Acute Encephalitis and Hydrocephalus in Dogs Caused by Canine Parainfluenza Virus

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Abstract. Gnotobiotic puppies were inoculated intracerebrally with a strain of canine parainfluenza virus (CPI-78-238). Four of eight dogs developed histological evidence of acute encephalitis. Clinical signs of encephalitis were seen in two of these four dogs; one had signs and lesions of interstitial pneumonia. Of six inoculated dogs observed for six months after infection, five developed internal hydrocephalus. Virus was reisolated from two dogs with acute encephalitis but not from dogs with hydrocephalus. Hemagglutination-inhibition antibodies persisted throughout the observation period of six months at high levels in the serum and cerebrospinal fluid of hydrocephalic dogs.

Infectious disease is the most common cause of neurological disorders in dogs [20]. Viruses known to cause encephalitis in dogs include canine distemper, rabies, infectious canine hepatitis, Aujeszky’s disease, and canine herpes infection in puppies. In infectious canine hepatitis, the infection develops rapidly and parenchymal lesions are minimal and limited to endothelial cells with intranuclear inclusion bodies. Canine herpes virus infection can cause meningoencephalitis in infected pups up to one month old. Both rabies and pseudorabies occur rarely and show well-characterized lesions [10]. Canine distemper is a naturally occurring disease in which demyelinating encephalitis sometimes accompanies systemic disease [32].

Hydrocephalus is the most common canine congenital anomaly of the nervous system. Dwarf and brachycephalic breeds may have hereditary predisposition for developing hydrocephalus [42]. In addition, it has been suggested that viral infection of ependymal cells may result in hydrocephalus [10].

In 1978 a viral agent was isolated from the cerebrospinal fluid of a dog with neurological dysfunction [12]. This isolate (78-238) was further characterized in our laboratory as a SV-5-like virus and distinguished from other SV-5 viruses by its biological properties [3]. Since the first isolation of a canine parainfluenza virus from a dog with a respiratory disease [5], canine parainfluenza viruses in dogs have been manifested as infections restricted to the respiratory tract [2]. Clinical signs vary from
a dry cough [2] to pneumonia [41]. Mortality rates are low. Serologic studies have shown that a variable but high number of dogs have been exposed to canine parainfluenza [4, 6, 7]. In addition, a vaccine for canine parainfluenza virus has been developed [11]. These data, however, have not shown any correlation between SV-5 infection and central nervous system involvement.

The purposes of this study were to determine the virulence of CPI-78-238 for gnotobiotic puppies after intracerebral inoculation; to determine the morphological features of acute and chronic neurologic disease induced by CPI-78-238; to reisolate the virus from infected dogs; and to monitor the hematological and serological responses to viral antigen after infection.

Materials and Methods

Virus and passage history

The viral variant (78-238) of canine parainfluenza virus isolated from the cerebrospinal fluid of a dog with posterior paresis [12] was used in this study. The isolate, designated CPI-78-238, after eight passages in African green monkey (Vero) cells was inoculated intracerebrally (0.2 ml of 10^{0.8} tissue culture infective dose [TCID] \_50/0.025 ml) into gnotobiotic dogs. For the second experiment, the viral inoculum was prepared from the brain of experimentally infected dogs and contained 10^{6.8} TCID\_50/ml of brain suspension when titrated in Vero cells. Further inocula for in vivo experiments consisted of progeny virus from the first Vero cell subpassage of brain suspension (brain split passage I at 10^{5.75} TCID\_50/ml) in Vero cells and from the second subpassage (brain split passage II 10^{6.25} TCID\_50/ml).

Cell cultures and viral titrations

Vero cells were maintained in minimal essential medium with Earle's balanced salt solution. Growth medium consisted of minimal essential medium Earle's supplemented with 10% fetal calf serum, 2 mM glutamine, 1% antibiotics (100 Units penicillin G/ml and 100 \( \mu \)g streptomycin/ml) and 1% NaHCO\_3 (8.8% wt/vol). Virus titrations (50% TCID) were done in microtiter plates as described previously [3]. In addition, 20% (wt/vol) organ suspensions were inoculated in 10-fold dilutions (10^{-6} to 10^{-7}) onto Vero monolayers in 25 cm\^2 plastic flasks with four flasks per dilution. To detect virus, both a hemadsorption test with canine erythrocytes and the appearance of a 78-238-specific cytopathic effect in form of multinucleated giant cells were used [3]. Cells were subcultured one week after infection and cell cultures without hemadsorption or cytopathic effect after two weeks were considered negative.

