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# Virulent and Attenuated Canine Distemper Virus Infects Multiple Dog Brain Cell Types In Vitro

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*ABSTRACT* Canine Distemper Virus (CDV) produces an encephalitis in dogs that varies with viral strain. We have studied the cell tropisms of two virulent strains (CDV-SH and CDV A75-17) and an attenuated strain, Rockborn (CDV-RO), in cultured canine brain cells. Infected cell types were identified by double immunofluorescent labeling of specific cell markers and viral antigens. All viral strains studied produced infection in astrocytes, fibroblasts, and macrophages. Neurons were not infected by CDV A75-17 but were rapidly infected by CDV-SH and CDV-RO. Multipolar oligodendrocytes were very rarely infected by any of the virus strains. In contrast, a morphologically distinct subset of bipolar oligodendrocytes were commonly infected by CDV-SH and CDV-RO. The kinetics of infection in the astrocytes, oligodendrocytes, neurons, and macrophages varied between strains. Both CDV-SH and CDV-RO rapidly infected bipolar oligodendrocytes, astrocytes, neurons, and macrophages by 14 days post infection while infection by CDV A75-17 was delayed until after 28–35 days post infection. The differences in the growth kinetics and cell tropisms for some brain cells, exhibited by the three viral strains examined in this in vitro study, may relate to the different CNS symptoms that these strains produce in vivo.

## INTRODUCTION

Canine Distemper Virus (CDV) serves as an animal model of diseases that are characterized by demyelination and persistent infection of the central nervous system (CNS). Two virulent strains, CDV-Snyder Hill (CDV-SH) and CDV A75-17, extensively studied in this and other laboratories, produce contrasting forms of Canine Distemper Encephalitis (Appel, 1969; Summers et al., 1979, 1984). The A75-17 strain typically produces a subacute to chronic disease of white matter with prominent glial cell infection and demyelination in the CNS while the Snyder Hill strain (CDV-SH) results in an acute CNS gray matter disease with neuronal infection. Attenuated strains of this virus, such as CDV-Rockborn (CDV-RO), are used for vaccinal purposes in dogs. Given the in vivo biological differences between these CDV strains, the question arises as to whether the differences in disease phenotypes are related to differences in CNS cell tropisms. In the experiments reported here we have examined growth properties of these different CDV strains in specific CNS cell types. Owing to the obvious importance of the myelin forming cell, the oligodendrocyte, during CNS demyelination, we have focused much of our attention on this cell in these studies.

## MATERIALS AND METHODS

### Brain Cell Culture

Dissociated cerebral or cerebellar cultures were established from 1-to-2-day-old specific-pathogen-free

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beagle dogs from the Baker Institute colony. The method for derivation of mixed brain cells from cerebrum was a modification of the McCarthy and deVellis (1980) technique as previously described (Pearce-Kelling et al., 1990). For comparison, cerebellar tissue was cultured by a method of Zurbriggen and co-workers (Zurbriggen et al., 1984). Briefly, the cerebellum was minced with scalpel blades and transferred to a centrifuge tube; 10 ml of Dulbecco's modified Eagles medium containing 4.5 g/L glucose were added, the tissue was triturated through a 5.0 ml pipet ten times, and the suspension was allowed to settle. The supernatant fluid was saved and these steps were repeated until 45 ml of cell suspension were obtained. To this, 5 ml of FCS was added. Five 10 ml aliquots of the cell suspension ($1.5 \times 10^6$ live cells per ml) were dispensed into Petri dishes (Falcon 100 × 20 mm). Ten glass coverslips, 22 mm in diameter (Roboz Brand), had been adhered to the bottoms of these dishes with sterile silicon (Dow, Corning).

After 4 days in vitro (div) culture media for all cultures were replaced. The media in both culture systems were replaced three times a week and the cultures were incubated at 37°C in the presence of 5% $CO_2$.

### Infection of Cultures With CDV Strains SH, A75-17, and RO

CDV strains SH and A75-17 were grown in PHA-stimulated dog thymocyte cultures (Friedlander et al., 1985) and stored at −70°C in 7% DMSO, 20% FCS, and RPMI 1640 medium. CDV-SH and CDV A75-17 inocula were titrated in dog lung macrophages (Appel and Jones, 1967). The avirulent strain CDV-RO was propagated and titrated in dog kidney cells (Appel, 1978). Cultures were infected when they neared confluency at 7–10 div. For inoculation of cerebral cultures, 0.4 ml containing $10^3$ Tissue Culture Infectious Dose 50 per ml of CDV-SH, CDV A75-17, and CDV-RO were adsorbed with the cultures in each tube for 2 h followed by the addition of 0.6 ml of media. Cerebellar cultures were incubated with 5.0 ml per Petri dish of the same inoculum for 2 h followed by the addition of 5 ml of media. Cultures were maintained for up to 63 days post inoculation (dpi) (70 div). During this period any cytopathic effect (cpe) caused by the virus in live cultures was recorded and at weekly time points coverslips were removed, fixed, and stained for immunofluorescent evaluation.

