INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in “sectioning” the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again — beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from “photographs” if essential to the understanding of the dissertation. Silver prints of “photographs” may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms
300 North Zeeb Road
Ann Arbor, Michigan 48106
McCULLOUGH, D.V.M., Clair Bruce, 1944-
STUDIES IN EXPERIMENTAL CANINE DISTEMPER
VIRUS-INDUCED DEMYELINATION AND LYMPHOID
DEPLETION.

The Ohio State University, Ph.D., 1973
Health Sciences, pathology

University Microfilms, A XEROX Company, Ann Arbor, Michigan
STUDIES IN EXPERIMENTAL CANINE DISTEMPER VIRUS-
INDUCED DEMYELINATION AND LYMPHOID DEPLETION

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By

Clair Bruce McCullough, D.V.M., M.Sc.

The Ohio State University
1973

Approved by

[Signature]

Department of Veterinary
Pathobiology
ACKNOWLEDGEMENTS

I thank my advisor Dr. Adalbert Koestner for his guidance and encouragement during my graduate training. I also wish to express my appreciation to Drs. R. A. Griesemer, J. A. Shadduck, and C. C. Capen for their contributions to my education. In addition, I thank my colleague, Dr. Steven Krakowka for his helpfulness.

My special gratitude is extended to the staff of the Germfree Life Laboratory - Charles Long, John Terrell, Larry Berkwitt, Dave Long, and Pat McAfee. I also thank the many other employees of the Department of Veterinary Pathobiology for their generous assistance.

Most of all, I express my thanks to my wife, Karen, for her unlimited support and understanding.
VITA

May 3, 1944 Born - Kenton, Ohio

1967 . . . . B.Sc., The Ohio State University, Columbus, Ohio
1969 . . . . D.V.M., The Ohio State University, Columbus, Ohio
1971 . . . . M.Sc., The Ohio State University, Columbus, Ohio
1969-1973 N.I.H. Trainee, Department of Veterinary Pathobiology, The Ohio State University

PUBLICATIONS


FIELDS OF STUDY

Major Field: Veterinary Pathobiology

Studies in Oncology and Neuropathology. Professor Adalbert Koestner
Studies in Gnotobiology. Professor Richard A. Griesemer
Studies in Infectious Diseases. Associate Professor John A. Shadduck
# CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>11</td>
</tr>
<tr>
<td>VITA</td>
<td>iii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>vi</td>
</tr>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>I. DEMYELINATING ACTIVITY OF DISTEMPER VIRUS ISOLATES IN GNOTOBIOTIC DOGS</td>
<td>1</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>3</td>
</tr>
<tr>
<td>Results</td>
<td>5</td>
</tr>
<tr>
<td>Discussion</td>
<td>8</td>
</tr>
<tr>
<td>Summary</td>
<td>12</td>
</tr>
<tr>
<td>II. EXPERIMENTAL CANINE DISTEMPER VIRUS INDUCED LYMPHOID DEPLETION</td>
<td>18</td>
</tr>
<tr>
<td>Introduction</td>
<td>18</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>19</td>
</tr>
<tr>
<td>Results</td>
<td>21</td>
</tr>
<tr>
<td>Discussion</td>
<td>25</td>
</tr>
<tr>
<td>Summary</td>
<td>30</td>
</tr>
<tr>
<td>III. EXPERIMENTAL CANINE DISTEMPER VIRUS INDUCED DEMYELINATION</td>
<td>61</td>
</tr>
<tr>
<td>Introduction</td>
<td>61</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>62</td>
</tr>
<tr>
<td>Results</td>
<td>64</td>
</tr>
<tr>
<td>Discussion</td>
<td>69</td>
</tr>
<tr>
<td>Summary</td>
<td>73</td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td></td>
</tr>
</tbody>
</table>
TABLES