Maintenance and inoculation of gnotobiotic dogs

A total of 19 colostrum-deprived gnotobiotic dogs from four separate litters were used in this study. Dogs were delivered by cesarian section and maintained in sterile flexible plastic isolation units [24, 29]. Each litter was infected and handled as a separate group. Most dogs (n=16) were inoculated intracerebrally into the left cerebral hemisphere when six days old. Two dogs were infected intracisternally when five months old. One dog served as an uninoculated control. Of the pups inoculated at six days old, six pups from the first litter (dogs 1 through 6) received 0.2 ml of CPI-78-238 (10^{6.8} TCID\_50/0.025 ml). They were killed 12 days post-inoculation (dogs 5 and 6), four weeks post-inoculation (dogs 3 and 4), and six months post-inoculation (dogs 1 and 2). Three pups from the second litter (dogs 7, 8 and 9) received 0.2 ml brain suspension containing 10^{6.8} TCID\_50/ml prepared from the brain of dog 6 from litter 1. Pup number 7 was killed four weeks post-inoculation. The two remaining dogs (dogs
8 and 9) were reinoculated with brain suspension at three months of age. Three months later, at six months of age, these same dogs received 1.0 ml Vero cell passed brain suspension (brain split passage II) containing $10^{6.26}$ TCID$_{50}$/ml intracisternally. They were killed at nine months (dog 8) and 12 months (dog 9). The third litter (dogs 10 through 15) received 0.2 ml brain split passage I containing $10^{5.76}$ TCID$_{50}$/ml when six days old and they were killed between 11 and 24 days post-inoculation. The fourth litter (dogs 16 through 18) received 0.2 ml brain split passage II at six days of age and were killed on days three (dog 16), five (dog 17), and seven (dog 18) post-inoculation.

Clinical and immunological studies

Dogs were examined three times daily for evidence of neurological dysfunction. Unclotted blood samples were collected weekly and evaluated for total and differential leukocyte counts, hemoglobin and packed cell volume, and total serum protein. Sera were collected from the pups one day before infection and at weekly intervals post-inoculation. In addition, sera from the pregnant bitches were collected in the last two weeks of pregnancy. Cerebrospinal fluid was collected by cisternal puncture [46] from dogs three months old or older.

Sera were tested for hemagglutination-inhibition and virus-neutralizing antibody titers. Cerebrospinal fluids were tested for antibody by hemagglutination-inhibition and evaluated for total and differential cell counts, color, specific gravity, and total protein. The method of preparing viral antigen, serum, and erythrocytes for hemagglutination-inhibition tests have been described [3]. Hemagglutination-inhibition and hemadsorption tests were done by recommended methods [9].

Virus neutralization tests

Virus neutralization tests on sera were performed in microtest plates (No. 3596, Costar, Cambridge, Mass.). Sera were heat-inactivated for 30 minutes at 56°C. Twenty-five microliters of two-fold dilutions of serum, four replicates each, were mixed with an equal volume of virus, containing 100 TCID$_{50}$/0.025 ml in plastic transfer plates (No. 220-43, Cook Laboratory Products, Alexandria, Va.). After incubation for one hour at 37°C, the mixtures were transferred to a 96-well microtest plate, containing 15,000 Vero cells/well in 0.1 ml growth media. Cultures were maintained at 37°C and results were read after seven days.

Tissue study

Four dogs (dogs 1, 2, 5 and 6) were killed and samples from various tissues, including brain and spinal cord, were collected and fixed in 10% buffered formalin. Twelve dogs were perfused with 4% (wt/vol) paraformaldehyde followed by 5% (vol/vol) glutaraldehyde [30] for subsequent light and ultrastructural evaluation. Removed organs were stored in 5% glutaraldehyde at 4°C. For histological examination, tissue was dehydrated, embedded in paraffin, cut at 6 μm and stained with hematoxylin and eosin (HE). Brains from the three remaining dogs were snap-frozen in liquid nitrogen for immunofluorescence studies.

