Tubule-like structures, seen as parallel bundles of filaments or as a lattice (inset), are characteristically found in HSV 2-infected nuclei. (c) Bundles of interwoven filaments are found in HSV 1-infected nuclei. (d) Fine fibrils associated with nucleocapsids in HSV 1 infection may be of similar origin to fibrils in the bundle seen in (c). (e) Rod-shaped structures with an electron-dense centre and granular fuzz along the side occur in the nucleus of cells infected with HSV 1 or HSV 2.

In HSV 1-infected cells, 10 to 20% of the nuclei in the CNS portion contained bundles of interwoven fine fibrils (Fig. 2c). They were less common in PNS cell nuclei. The individual fibres resembled those associated with clumps of nucleocapsids seen in HSV 1-infected cells (Fig. 2d).

In cells infected with HSV 2, about 70% of the CNS nuclei and about 60% of the PNS nuclei contained tubule-like structures which appeared either as a bundle of parallel filaments (Fig. 2b) or as a lattice (Fig. 2b, inset) depending upon the plane of section. Briefly, at 48 h p.i., more than 80% of the CNS neuronal and PNS fibroblast nuclei, 50 to 70% of oligodendrocytic, astrocytic, satellite cell and Schwann cell nuclei, and about 20% of PNS neuronal nuclei contained these tubules. The tubules had a diam. of 18 to 20 nm, a centre–centre spacing of approx. 28 nm and could be up to 3 μm in length.
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Fig. 3. Electron micrograph of peripheral neuron (N) and three satellite cell nuclei (S) in a culture 36 h p.i. with HSV 1. Two of the satellite cell nuclei are in a syncytium with the neuron.

Location of enveloped virus particles

In cultures infected with either HSV 1 or HSV 2, enveloped virus was found in the cytoplasm of all cell types after 24 h of infection. Neurons, oligodendrocytes and astrocytes in the CNS portion of the culture contained similar amounts of enveloped virus in their cytoplasm. In PNS, neurons and fibroblasts infected with either virus appeared to contain numerous enveloped virus particles (often as many as 70% of the total particles). There were more enveloped viruses in Schwann cells and satellite cells in cultures infected with HSV 2 than in cultures infected with type 1 virus.

Enveloped nucleocapsids were also found in the nucleus of some cells where they were present either alone or in groups, usually membrane-bound, or between the inner and outer nuclear membrane (Fig. 1 b). This nuclear enveloped virus was more common in the CNS than the PNS, and astrocytes often appeared to contain more enveloped virus in the nucleus than in the cytoplasm.

Intracytoplasmic nucleocapsids

Unenveloped virus nucleocapsids were found grouped in the cytoplasm of about 20% of all the astrocytes in cultures infected for 24, 36 or 48 h with HSV 1. They were occasionally found in the cytoplasm of astrocytes infected with HSV 2, but were rarely seen in the cytoplasm of any other CNS or PNS cells infected with either virus. In HSV 1-infected astrocytes (Fig. 1 b), the cytoplasmic nucleocapsids were usually dense-cored and associated with vesicles that were 60 to 80 nm in diam. Cytoplasmic nucleocapsids were not associated with microtubules, but some were in close proximity to portions of thickened membranes.

Syncytial formation

Syncytia were occasionally found in both the CNS and PNS components of cultures infected with HSV 1, but unequivocal syncytia were not seen in HSV 2-infected cultures.
Syncytia involving 2 or 3 neurons or, more rarely, oligodendrocytes were found, but syncytia composed of astrocytes were more common (Fig. 1a, b). A syncytium containing what appeared to be a neuronal as well as an astrocytic nucleus was seen in one instance.

In the PNS, fusion developed almost exclusively between a neuron and its surrounding satellite cells (Fig. 3). Syncytia containing more than one neuronal nucleus were not found. The nuclei of satellite cells involved in the syncytia did not always show evidence of virus infection.

**DISCUSSION**

We have shown that in an organotypic nerve cell culture system, all cells in both the CNS (neurons, astrocytes and oligodendrocytes) and the PNS (neurons, fibroblasts, satellite and Schwann cells) components can support replication of HSV 1 and HSV 2 and produce enveloped virus particles. HSV 2 infection spread more rapidly than did HSV 1 infection and both viruses caused more cell death in the CNS portion than in the PNS portion. Syncytia were found in both the CNS and PNS components of cultures infected with HSV 1, but unequivocal syncytia were not seen in HSV 2-infected cultures. Fusion was found between neurons and their associated satellite cells in the PNS component. In the CNS component, fusion was seen between neurons, but more commonly involved the astrocytes.

HSV 1 infection was characterized by the presence of bundles of fine filaments in the nuclei of infected cells, and by the formation of syncytia. The bundles of filaments, shown here to be typical of type 1 virus infection, were not found frequently in infected PNS nuclei, but were present in 10 to 20% of infected CNS nuclei. They may represent a special form of virus DNA accumulation within the nucleus late in infection (Luetzeler & Heine, 1978). The formation of syncytia has been reported previously in organotypic cultures of CNS (Feldman et al. 1968; Leestma et al. 1969; Dubois-Dalcq et al. 1972; Ecob-Johnston et al. 1978) and of the PNS (Feldman et al. 1968; Hill & Field, 1973) but in none of these cases were the syncytia thought to involve neurons. The only previous report in which neurons were involved in syncytial formation was after HSV 2 infection of rat PNS (Ziegler & Pozos, 1977). This is the first report of neurons being involved in syncytia after HSV 1 infection of organotypic cultures. Unlike Ziegler & Pozos (1977), however, our electron microscopic studies did not reveal syncytia with more than one neuronal nucleus.