Chapter I

Table
1. Inoculation of gnotobiotic dogs with cerebellar suspensions prepared from naturally occurring cases of demyelinating distemper .......................... 13
2. Virulence of R252 CDV after one passage in gnotobiotic dogs ........................................ 14
3. Intraperitoneal inoculation of gnotobiotic dogs with lyophilized preparations of French isolate (#720-2212) CDV .................................................. 16
4. Effect of route of inoculation on virulence of R252 CDV after one passage in dogs ............ 17

Chapter II

Table
5. Hassall's corpuscles per thymic lobule in gnotobiotic dogs infected with R252 CDV .......... 55

Chapter III

Table
6. Effect of R252 CDV on the central nervous system (CNS) of gnotobiotic dogs ............. 102
# ILLUSTRATIONS

## Chapter II

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Means of absolute lymphocyte counts of gnotobiotic dogs infected with R252 CDV</td>
<td>31</td>
</tr>
<tr>
<td>2.</td>
<td>Means of absolute neutrophil counts of gnotobiotic dogs infected with R252 CDV</td>
<td>33</td>
</tr>
<tr>
<td>3.</td>
<td>Means of absolute eosinophil counts of gnotobiotic dogs infected with R252 CDV</td>
<td>35</td>
</tr>
<tr>
<td>4.</td>
<td>Means of total leukocyte counts of gnotobiotic dogs infected with R252 CDV</td>
<td>37</td>
</tr>
<tr>
<td>5.</td>
<td>Thymus weights of gnotobiotic dogs infected with R252 CDV</td>
<td>39</td>
</tr>
<tr>
<td>6.</td>
<td>Thymus weight/body weight ratios of gnotobiotic dogs infected with R252 CDV</td>
<td>41</td>
</tr>
<tr>
<td>7.</td>
<td>Thymus of uninfected control dog</td>
<td>43</td>
</tr>
<tr>
<td>8.</td>
<td>Thymus of dog of Group I</td>
<td>45</td>
</tr>
<tr>
<td>9.</td>
<td>Thymus of dog of Group I</td>
<td>47</td>
</tr>
<tr>
<td>10.</td>
<td>Viral nucleoprotein in the cytoplasm of thymocytes of dog of Group I</td>
<td>49</td>
</tr>
<tr>
<td>11.</td>
<td>Viral nucleoprotein in the cytoplasm of a thymocyte of dog of Group I</td>
<td>51</td>
</tr>
<tr>
<td>12.</td>
<td>Necrotic cell in thymus of dog of Group I containing viral nucleoprotein</td>
<td>53</td>
</tr>
<tr>
<td>13.</td>
<td>Hassall's corpuscle in thymus of dog of Group III</td>
<td>56</td>
</tr>
<tr>
<td>14.</td>
<td>Germinal center in thymus of dog of Group III</td>
<td>58</td>
</tr>
<tr>
<td>15.</td>
<td>Lymph node of dog of Group I</td>
<td>60</td>
</tr>
</tbody>
</table>
ILLUSTRATIONS (Continued)

Chapter III

Figure | Description | Page
--- | --- | ---
16. | Demyelination and astrocytosis in cerebellum of dog of Group I | 74
17. | Intranuclear viral inclusions in cerebellar astrocytes of dog of Group I | 76
18. | Demyelination and moderate astrocytosis in cerebellum of dog of Group I | 78
19. | Necrosis of cerebellar gray matter of dog of Group I | 80
20. | Demyelination and nonsuppurative inflammation in medulla of dog of Group II | 82
21. | Intranuclear viral inclusions in astrocytes near perivascular cuff in cerebellum of dog of group II | 84
22. | Demyelination of optic chiasma of dog of Group II | 86
23. | Demyelination and nonsuppurative inflammation of anterior medullary vellum of dog of Group II | 88
24. | Plasma cell near small venule in cerebellum of dog of Group II | 90
25. | Demyelinated axon surrounded by macrophage in cerebellum of dog of Group II | 92
26. | Demyelinated axon, macrophage and edema in cerebellum of dog of Group II | 94
27. | Intracytoplasmic and intranuclear viral nucleoprotein in astrocyte in cerebellum of dog of Group II | 96
28. | Demyelinated and remyelinating axons and edema in cerebellum of dog of Group II | 98
29. | Remyelination of axon in cerebellum of dog of Group II | 100
CHAPTER I
DEMYELINATING ACTIVITY OF DISTEMPER VIRUS
ISOLATES IN GNOTOBIOTIC DOGS