Immunofluorescence

For immunofluorescence tests, one-half of the brain was excised from several dogs under deep ultrashort barbiturate anesthesia [30]. Small pieces (1.0 × 1.0 × 0.5 cm) of brain embedded in O.C.T.-compound (Miles Laboratory, Tissue-Tek, Naperville, Ill.) were immersed quickly in liquid nitrogen and stored at −70°C until use. Blocks of frozen brain were sectioned at 6 μm on a cryostat, mounted on glass slides, briefly air-dried and fixed for 10 minutes in acetone. Paraffin-embedded formalin-fixed brain tissue was used for immunoflu-
orescence as described elsewhere [45]. As a technical control, formalin-fixed paraffin-embedded brain sections from dogs experimentally infected with canine distemper virus [25], which were immunofluorescence-positive using unfixed frozen sections, were stained with an anti-canine distemper virus conjugate.

Vero cells inoculated with tissue suspensions for virus reisolation were grown on glass microscope slides (No. 4804, Lab-Tek Products, Naperville, Ill.) and examined for virus antigen by both direct and indirect immunofluorescence methods. Procedures for conjugating and using a fluorescein isocyanate conjugated anti-78-238 serum have been described [26]. Convalescent sera against 78-238 from experimentally infected gnotobiotic dogs were taken as primary reagent for the indirect test. To determine specificity, the test was done on uninfected monolayers, on brain sections of uninfected dogs, or with pre-inoculation sera instead of post-inoculation sera. In addition, 0.025% Evans blue [27] was used to reduce non-specific fluorescence.

**Virus reisolation**

Tissues removed aseptically from dogs that had been killed or deeply anesthetized were minced and homogenized as a 20% (wt/vol) suspension in minimal essential medium Earle’s. After centrifugation for 10 minutes at 300 times gravity, the sediment was discarded and aliquots of the supernatant were stored at −70°C.

Specimens of brain, spinal cord, liver, lung, heart, and kidney were tested for infectious virus. Throat specimens were taken one day before infection and four times post-inoculation at three-day intervals. Sterile cotton swabs were placed into glass tubes containing 2 ml minimal essential medium Earle’s, rinsed in this medium thoroughly and thereafter discarded; the remaining medium was stored at −70°C until use. Leukocyte-plasma suspensions were used for detecting viremia. Blood was collected three times within 14 days post-inoculation. Ten milliliters of heparinized blood (20 units/ml) was centrifuged at 250 times gravity for 10 minutes. Plasma was stored at 4°C while the remaining cellular sediment was processed further. The sediment was resuspended in 5 ml sterile 5% dextran solution and incubated for 30 minutes at room temperature. The leukocyte-rich supernatant was aspirated and leukocytes were washed once in phosphate buffered saline for 10 minutes at 250 times gravity. Cellular sediment was resuspended into the plasma, aliquoted and stored at −70°C until titrated. Brain explants were established and grown in 25-cm² plastic flasks as described elsewhere [33].

After removal of the medium, preconfluent monolayers of Vero cells were infected with 0.5 ml organ suspension, plasma-leukocyte suspension, or throat swabs. After a two-hour incubation period at 37°C, cells were washed once with phosphate buffered saline, 5 ml growth media was added, and cells were maintained at 37°C. Cells were observed weekly for the presence of cytopathic effect, and passed weekly for a period of three weeks. In addition, hemadsorption tests were done weekly. Specimens that showed neither hemadsorption nor cytopathic effect after three sub-passages were considered negative. Presence of virus in cultures showing both cytopathic effect and hemadsorption was confirmed by immunofluorescence.

Virus reisolation was also done on brain explants prepared from experimentally infected dogs. Confluent monolayers of brain explants were split (1:3) in 25-cm² flasks, and two of these brain explant cell cultures were co-cultivated with Vero cells (ratio 1:1). One of these co-cultures was treated with polyethylene glycol to induce cell fusion [40]. Briefly, monolayers were washed once in phosphate buffered saline and then incubated with 40% (wt/vol) polyethylene glycol (6000 daltons) solution for three minutes. After aspiration, the polyethylene glycol-treated cells were washed with phosphate buffered saline, growth medium was added, and cultures were maintained at 37°C and monitored for virus as described above.
Parainfluenza Virus in Dogs

Fig. 1: Half coronal section of brain from dog 1, infected with CPI-78-238 six months previously. Lateral ventricle dilated; cerebrum shows severe pressure atrophy. Bar = 1 cm.