### Identification of CDV Infected Cell Types

CDV antigen was localized within specific cell types by double immunofluorescence. The primary antisera used to identify these cells and their conditions for fixation and staining are shown in Table 1. Cultures were fixed for 10 min in the appropriate fixative (see Table 1) and washed in phosphate-buffered saline (PBS). When possible, acetone was employed as a fixative because this produced optimal immunofluorescent detection of CDV. Incubation with primary antisera against the specific cell markers was followed by two 5 min rinses in PBS before the secondary incubation. Secondary antibody was either goat anti-mouse immunoglobulins or goat anti-rabbit immunoglobulins (depending on the source of the primary antibody) conjugated to rhodamine and diluted in PBS 1:20. Viral antigen within the labelled cells was then identified with fluoresceinated anti-CDV hyperimmune dog serum prepared in this laboratory by the methods of The and Feltkamp (1970), which was applied as a final step. Each incubation with antibody in all procedures was performed for 30 min at room temperature. Stained coverslips were washed in PBS, dipped in distilled water, and mounted in gelvatol to preserve fluorescence (Johnson et al., 1982). Negative controls included stained uninfected cultures and the omission of primary antisera during the staining procedure. Labeled cells were viewed on a Leitz epifluorescent microscope equipped with rhodamine (530–560 nm) and fluorescein (470–490 nm) filters.

Macrophages were identified by their rosetting and subsequent phagocytosis of IgG-coated sheep red blood cells (SRBC) as described by Stoddart and Scott (1988). Cultures were fixed for 30 sec in buffered methanol (Dif-Quik, Fischer Scientific Products) and stained for viral proteins with anti-CDV-FITC as described above. In a few replicate cultures, viral protein was also demonstrated in macrophages by standard peroxidase-anti-peroxidase (PAP) procedures (Sternberger, 1979) using a monoclonal antibody (clone number 4.272 diluted 1:1,000) directed against the NP protein of CDV (Baumgartner et al., 1989).

## RESULTS

### Description of Cultures

The cerebral cultures reached confluency between 7 and 10 div and consisted of a variety of cell types including macrophages, astrocytes, fibroblasts, neurons, and oligodendrocytes. The cerebellar cultures reached confluency earlier, between 4 and 7 div, and contained a denser population of similar types of cells as the cerebral cultures.

Two types of oligodendrocytes were present in both culture systems. GC labeled cells exhibiting a bipolar morphology (Fig. 1A) comprised a small percentage (less than 5%) of the total cell population and were more common in cerebral cultures than in cerebellar cultures. The number of bipolar GC+ cells dropped from an average of approximately 100 per coverslip (in cerebral cultures) during the first 28 div to less than 20 per coverslip beyond 28 div.

A second type of oligodendrocyte was strikingly different in morphology from the GC+ bipolar cells. These were GC+ cells with small, round, and birefringent cell bodies and many finely branching processes as shown in

*TABLE 1. Cell markers used for cell type identification*

| Cell marker | Host species | Fixation | Dilution | Source | Cell type identification | References |
|---|---|---|---|---|---|---|
| Glial fibrillary acidic protein (GFAP) | Rabbit | Acetone | 1:50 | DAKO Corp. | Astrocytes | Eng, 1971 |
| Galactocerebroside (GC) | Mouse | Formalin | 1:100 | Dr. B. Ranscht[a] | Oligodendrocytes | Raff, 1978 |
| Neurofilament protein (RT97) | Mouse | Acid/alcohol | 1:500 | Dr. J. Wood[b] | Neurons | Wood, 1981 |
| Fibronectin-TRITC | Goat | Acetone | 1:50 | Cappel | Fibroblasts | Schachner, 1978 |
| IgG-coated sheep red blood cells | — | Dif-Quik | 1:200 | Sigma | Phagocytic macrophages | Stoddart, 1988 |