Introduction

Canine distemper is a naturally occurring viral disease in which demyelination of the central nervous system sporadically accompanies systemic disease (1). The demyelinated lesion in affected dogs is similar to that found in human patients with subacute sclerosing panencephalitis (SSPE) and other demyelinating diseases (2-5). In addition to being antigenically related, canine distemper virus (CDV) shares several biophysical and biochemical characteristics with measles virus and is grouped with it under the paramyxoviruses (6). Measles virus is known to be associated with the pathogenesis of SSPE (2).

Distemper has been extensively studied, but most investigations focused attention to the acute phase of the disease and the development of humoral immunity against the virus (7,8). Exploration of the pathogenesis of CDV-induced demyelination and characterization of this disease process as a model for human demyelinating diseases has been greatly impeded because of the low incidence of demyelination following experimental CDV infection. Gillespie and Rickard (9) reported 2 cases of demyelinating encephalitis in 119 dogs used during their development of the neurovirulent Snyder-Hill strain of CDV, Appel (10) had 2 cases among 55 dogs in his study of the pathogenesis of distemper, in which he exposed
dogs to St. Joseph (Snyder-Hill) strain CDV by aerosol, and Cornwell, et al. (11) described 2 cases in 16 dogs infected by exposure to ferrets infected with an unspecified strain of CDV. In the only reported study employing germfree dogs, Gibson, Griesemer, and Koestner (12) found no evidence of demyelination in 12 dogs infected with either Snyder-Hill or Lederle CDV. In contrast, Reculard and Guillon (13) recently reported that CDV isolates from naturally occurring cases of distemper differed in their ability to induce demyelination. Of their 5 isolates 1 consistently induced demyelination in a higher percentage of experimentally infected dogs (14).

Progressive multifocal leukoencephalopathy, a demyelinating disease of man thought to be caused by a papova virus, occurs almost exclusively in immunosuppressed patients (3). Further, abnormally weak delayed hypersensitivity responses have been reported for some patients with SSPE (14). In conventional dogs inoculated with attenuated vaccine strain CDV immunosuppression leads to fatal infection (15,16). Study of the effect of immunosuppression on CDV infection of gnotobiotic dogs has not been reported.

It was the objective of this study to select the distemper virus isolate which would most consistently induce demyelination in gnotobiotic dogs either alone or in conjunction with immunosuppression, and to determine the effect of route of inoculation and age of the dogs upon the induction of demyelination. The gnotobiotic animal was chosen as an ideal host for the study of distemper virus infection because complicating factors such as acquired active and passive immunity and secondary bacterial infections were minimized (17).
MATERIALS AND METHODS

Dogs. Unconditioned pregnant bitches in late gestation were obtained from a local dealer. The methods of Griesemer and Gibson (18) were used to deliver pups into flexible plastic isolators where they were raised as gnotobiotes¹. Swabs of the isolator interiors were taken weekly and cultured in thioglycolate broth at 22 C and 37 C. All pups were treated for nematodes by administering 6 weekly doses of the larvacide diethylcarbamazine (CaracideR-American Cyanamid Co., Princeton, N.J., 50 mg/kg) starting at 3 weeks of age (18).

A total of 67 gnotobiotic pups representing 12 litters were used. In studies of the R252 CDV, 1 or 2 pups per litter were housed in a separate isolator and served as uninfected controls. The numbers of pups inoculated with each isolate and the routes of inoculation are indicated in the results.

Viruses. Portions of cerebellar tissue from 2 naturally occurring cases of demyelinating distemper (R252 and P3977) had been stored at -90 C for 12 and 18 months, respectively, prior to their preparation as 20% suspensions. Clarification of the suspensions was not performed because of the small quantities available.