Results

Neurological signs

Dog 6, which received the original isolate of CPI-78-238, developed serial myoclonic jerks (one per minute) eight days post-inoculation. The dog showed progressive central nervous system depression, including anorexia, and was moribund within four days following onset of signs. During this time the puppy lost weight from 320 g to 265 g. Dog 5, from the same litter, developed severe convulsions 11 days post-inoculation and was moribund on the following day. This same dog developed severe inspiratory-expiratory dyspnea. Clinical signs related to either the central nervous system or respiratory tract were not seen in the other dogs.

Lesions

In the acute stage of infection, no gross lesions were seen in the brain or spinal cord. Other organs were unchanged, except the lung of dog 5, which was distended and consolidated. Examination of the convalescent dogs between 4½ weeks and six months post-inoculation showed that 83% of the dogs had internal hydrocephalus. Moderate gross enlargement of the lateral and third ventricles were present in dog 4, killed 32 days post-inoculation, in dog 8, killed three months post-inoculation, and in dog 9, killed six months post-inoculation. Dog 9 had dilation of the Sylvian aqueduct. Severe hydrocephalus with enlargement of the lateral and third ventricles and thinning of the cerebral tissue was present in dogs 1 and 2 (fig. 1). The fourth ventricle was unchanged in all dogs.

A total of eight dogs were used to evaluate the ability of 78-238 to cause acute encephalitis. Of these eight, four (50%) had clinical or histological evidence of encephalitis. Dogs 5 and 6, which had acute fatal encephalitis, had extensive lesions
in the brain. In dog 5, lesions were restricted chiefly to the forebrain. The olfactory lobe had multifocal areas of necrosis and accompanying inflammation. Affected neurons showed central chromatolysis, karyolysis and necrosis. Reactive astrocytosis, microgliosis, capillary proliferation and neutrophilic infiltrates accompanied these changes (fig. 2). The temporal lobe had large areas of poliomalacia, status spongiosus, and microgliosis in the white matter. Perivascular accumulations of mononuclear cells accompanied the gliosis. The meninges overlying these lesions were infiltrated with lymphocytes and monocytes. The spinal cord had focal neuronal necrosis with reactive gliosis and mild nonsuppurative meningitis. The acute lesions seen in dog 6 were qualitatively similar to those of dog 5 except that, in addition to the temporal lobe, the mesencephalon and medulla oblongata were involved. Encephalomalacia with microgliosis and astrocytosis was especially prominent in areas adjacent to the Sylvian aqueduct.

Two dogs without clinical evidence of neurological involvement (dogs 11 and 14)
were killed on days 14 and 11, respectively. One large area of poliomalacia accompa-
nied by a moderate perivascular lymphoplasmacytic cellular infiltrate and reactive
microgliosis was seen in the temporal cerebral cortex in one dog. Only a few focal
areas of microgliosis in the subcortical grey region of the temporal lobe were found
in the other dog. Both dogs had nonsuppurative leptomenigitis.

The only significant extraneural lesion occurred in the lung of dog 5. Histological
examination showed interstitial pneumonia with thickening of the alveolar walls.
Pulmonary tissue from other dogs was normal. Lymphatic tissues, including the
thymus, were well developed and within normal limits for gnotobiotic dogs.

The second group of dogs ($n = 6$) infected with 78-238 and brain split passage II
were maintained for one to six months post-inoculation. From these dogs, a composite
picture of developing internal hydrocephalus was constructed. Lesions were restricted
to the ventricular and periventricular regions in the brain. Two dogs (dogs 3 and 4)
were killed and examined one month post-inoculation. Dog 4 had moderate spon-
giform degeneration along the lateral ventricles. The ependymal cells overlying this
change were normal, although the ependymal cell lining was missing in focal areas.
Hematogenous inflammatory cell infiltrates were not seen in these areas. Dog 3 had
no lesions in the central nervous system.