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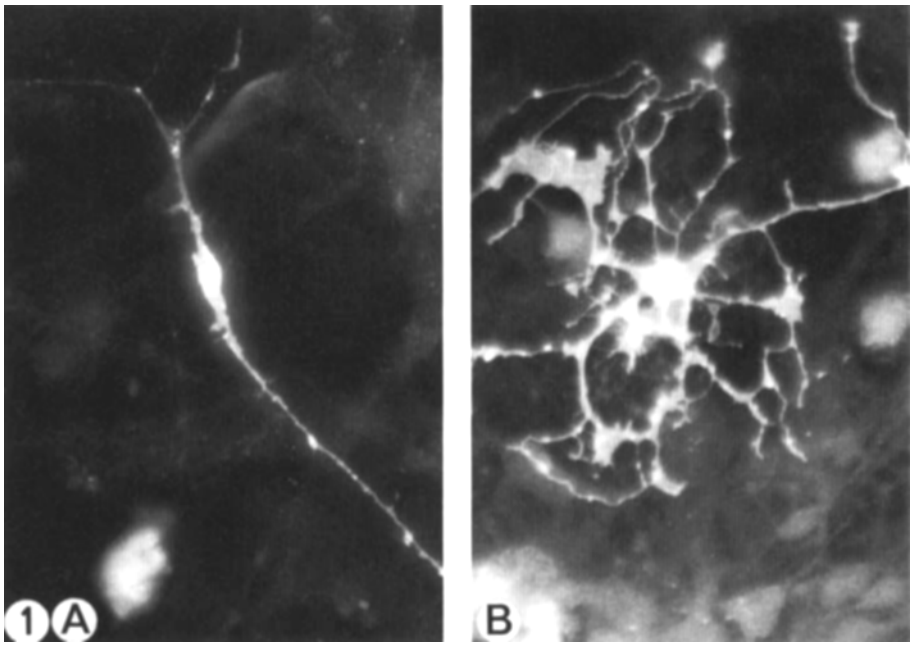


Fig. 1. Galactocerebroside (GC) immunofluorescence of a bipolar (A) and multipolar (B) oligodendrocyte in cultured canine brain cell cultures. A: Bipolar GC+ cells are more prevalent in the cerebral cultures and were common until 28 div. B: Multipolar GC+ cells were more prevalent in cerebellar cultures and persisted until the last time points sampled (56 div) × 500.

Figure 1B. These cells appeared typical of oligodendrocytes as illustrated in comparable studies in mice and rats (Schachner et al., 1981; Kachar et al., 1986). The multipolar oligodendrocytes were more prevalent and persisted longer in the cerebellar cultures than in cultures derived from the cerebrum and were observed from 7 to 56 div (the last time point sampled for cerebellar cultures), however they comprised less than 5% of the total cell population in these cultures. In the cerebellar culture, multipolar oligodendrocytes composed a higher percentage of the total GC+ population than did bipolar oligodendrocytes. Cerebellar cultures were seeded at three times the density of the cerebral cultures and typically many hundreds of multipolar GC+ cells were present compared to the less than 50 bipolar GC+ cells observed. In contrast, the cerebral cultures contained predominantly bipolar oligodendrocytes (generally fewer than 20 multipolar oligodendrocytes were observed on each coverslip). The multipolar oligodendrocytes were often clustered together and were common in areas of high cell density, such as in fibrocytic regions of the cultures.

Two morphological subsets of neurons were labeled by monoclonal antibody RT97 directed against the 200 kD neurofilament protein. The first type was a small bipolar cell that was very common (many hundreds per coverslip) during the first 14 div; the processes of these neurons typically were beaded (Fig. 2A). The second neuronal type (Fig. 2B) bore a large cell body and had longer processes with a few distal branches. This cell

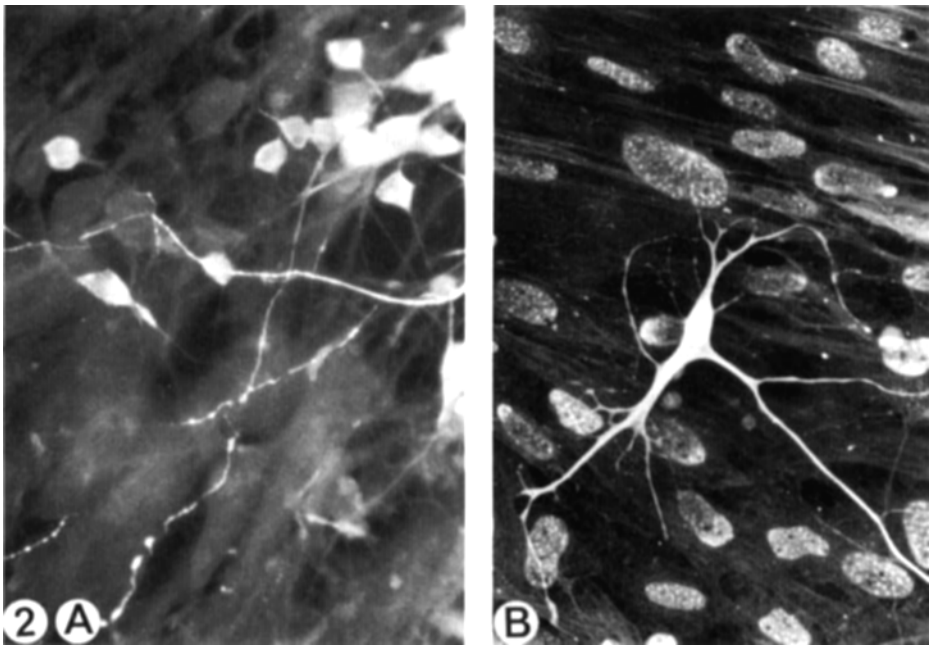


Fig. 2. Neurofilament (RT97) staining of neurons in canine brain cell cultures. A: Small-bodied, bipolar neurons which lived only for the first 14 div. B: Large neurons with long, multibranched processes which survived for long periods of time in culture. (Histones within the nuclei of other cells below the neuron stain due to a cross reaction with the antibody.) × 500.

type remained at a low density in both culture systems (less than 20 per coverslip) up to the last time points sampled.