In subsequent studies of the R252 isolate we utilized a 10% suspension of cerebellar tissue collected from previously infected dogs (R6411, R6412, and R6413). This suspension was clarified by low speed centrifugation.

¹ The terms gnotobiotics and gnotobiotes as used in this paper is not intended to connote absolute "germfreeness" since most isolators became monocontaminated with airborne bacteria (non-pathogens) at some time during the experiments.
gation and 1.0 ml aliquots were stored in liquid nitrogen. The insusceptibility of available tissue culture cells to direct inoculation of this suspension precluded in vitro quantitation of the infectious virus present in the inoculum.

Snyder-Hill (SH) strain CDV was obtained as a 10% tissue suspension (Lot No. 69-1) from Mr. S. J. Musser, Phillips-Roxane, Inc., St. Joseph, Mo. In that laboratory, the SH strain produces a rapidly fatal form of nervous distemper in susceptible, conventionally housed dogs when inoculated intracerebrally (16).

A glial tissue-culture adapted (GA) strain of CDV was made available by Dr. John Long of this department. The virus, originally started from the Lederle strain, had been passaged 5 times in glial tissue-cultures derived from the brains of newborn puppies (19). The inoculum used was prepared from infected cells disrupted by alternate freeze-thawing and clarified by low speed centrifugation (19).

A strain of CDV (#720-2212-71) having reported ability to induce demyelination in dogs (13) was provided by Dr. P. Reculard, Pasteur Institute, Paris, France. Aliquots were supplied as lyophilized preparations of infected dog spleen and lymph node. In addition, portions of the brain from 2 dogs (R6132 and R6133) representing the first gnotobiotic dog passage of this isolate, were prepared as 10% suspensions.

Clinical Studies Rectal temperatures were taken twice daily, morning and late afternoon. Blood samples were collected by jugular puncture while the dogs were sedated with InnovarR (Pitman-Moore, Inc., Washington Crossing, New Jersey). Hemograms were usually obtained on each dog weekly, but this schedule was varied for some dogs, because of treatment or
absence of obvious disease. Dogs were observed daily for clinical signs of disease.

**Neutralizing Antibody Assay** Serum neutralizing antibody titers were determined in Leighton tube cultures of Vero cells infected with Vero-adapted CDV. Selected samples were tested as indicated in the results. All sera were heat-inactivated at 56°C for 30 minutes prior to testing. Four-fold dilutions of serum were exposed to 50 TCID_{50} of virus at 37°C for 30 minutes. Three tubes were used per dilution. The titers are expressed as the reciprocals of the highest dilutions showing complete protection against viral CPE. Serum from a dog hyperimmunized against D-Vac^R vaccine (Bio-Geutic Laboratories, Inc., St. Joseph, Mo.) was included in each test as a positive (antibody) control. Tests were repeated if extreme deviation of the titer of this standard serum occurred.

**Immunosuppression** Methotrexate (MTX, American Cyanamid Company, Pearl River, New York) or cyclophosphamide (CTX, Cytoxan^R, Mead Johnson Laboratories, Evansville, Indiana) was administered to several dogs following CDV inoculation. Every third day, doses of 1.0 mg/kg MTX or single or weekly doses of 30-40 mg/kg CTX were used.

**RESULTS**

Bacterial monocontamination was detected in all isolators at some time during the experiments. No correlation could be made between the development of lesions in the nervous system and the presence of specific bacteria or the time at which contamination occurred. Bacterial contamination did not alter the clinical appearance of any dogs either infected or controls. Rectal temperatures provided no clear indication of disease
progress or severity.

**R252 Isolate** In the initial studies of the R252 isolate 4 dogs were used (Table 1). One dog (R6101) was asymptomatic but lymphopenic on PID 21 when methotrexate (MTX) treatment was initiated. The day after the second dose of MTX (PID 24), the dog developed convulsions. Its condition deteriorated and death occurred on PID 29. Generalized lymphoid depletion was the only lesion observed. No demyelination was found.