HSV 2 infection was characterized by the presence of tubule-like structures in about 70% of the infected CNS nuclei and about 60% of infected PNS nuclei. The tubule-like structures have been seen in cell lines (Schwartz & Roizman, 1969; Mori et al. 1973; Oda & Mori, 1976; Young et al. 1977; Atkinson et al. 1978), organotypic nerve cell tissue culture (Dubois-Dalcq et al. 1972; Ecob-Johnston et al. 1978), embryonated eggs (Couch & Nahmias, 1969) and experimental animals (Murphy et al. 1967) infected with HSV 2. These tubule-like structures were found in all cell types in HSV 2 infection in our study but had appeared infrequently in peripheral neurons (Ecob-Johnston & Whetsell, unpublished results). Of infected neuronal nuclei in the PNS, only 20% contained structures whereas at least 50% of all nuclei contained them. This lower proportion may reflect a host cell–virus interaction of the peripheral neuron which is different from that of other cell types in this system. The role of the tubule-like structures in virus replication, however, remains obscure.

In cultures infected with both HSV 1 and HSV 2, nucleocapsids appeared to acquire an envelope at the inner nuclear membrane and, occasionally, at the cytoplasmic membrane. Enveloped virus particles were found either free or within vesicles in the cytoplasm. Our results agree with those of Nii et al. (1968) and Atkinson et al. (1978) that enveloped or naked nucleocapsids in the cytoplasm invariably contain dense-cores. Friedman et al. (1976) noted naked nucleocapsids (associated with small vesicles) in the cytoplasm of Vero
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cells infected with *Herpesvirus saimiri*. These were present in cells in which there was no nuclear evidence of virus replication, leading these authors to speculate that *Herpesvirus saimiri* developed in the cytoplasm. Cytoplasmic nucleocapsids in our study were only present in cells with nuclear nucleocapsids or enveloped virus particles and were probably derived from the nucleus.

In comparing the effect of HSV infection in organotypic cultures of nervous system to effects of HSV infection in nervous system of whole animals, a consideration of differences between the two experimental models indicates the value of making such comparisons. In the culture system, all of the cellular components are more readily available to virus infection than in the intact animal. Also, the cultures, growing and developing in isolation, apparently are unable to mount an immune response against the virus infection. These two features, especially, facilitate closer examination of certain aspects of the response of neurons and supporting cells to HSV infection in both CNS and PNS. Specifically, the cultures permit study of whether all cell types, when exposed to the virus in the absence of immunological defences, can actively support virus infection and whether there are differences in the responses of the different cell types during the course of the infection. Though it is the primary intent of this communication to examine those two questions in the organotypic culture model, useful comparisons can be made between the culture studies and studies of HSV infections in animals. Using both HSV 1 and HSV 2, Kristensson *et al.* (1978), inoculated mice either via foot pad or cornea and noted that intra-axonal transport of virus occurred to the ipsilateral ganglia in either case and that both HSV 1 and HSV 2 invaded the CNS equally well. Although there was evidence of virus replication in neurons and Schwann cells of the PNS, cellular destruction was almost totally confined to the CNS; similar observations have also been reported recently in rabbits (Townsend & Baringer, 1978). Kristensson *et al.* (1978) has suggested that the PNS in the intact animal is protected by the availability of an immune system (Walz *et al.* 1976). In the absence of an immune response in our culture system the PNS was not spared, as it is in vivo, although both viruses caused less devastation there than they did in the CNS components.

When rabbits were given an intra-ocular injection of HSV 2, there was intra-axonal spread of virus and evidence of virus infections in neurons, oligodendrocytes and astrocytes (Kristensson & Wisniewski, 1978). No investigation of PNS was reported, but these studies indicate, as do our studies, that oligodendrocytes, astrocytes and neurons can actively support the virus infection.

In the tissue culture model we have used, a model of PNS and CNS in which all cell types are fairly readily available for virus infection and in which there is no immunological response capability, the cellular response to infection with either virus appears to be characteristic of the particular host-cell type involved. In the PNS fragment, neurons were the site of greatest production of enveloped virus and appeared to survive at least 24 h after virus was first detected by electron microscopy in their nuclei. Fibroblasts similarly produced an abundance of enveloped particles, but infected fibroblasts did not survive as long as infected neurons. Satellite cells and Schwann cells appeared more resistant to HSV 2 than to HSV 2 infection.

In the CNS fragment, after an initial reduction in numbers, the remaining neurons appeared to survive in an infected state for at least 24 h. Oligodendrocytes, however, appeared to succumb to virus infection very quickly, their numbers rapidly decreasing throughout the progression of the infection. Astrocytes responded in a unique way to virus infection in that they appeared to swell and to develop large bundles of glial filaments as early as 24 h p.i. By 48 h p.i., there was an actual increase in numbers of astrocytes in the cultures; in some cases the cultures were almost entirely composed of astrocytes. In the nuclei of these cells, aggregates of nucleocapsids were frequently observed near the nuclear
membrane, and buds of nucleoplasm, often containing dense-cored virus particles, were seen projecting from the infected nuclei. In addition, groups of dense-cored but unenveloped virus particles, always associated with small vesicular structures, were found in the cytoplasm of many of these infected astrocytes. These naked nucleocapsids may have arisen from particles which acquired an envelope at the nuclear membrane and have subsequently undergone a process of de-envelopment such as that described for Lucké frog virus (Stackpole, 1969). An alternative hypothesis may be that the nuclear buds containing the virus nucleocapsids disintegrate, releasing naked nucleocapsids into the cytoplasm. In any case, there is evidence to suggest that astrocytes, in this system, deal with virus infection differently from other cellular elements and have the unique ability to contain or modify the virus replication so that the cells not only survive virus infection, but are still able to react to the noxious stimulus.

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