Three other cell types were prominent in these cultures. Astrocytes, defined by glial fibrillary acidic protein (GFAP) labeling, composed the major cell population in both culture systems throughout the course of the experiments. Three morphologies of GFAP-positive cells were observed: a fibrillary type of astrocyte with a small perikaryon and long thin processes (Fig. 3A), a broad protoplasmic type cell with fewer, shorter processes, and also a small bipolar type of cell, perhaps representing a newly differentiated astrocyte from a progenitor lineage.

Macrophages were identified by their rosetting and phagocytosis of IgG-coated SRBC (Fig. 3B), which frequently numbered more than ten per cell and completely filled the cytoplasm. Macrophages were typically round to epithelioid and had a few stout or no processes. They persisted in large numbers (estimated at thousands per coverslip) in both culture systems throughout the course of the experiments (to 70 div).

Fibroblasts, defined by labeling of extracellular fibronectin, were more prevalent in the first few weeks of culture but diminished by 4 weeks in vitro. Removal of meningeal tissue reduced the density of fibroblast growth in the cerebral cultures. The cerebellar cultures, from which the meninges were not extensively removed, contained a higher density of fibroblasts.

## CDV in Cultured Canine Brain Cells

Double immunofluorescent staining of different brain cell types allowed identification of infected cell types. Some fixatives (Table 1) were more compatible with concurrent viral staining than others. The fixative requirements of oligodendrocytes and neurons (formalin and acid/alcohol, respectively) diminished the intensity of viral staining such that it was often not possible to determine if some of these cells were CDV positive. Another difficulty in determining if oligodendrocytes were infected arose because these cells frequently grew above other infected cell types such as astrocytes. For these reasons a quantitative comparison of the infection of various brain cells was not feasible.

## CDV in Canine Oligodendrocytes

CDV proteins were commonly detected only in the bipolar type of oligodendrocytes that were present in both cultures systems. Although infection of these cells was often indeterminable (due to technical reasons stated above) careful examination of the proper cell stratum allowed observation of unequivocal infection in bipolar GC+ cells. An average of 1,000 bipolar oligodendrocytes were observed in each of three experiments. By 21 dpi, the majority of these cells were infected by CDV. Two bipolar oligodendrocytes are shown staining with GC (Fig. 4A). Each is infected with CDV RO (Fig. 4B). As

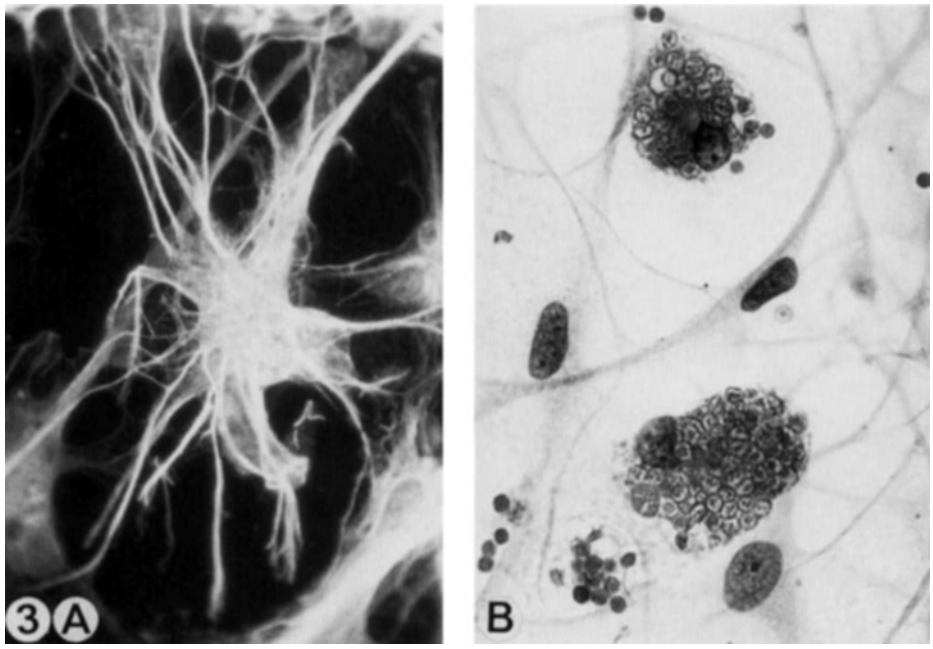


Fig. 3. A: Glial fibrillary acidic protein (GFAP) immunofluorescence of a fibrous astrocyte in cultured canine brain cells. × 500. B: Two macrophages in canine brain cells cultures which have phagocytized IgG-coated sheep red blood cells. Counterstained with hematoxylin. × 875.