The remaining 3 dogs were not treated. Two dogs (R6412 and R6413) remained asymptomatic until PID 43 and 39 when convulsions first occurred. Debilitation was rapid and the dogs were euthanatized on PID 49 and 43, respectively. The third dog (R6411) had signs of motor disturbance from PID 21 until PID 70 when it was euthanatized due to severe convulsions. Absolute lymphopenia was present in these dogs throughout the course of the disease. Demyelination associated with astrocytosis and sometimes with necrosis and gitter cells was found in cerebellar and medullary white matter. Hematogenous inflammatory cells were not present. Eosinophilic intranuclear inclusions were frequently observed in swollen astrocytic nuclei in the demyelinated areas. Similar inclusions were found in the mucosa of the stomach and urinary bladder. Thymic atrophy and lymphoid depletion were severe. Neutralizing antibodies against CDV were not detected in these three dogs.

The results of studies to determine the effects of age at inoculation and route of inoculation on induction of demyelination by R252 CDV are presented in Table 2. Intracerebral inoculation of 1 week old pups resulted in death by PID 28. Convulsions occurred for 1-3 days prior to
death. Demyelination was not found, but widespread necrosis of single neuronal cells was observed in 2 pups. The medullary portions of the thymuses in these pups were necrotic and many cells contained eosinophilic intranuclear inclusions. Other lymphoid tissues were moderately depleted.

Pups inoculated at 4 or 8 weeks of age by the routes indicated in Table 2 had absolute lymphopenia which persisted until death or spontaneously returned to normal by 7 weeks post infection. Demyelination similar to that in the 3 original dogs was found in 8 dogs, (4 intracerebral, 1 intradermal, 1 intratracheal, and 2 contact exposed) which died between PID 27 and 54. Five dogs (1 intracerebral, 1 intraspinal, 1 intravenous, and 2 contact exposed) which survived the total observation period had areas of demyelination heavily infiltrated by mononuclear inflammatory cells (lymphocytes, monocytes and plasma cells). Perivascular cuffs of similar cells were prominent throughout affected areas. The number of intranuclear viral inclusion bodies within and in the vicinity of demyelinating lesions differed from area to area.

Selected serum samples indicated that neutralizing antibodies against Vero-adapted CDV were not present in dogs which became moribund, while titers of 64 to 256 were detected in dogs which survived the course of the experiment. Pre-inoculation serum samples were negative.

Spontaneous Case P3977 A suspension of cerebellar tissue from a histologically confirmed case of demyelinating distemper (P3977) did not induce disease in the 1 dog which survived the inoculation procedure. This material was not studied further.
Snyder Hill (SH) CDV Two dogs were inoculated with SH-CDV intracerebrally. Others were inoculated intraperitoneally and then treated with methotrexate. These dogs developed convulsions and died by PID 10. No neural lesions were found.

Glia Tissue-Culture Adapted (GA) CDV Four dogs were inoculated intracerebrally with GA-CDV. Two were treated with methotrexate and died by PID 10. No neural lesions were found. The 2 untreated dogs remained healthy. No neural lesions were found when examined on PID 62.

French Isolate (#720-2212-21) CDV Seventeen dogs were used in the study of this isolate. Six dogs were inoculated intraperitoneally with the lyophilized inoculum (Table 3). Two of these dogs remained asymptomatic, 2 (R6132 and R6133) died and had multifocal cerebellar demyelination, and 2 died without demonstrable neural lesions. The latter 2 had been treated with immunosuppressants.

Suspensions prepared from neural tissues of the affected dogs failed to induce disease in 11 dogs inoculated intraperitoneally, intraspinally, or intracerebrally. Convalescent serum neutralizing antibody titers ranged from 64 to 256.

DISCUSSION

The present studies demonstrated that CDV in brain tissue of a dog (R252) with naturally occurring demyelinating distemper was capable of inducing central nervous system (CNS) demyelination in gnotobiotic dogs. In addition, 35% (9/26) of the dogs inoculated with cerebellar tissue from dogs experimentally infected with R252 CDV also developed lesions of demyelination (Table 4). This inoculum failed to infect tissue culture cells following direct inoculation, therefore, an in vitro infec-
tivity titer could not be determined. Infection of dogs with R252 CDV by contact exposure gave a greater incidence (4/6) of demyelination as compared to their parenterally infected littermates. Sera of dogs which survived infection with R252 CDV neutralized Vero-adapted CDV in vitro at titers of 64 to 256, but neutralizing antibodies were not detected in the sera of moribund dogs.