Dog 8 (killed and examined three months post-inoculation) had extensive spon-
giform degeneration of the subependymal region of the lateral ventricle extending

**Fig. 4**: Section through Sylvian aqueduct of dog 1, six months post-inoculation. Lumen
partially occluded.

**Fig. 5**: Section of mesencephalon, five days post-inoculation with brain split passage II.
Virus-specific immunofluorescence in ependymal cells of Sylvian aqueduct. A = aqueductal
lumen, B = brain parenchyma, arrow = fluorescent foci of CPI-78-238 in ependymal cells.
from the temporal lobe to the parietal lobe of the cerebral white matter (fig. 3). The ependymal lining cells were flattened and discontinuous. These changes were present throughout the ventricular system including the fourth ventricle. There was no stenosis of the aqueduct of Sylvius, and inflammatory cells were not seen.

Three dogs (dogs 1, 2 and 9) were killed six months after inoculation. All three had moderate to severe internal hydrocephalus. Dogs 1 and 2 had moderate subependymal edema and flattened and distorted ependymal lining cells in all ventricles, and the Sylvian aqueduct was partially occluded in both (fig. 4). Ependymal cells were distorted and formed pseudo-rosettes. Partial adhesion of the lining of the aqueduct divided the canal into dorsal and ventral channels. Serial sections through this region, however, showed that complete occlusion of the aqueduct never occurred. Dog 9 had flattened ependymal cells in all four ventricles.

Half-coronal central nervous system tissue sections from all major areas of the brain were examined for the presence of virus antigen by direct and indirect immunofluorescence methods. In three dogs (dogs 16, 17, and 18) killed three, five and seven days post-inoculation, viral antigen was seen in ependymal cells by immunofluorescence techniques. Maximum cytoplasmic fluorescence was present five days post-inoculation (fig. 5). Immunofluorescence staining showed that virus was occasionally present in meningeal cells and neurons. Viral antigen was not found in nervous tissues from hydrocephalic dogs or in formalin-fixed tissues after trypsin digestion.

**Development of antibodies**

Dogs inoculated with 0.2 ml brain suspension and three months later with 1.0 ml brain suspension did not produce antiviral antibodies. It was concluded that the low dose of viral inoculum used did not result in virus infection in these dogs (table I). After these dogs were inoculated with brain split passage I, hemagglutination inhibiting antibodies developed within one week, reached peak values seven weeks post-inoculation, and then declined, but were still detectable at significant levels for six months. Virus-neutralizing antibodies were found two weeks post-inoculation. They subsequently increased and attained their highest level after five weeks. Dogs infected with brain split passage I1 when five months old developed measurable hemagglutination inhibition antibodies within one week. Peak titers were reached two weeks post-inoculation and remained elevated for four weeks, before a gradual decline. As before, these antibodies were still present at significant levels six months post-inoculation (fig. 6).

The ratio of hemagglutination inhibition antibody titers in the cerebrospinal fluid to hemagglutination inhibition antibody titers in paired sera was calculated by the following formula: ratio = reciprocal titer in serum ÷ reciprocal titer in CSF. In the first four weeks post-inoculation ratios ranged between 60 and 40. In the following 15 weeks this ratio was between 24 and 10. Thereafter, ratios of 7.5, 5.0, and 3.0 were found. These values reflect persistent high titers of hemagglutination inhibition antibodies in cerebrospinal fluid of hydrocephalic dogs.
Table I. Summary of results of infection of gnotobiotic dogs with various inocula of canine parainfluenza virus

<table>
<thead>
<tr>
<th>No. of dogs</th>
<th>Source and dose of virus</th>
<th>Seroconversion</th>
<th>Virus reisolation</th>
<th>Signs/lesions acute encephalitis</th>
<th>Hydrocephalus</th>
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<tbody>
<tr>
<td>6</td>
<td>78-238</td>
<td>+</td>
<td>+</td>
<td>2/6</td>
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<td>BS</td>
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<td>10⁵.₅TCID₅₀/0.2 ml</td>
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<td>2</td>
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<td>10⁶.₂⁵TCID₅₀/ml</td>
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¹ ND = not done.
² Dogs had cerebrospinal fluid changes compatible with viral encephalitis.

TCID = tissue culture infective dose; BS = brain suspension; BSP = brain split passage.

Hematological findings

Dogs that survived 78-238 infection developed a relative (45% to 60%) and absolute lymphocytosis (4500–5200 lymphocytes/μl). Lymphocytosis was most evident in convalescent dogs five weeks post-inoculation and declined thereafter.