shown here, viral staining is typically observed primarily in the perinuclear areas as well as along the cell processes. Intranuclear inclusions were not as frequently seen as in infected astrocytes (Pearce-Kelling et al., 1990). Infection by both CDV-SH and CDV-RO in bipolar oligodendrocytes was evident by 7 dpi in both culture systems. CDV A75-17 infection was not observed in bipolar oligodendrocytes until beyond 35 dpi, by which time point most of these cells had disappeared from the cultures, particularly those derived from the cerebrum. CDV was very rarely observed in the multipolar GC+ oligodendrocytes. No cpe (e.g., no alteration in morphology) nor reduction in the number of either type of oligodendrocytes compared to control cultures was noted in infected cultures up to 49 dpi.

### CDV in Canine Neurons

CDV-SH and CDV-RO infection was observed only in the large, long-lived neurons. Neurofilament staining is shown in Figure 5A and staining of viral protein is illustrated in this neuron in Figure 5B. No CDV A75-17 infected neurons were observed in these experiments. No CDV was seen in the small bipolar neurons but they did not remain in the cultures beyond 14 dpi.

### CDV in Other Cell Types: Astrocytes, Macrophages, and Fibroblasts

We have previously described in detail the infection of astrocytes by different strains of CDV (Pearce-Kelling et al., 1990). Briefly, all three strains of CDV employed in these experiments infected astrocytes (Fig. 6A,B) but differences in their rates of growth and cytopathic effects on the astrocytes were found. CDV-SH typically infected the majority of astrocytes by 14 dpi, whereas a similar level of infection by CDV A75-17 was not attained until after 28–35 dpi (Pearce-Kelling et al., 1990). Neither of these virulent strains caused cytolysis of the astrocyte population. In contrast, CDV-RO produced a rapid and cytolytic infection by 14 dpi.

Macrophages were among the first cell types infected by both of the virulent strains of CDV as well as by CDV-RO (Fig. 7); their kinetics of infection were similar to the rates of infection observed in other brain cells and as previously described in detail in the astrocytes (Pearce-Kelling et al., 1990). The percentage of CDV infection in approximately 1,000 macrophages was determined during the first 5 weeks post inoculation for each of the three viral strains. By 21 dpi, both CDV-SH and CDV-RO had infected over 90% of the macrophages while CDV A75-17 had infected only 8%. By 35 dpi, the percentage of CDV A75-17 positive macrophages had risen to over 90%. Viral inclusions were observed both in the cytoplasm and nuclei of these cells. Fusion of infected macrophages was occasionally observed. In PAP-stained preparations, cytoplasmic staining was seen between ingested SRBC (Fig. 7).

Fibroblasts, defined as those cells surrounded by fibronectin staining, were readily infected by all three strains of CDV. The rates of infection by the different viral strains in fibroblastic areas of the culture were

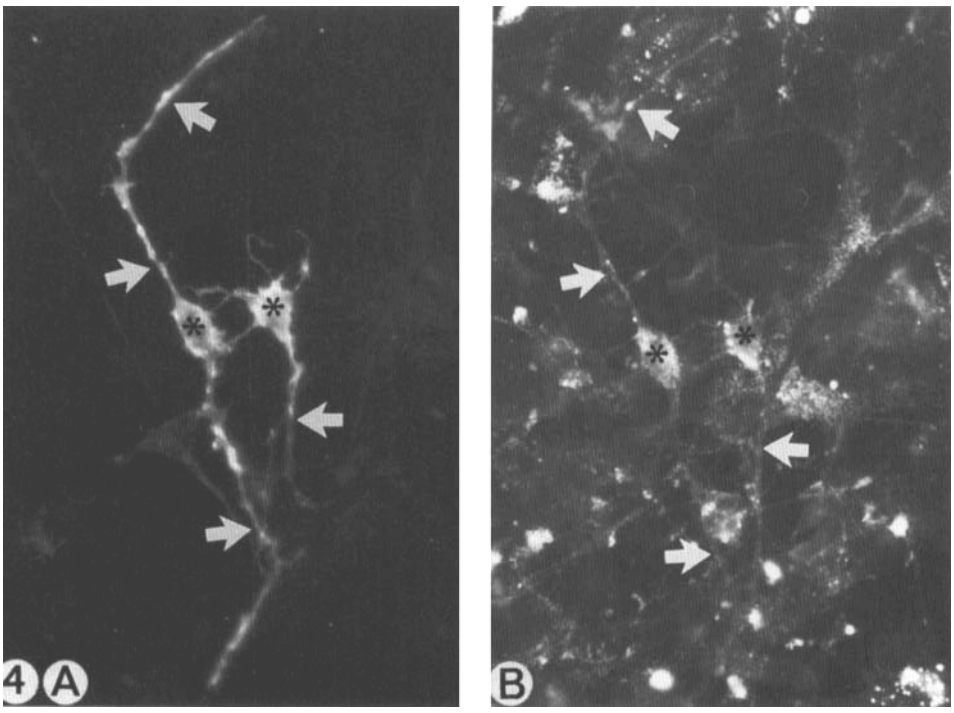


Fig. 4. Two bipolar oligodendrocytes infected with CDV-RO. **A**: GC immunofluorescence (rhodamine). Note the two cell bodies (asterisks) and their processes (arrows). **B**: The same field showing CDV immunofluorescence (fluorescein). The oligodendrocytes (asterisks and arrows) frequently grew on top of other infected cells as shown here. × 500.

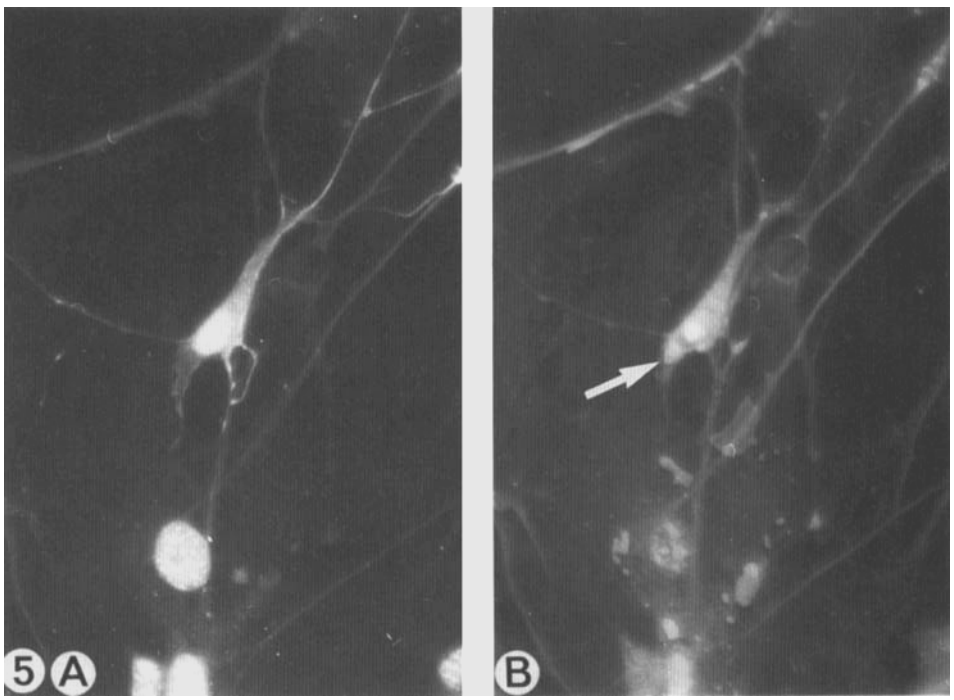


Fig. 5. A neuron infected with CDV-SH. **A**: Neurofilament immunofluorescence (rhodamine) of a large neuron. **B**: The same field showing viral staining (fluorescein). Note a cytoplasmic inclusion (arrow). The decreased intensity of viral immunofluorescence is due to the acid alcohol fixation requirements of the neurofilament antigen. × 500.

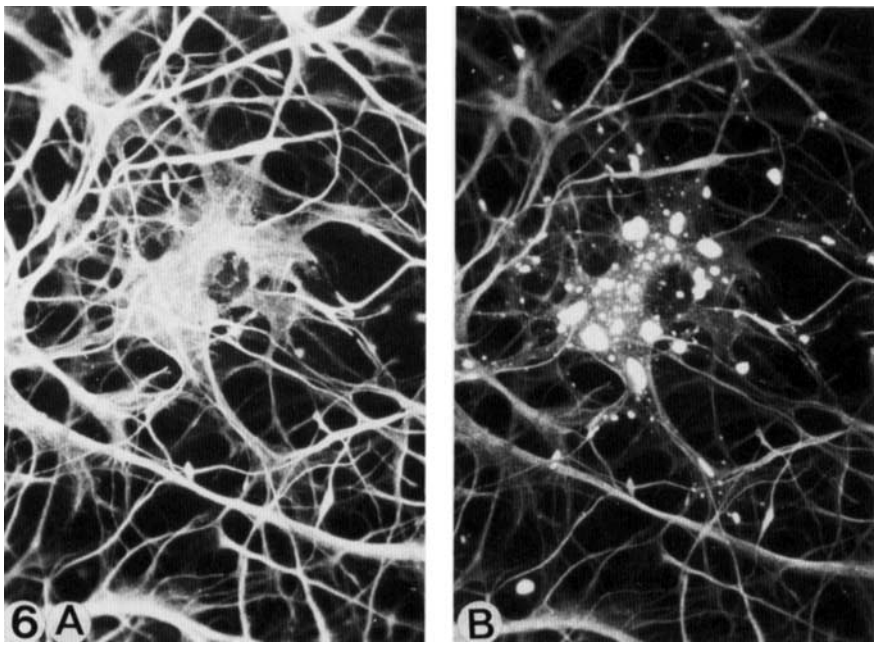


Fig. 6. An astrocyte infected with CDV-SH. A: GFAP immunofluorescence (rhodamine). B: The same field showing viral staining (fluorescein) within the astrocyte. Both cytoplasmic and intranuclear CDV inclusions are well stained following acetone fixation (as opposed to formalin and acid alcohol fixation as seen in Figs. 4 and 5). × 500.

similar to the kinetics observed in the astrocyte population; i.e., CDV-SH and CDV-RO caused rapid and widespread fibroblast infections while CDV A75-17 produced a slow but eventually a total infection.