Virus reisolation

Virus was recovered from tissue of two dogs (dogs 5 and 6). Virus was reisolated from the brain and spinal cord of both dogs, and from lung tissue of dog 5. Spinal cord and lung tissue of dog 5 contained 10³.₀ TCID₅₀/2 ml organ suspension, and brain tissues of dogs 6 and 5 and spinal cord of dog 6 contained 10³.₅ TCID₅₀/0.5 ml organ suspension. Virus was not recovered from tissue of the remaining acutely infected or hydrocephalic dogs, nor from throat swabs, blood, or explant cultures.

Discussion

This is the first description of multiple cases of neurological dysfunction induced by a canine parainfluenza virus (78-238) in dogs under experimental conditions. Acute encephalitis was found in four of eight dogs, and internal hydrocephalus occurred in five of six convalescent dogs. Gnotobiotic dogs were used to ensure neonatal susceptibility to infection and to preclude external contamination with other viral and bacterial pathogens.

Acute encephalitis was found within three weeks post-inoculation in four dogs.
Fig. 6: Development of hemagglutination inhibition antibody titers in serum and cerebrospinal fluid following canine parainfluenza infection expressed as mean titers of all dogs. Serum antibody titer (broken line); cerebrospinal fluid antibody titer (solid line).

Two dogs showed clinical signs of severe encephalitis, which in one was associated with interstitial pneumonia. Two dogs had histological evidence of encephalitis without clinical signs. The other four dogs were clinically and histologically normal. Histological evaluation of the lesions showed that they differed from other recognized virus-induced encephalitides in newborn pups. Canine distemper virus may produce focal cerebrocortical necrosis without any inflammatory reaction in neonatal pups [25, 34]. No perivascular cuffs are seen and extraneural lesions are thymic atrophy and interstitial pneumonia [23]. In addition, canine distemper virus-specific inclusion bodies are present in most cases [1, 20]. Canine herpesvirus infection in newborn pups is characterized by focal granulomatous encephalitis, interstitial pneumonitis and segmental renal necrosis with dysplasia [38]. Areas of destruction in the brain frequently are associated with basophilic viral inclusions in cell nuclei [39]. Infectious canine hepatitis is characterized by widespread hemorrhages, hemostatic defects and hepatocellular necrosis. Intranuclear basophilic viral inclusion bodies are visible in endothelial cells and hepatocytes [22]. Our CPI-78-238-infected newborn pups had encephalomalacia, laminar cortical necrosis, and a significant inflammatory response. No inclusion bodies were found. Extraneural lesions in these dogs were rare. An interstitial pneumonia was found in one dog; no lesions were found in other tissues, however.

Mumps is the virus most commonly associated with viral meningitis and encephalitis in man [34]. An encephalitic syndrome in dogs has been described and related to mumps virus infection [44]. Serum titers to mumps showed fluctuations in two dogs, and in one a definite rise paralleled exacerbation of clinical signs. The
significance of mumps virus findings should be interpreted cautiously, particularly in view of SV-5 antibodies in guinea pig serum commonly used as a source of complement in complement-fixation procedures [4]. A cross-reaction between mumps virus and SV-5 hyperimmune serum has been demonstrated [41]. Judging by the data presented in that study, it is possible that the mumps virus-related encephalitic syndrome described by these authors was, in reality, caused by canine parainfluenza virus.

Immunofluorescence findings of virus antigen in ependymal cells in the early stage of infection is substantiated by similar findings with paramyxoviruses in other species [21]. Our failure to demonstrate virus in convalescent animals confirms those results. Further, our results indicate that trypsin digestion of formalin-fixed tissue is not a useful tool for demonstrating either canine parainfluenza virus or canine distemper virus antigen in formalin-fixed tissues.

Other studies have not shown viremia in canine parainfluenza infection [10]. In our series, virus was not recovered from plasma or leukocytes. The apparent restrictive nature of canine parainfluenza infection [10] has led to the conclusion that canine parainfluenza virus causes only local infections of the respiratory tract. The first study on the isolation of SV-5 virus from dogs, however [5], reported isolation of the agent from spleen, kidney, and liver in two of seven dogs. Further, canine SV-5 virus has been isolated from rectal specimens [28]. In our studies, canine parainfluenza was reisolated from several tissues, though only from acutely infected dogs. Reisolation of canine parainfluenza virus from various organs including spinal cord, lung, and brain confirms the findings of others with SV-5 virus in mice, hamsters, monkeys, and man [8, 19]. Thus, it is apparent that under natural or experimental conditions, SV-5 viruses can spread to tissues throughout the body.

Our serologic studies confirm that infection with canine parainfluenza had occurred. Further, the ratio of hemagglutination inhibition titers in serum and cerebrospinal fluid from hydrocephalic dogs had diagnostic significance. In normal dogs, the ratio of globulin in cerebrospinal fluid to that in serum is 1:400 or more. Thus, a ratio of 400 or more could be used to indicate a respiratory infection without central nervous system involvement. Ratios of less than 60 were seen in our dogs with internal hydrocephalus. Elevated hemagglutination inhibition antibodies in cerebrospinal fluid may result from leakage of serum proteins through damaged parenchyma into cerebrospinal fluid compartments. Alternatively, these elevations in cerebrospinal fluid antibody may reflect virus or antigen persistence in the brain and a local immune response to them. We could not reisolate virus from any of our dogs with hydrocephalus, nor could unequivocal ultrastructural evidence of viral persistence be obtained [Baumgartner, et al., submitted for publication, 1981]. Others have reported similar negative findings [21, 44] in regard to virus reisolation from hydrocephalic animals, although inclusion bodies have been demonstrated in hydrocephalic hamsters after measles infection [35].

Internal hydrocephalus is considered chiefly a developmental disease in dogs [42].
A few reports have suggested that hydrocephalus may be a sequel to bacterial infection, especially with Streptococcus sp [17, 18, 36]. A number of members of the myxovirus group are known to induce hydrocephalus in laboratory rodents after intracerebral inoculation. These include parainfluenza 1, 2, and 3, mumps virus, influenza A, parainfluenza virus 6/94, measles virus, and respiratory syncytial virus [6, 15, 21, 43]. In addition, reovirus has been reported to cause hydrocephalus in hamsters, ferrets, rats, and mice [31].

This is the first report of the occurrence of internal hydrocephalus in dogs following a canine virus infection. Hydrocephalus occurred both with and without aqueductal stenosis. It has been suggested that hereditary predisposition for hydrocephalus exists in dwarf and brachycephalic breeds [42]. Congenital and developmental abnormalities of the beagle brain may arise spontaneously [14]. A mild form of hydrocephalus was found in a strain of beagles in association with partial agnathia or shortening of the lower jaw [13]. The syndrome of hydrocephalus and partial agnathia was termed low-grade octocephaly. None of our hydrocephalic beagles showed comparable conformation deficits. Deficiency of any nutrient in the diet as cause for hydrocephalus [37] can be excluded, because other gnotobiotic dogs raised in this laboratory have not developed hydrocephalus. Furthermore, many dogs received canine distemper virus viral inocula intracerebrally [32, 33] without subsequent development of hydrocephalus, indicating that mechanical factors associated with the inoculation procedure cannot account for development of hydrocephalus. As our study shows, the inability to recover virus from brains of hydrocephalic dogs, coupled with the lack of inflammatory change in the central nervous system ordinarily associated with virus infection, does not preclude a viral cause of canine hydrocephalus. Suggestive evidence for the natural occurrence of canine parainfluenza-induced encephalitis is indicated by the high incidence of canine parainfluenza in the dog population [3, 4, 10, 16]; the recovery of canine parainfluenza virus from various organs after naturally occurring infection [5, 12]; and reisolation of canine parainfluenza and SV-5 from various tissues in experimentally infected animals [8, 19]. Thus, canine parainfluenza infection in dogs must be considered as a potential cause of hydrocephalus. Additional studies are needed to evaluate the importance of canine parainfluenza in naturally occurring viral neurological disorders in dogs.

Acknowledgements

Supported by The State of Ohio Canine Research Funds and Public Health Service Grant A109022, from the National Institutes of Health. Dr. Baumgartner is supported by a scholarship from the Deutsche Akademische Austauschdienst, Bonn, West Germany.

The authors wish to acknowledge the excellent assistance of Addajane L. Wallace, David Long and Nancy J. Austin. The authors also thank Dr. S. E. Weisbrode, The Ohio State University, Goss Laboratory, for critical review of this manuscript.

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