## DISCUSSION

In these studies, the cellular composition of neonatal canine cultures was found to include two types of oligodendrocytes, astrocytes, macrophages (presumably resident microglial cells), fibroblasts, and two types of neurons (short and long-lived). In previous studies of cultured canine brain cells (Zurbriggen et al., 1984, 1987a), oligodendrocytes, astrocytes, macrophages, neurons, and fibroblasts were described, however only one type of oligodendrocyte was noted. The oligodendrocytes described in studies of Zurbriggen and Vandevelde appear similar to the multibranched GC+ cells we have observed (as shown in Fig. 1B) and which others have illustrated in the rat and mouse (McCarthy and de Vellis, 1980; Knapp et al., 1987). Although most of the brain cell types survived comparably when grown from either the cerebrum or the cerebellum, the multipolar oligodendrocyte was most successfully cultured by the cerebellar technique. Several factors may bear on this difference: 1) intrinsic differences between cerebral and cerebellar tissue, 2) the differences in cell density at time of plating, 3) the shorter preparation time for the cerebellar cultures, and 4) the absence of trypsin treatment for cerebellar tissue.

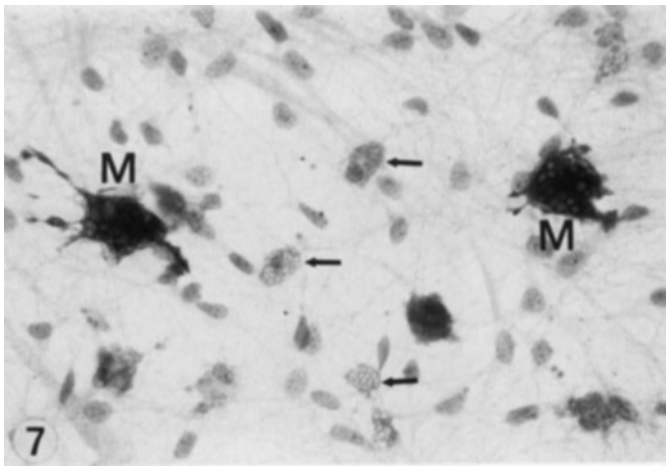


Fig. 7. Two macrophages infected with CDV A75-17. Fc-mediated phagocytosis of sheep red blood cells to identify macrophages was followed by staining of viral NP protein by routine PAP procedures and counterstaining with hematoxylin. Infected macrophages (M) and uninfected macrophages (arrows) × 350.

We have yet to elucidate what, if any, relationship exists between the two morphological subsets of GC+ cells in our cultures. Each may represent a different functional subtype of oligodendrocyte, or alternatively they may be related in a differentiation pathway. In recent years, tissue culture studies have yielded much information on the maturation of neuroglia from pro-

genitor cells. In the rat optic nerve (Raff et al., 1983) and sheep (Elder et al., 1988) and perhaps in other species, type I astrocytes arise from one progenitor cell while oligodendrocytes and type II astrocytes arise from a second common precursor called the O-2A progenitor. Raff and co-workers (1983) have described this O-2A progenitor as a mobile, bipolar cell. The bipolar GC+ cells we have observed in our cultures are of similar morphology, as are some of the GFAP+ cells present in these cultures. We have also observed a similar cell type, which was commonly infected by all strains of CDV, and which expresses the HNK-1 marker (data not shown). This antibody marks several glycoproteins found on a variety of CNS cell types and thus cannot be associated with a specific cell type. As these cultures are derived from neonatal tissue (animals less than 3 days of age), the presence of recently differentiated cell types is to be expected.

The contrasting CNS diseases produced by different virulent strains of CDV in vivo have been well documented (Summers et al., 1984). The two virulent strains produce differing forms of encephalitis: CDV-SH results in an acute, primarily gray matter disease, while CDV A75-17 causes a delayed onset of CNS signs marked by demyelination with more sporadic gray matter involvement (Summers et al., 1984). In this in vitro study we examined whether these strain-dependent differences could be clearly related to contrasting tropisms for cells of the CNS. For some cells, this did not appear to be the case. All cell types examined, except neurons and multipolar oligodendrocytes, supported infection with all three viral strains studied. CDV-RO and CDV-SH produced widespread infection in bipolar oligodendrocytes, macrophages, and astrocytes by 14 dpi. CDV A75-17 was also observed in all cell types except neurons and multipolar oligodendrocytes; however, the infection was restricted to small foci and low percentages of infected cell types until 28–35 dpi. We previously described the contrasting kinetics of astrocyte infection by different CDV viral strains (Pearce-Kelling et al., 1990). In these studies we observed rapid infection by CDV-SH and CDV-RO and more delayed growth with CDV A75-17. As our current study has shown, similar differences in the kinetics of infection occur in several other CNS cell types, including macrophages, fibroblasts, and bipolar oligodendrocytes. These differences in kinetics of growth of brain cells are in contrast to the equivalent growth rates that the two virulent strains exhibit in cultured dog lymphocytes and lung macrophages (Appel, unpublished data).

Interestingly, CDV A75-17 did not infect neurons during the time in which these cells were prevalent (0 to 14 div), whereas CDV-SH, which routinely causes gray matter disease, did infect neurons by 14 dpi. We have observed the infection of neurons in canine brain cell cultures by CDV A75-17 which has been modified by passaging it through brain cell cultures (Pearce-Kelling, unpublished data). This suggests that, given time, CDV A75-17 can infect neurons in culture. These observations further support our previous speculation that a rapid and widespread infection, especially of neurons, as occurs with CDV-SH, in vivo, may directly relate to neuronal destruction and acute death of the dog. In contrast, the slowly spreading infection, as occurs with CDV A75-17 in vitro, may reflect an adaptation of this strain to the CNS environment. This slow adaptive growth may also occur in the CNS of the infected dog and result in this strain's characteristic delayed onset of neurological signs.

We observed two morphologically distinct populations of oligodendrocytes that appear to have different susceptibilities to CDV infection. The multipolar oligodendrocytes appear to be refractory to infection, consistent with the observations of others (Zurbriggen, et al., 1986, 1987b,c). Recently Glaus and co-workers (1990) have reported that 2 to 3% of oligodendrocytes in culture were CDV infected. In multiple replicate experiments however, we observed CDV fluorescence in the majority of bipolar GC+ cells by 21 dpi in CDV-SH and CDV-RO infected cultures. In an effort to understand the observed differential response to CDV by the different subtypes of oligodendrocytes in our cultures, we are currently undertaking further experiments employing antibodies which mark different stages of oligodendroglial differentiation.

If the two subtypes of oligodendrocytes differ in their stages of maturation then one may speculate that their differential infection by CDV is due to a "window" of susceptibility. A similar virus-cell interaction has been reported with the murine JHM coronavirus, which, in vitro, will infect only immature rat oligodendrocytes (Beushausen and Dales, 1985). It remains to be established whether these in vitro experiments can be extrapolated to canine oligodendrocyte infection in vivo, which has infrequently been documented (Blakemore et al., 1989; Raine, 1976). Mature oligodendrocytes in the CNS may be relatively resistent to infection. In the course of these experiments we did not observe the degeneration and necrosis of multipolar oligodendrocytes in the infected cultures as was reported (Vandevelde et al., 1985; Zurbriggen et al., 1986, 1987b,c) and which occurred despite the lack of infection of these cells. In our cerebral and cerebellar cultures, GC+ oligodendrocytes comprised only a minor component of the cell populations in contrast to the large numbers of MAG+ oligodendrocytes reported in the studies of Vandevelde and Zurbriggen. It is possible that our failure to observe oligodendrocyte degeneration may be due to the study of different subsets of oligodendrocytes: those identified by MAG largely in immunoperoxidase procedures (Zurbriggen et al., 1986, 1987a,b,c) compared to those labeled by GC in our immunofluorescent studies.

Astrocytes are readily infected with CDV and we have reported on the kinetics of infection with different viral strains in these cells (Pearce-Kelling et al., 1990). Different functions have been ascribed to type I and type II astrocytes; for example, the latter seem to be involved in the integrity of the node of Ranvier which may bear on myelin stability (Raff, 1989). Type II astrocytes are preferentially marked by HNK-1 in rat optic nerve

(Raff, 1989), which may also be marking this subset of cells in our canine cultures. Type II astrocytes, plus the undifferentiated O-2A progenitor cell, can also be identified in rat brain cells with the monoclonal antibody A2B5 and we have, in preliminary studies, observed A2B5 positive astrocytes in canine cerebral cultures.

Our studies reported here do not directly link susceptibility to infection of specific CNS cells with specific viral biotypes of CDV. Rather, our observations suggest that a difference in the kinetics of infection within the spectrum of CNS cell types may be of significance. The infection of neurons, by CDV-SH but not by CDV A75-17, may be of particular importance in determining the course of CNS infection in the dog. In addition, our observations suggest that viral strain differences may be related not to susceptibility of general cell types (oligodendrocytes and astrocytes) but to the kinetics of infection of subpopulations within these pools.

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