The disease induced in 11 of the 35 dogs with R252 CDV differs from that generally associated with CDV infection (7), in that the prepatent period was prolonged for several weeks, peripheral lymphocytes remained significantly decreased throughout the course of disease, and serum neutralizing antibodies to CDV were not detectable. The areas of demyelination which developed in these dogs were associated with an abundance of intranuclear viral inclusions and marked astrocytosis, but hematogenous inflammatory cells were seldom found. In addition, the thymuses were atrophic and the lymphoid tissues depleted. The importance of these findings is not presently known, but they suggest that the inflammatory response to CDV infection of the CNS may be suppressed due to a concomitant depletion of lymphoid tissues. Further, these observations indicate that demyelination associated with CDV infection can occur in the absence of obvious hematogenous inflammation. This process may be similar to that which occurs in immunosuppressed patients with progressive multifocal leukoencephalopathy (20).

Five R252 CDV-infected dogs had CNS demyelination associated with hematogenous mononuclear inflammatory cell infiltration. They were clinically normal at the time of euthanasia except for 2 which had minimal neurologic disturbances. The presence of hematogenous mononuclear
inflammatory cell infiltrates in the CNS and the absence of demonstrable CDV inclusions in some areas of demyelination suggests these dogs may have been suffering additional myelin damage due to a host response initiated by the virus infection. The demyelination in these dogs is similar to that seen in most naturally occurring cases of demyelinating distemper. The immune responsiveness of R252-infected dogs to other antigens, including myelin, is presently being investigated in this laboratory.

Detailed descriptions of the clinical disease, the neural and lymphoid lesions, and the in vitro behavior of the R252 CDV will be presented in separate communications (21,22,23). The in vitro studies are based upon R252 CDV isolated from experimentally infected dogs by cocultivation of brain cells with Vero cells.

Our studies failed to determine if other isolates of CDV such as the Snyder-Hill or Lederle strains will induce demyelination more often by manipulation of the immune response of the host. The present studies along with past reports (9,10,12) suggest, however, that these CDV strains induce demyelination infrequently. The reason for the loss of demyelinating activity of the French CDV isolate after our initial successes cannot be explained at present.

In instances when demyelination has been reported in previous experimental cases, the lesion was observed in dogs which survived for periods considered to be longer than usual for experimentally infected dogs (7,10,11). Since gnotobiotic dogs are protected from overwhelming secondary bacterial infection, a common cause of acute death in conventional dogs infected with CDV (17), one could expect gnotobiotic dogs to
survive longer and subsequently develop a higher incidence of demyelinating lesions. The present study indicates that although longevity appears to be important, it is probably not the sole factor influencing virus induced demyelination. In fact, variations in the course of distemper may only be a reflection of differences in biological behavior of subpopulation of CDV, a concept supported by the recent work of Reculard and Guillon (13).

Variability also exists in measles virus infection of man, for in vitro investigations of viruses isolated from cases of SSPE suggest that even though these viruses are similar in many respects to classical measles virus they do possess distinct characteristics (24-27). Determination of whether these in vitro differences represent ability to affect myelin supporting cells directly (i.e. oligodendrocytes) or indirectly by immune mechanisms has not been made because of the inability to experimentally induce demyelination with these viruses (16). In light of the known relationships between measles and distemper viruses (9), studies now in progress to elucidate the mechanism(s) by which R252 CDV induces demyelination in gnotobiotic dogs, should prove important in understanding virus-induced demyelinating diseases of man.
Demyelination was induced in 16 of 35 gnotobiotic dogs by infecting them with canine distemper virus (CDV) from a naturally-occurring case of demyelinating distemper (R252). Infection of dogs with R252 CDV by contact exposure caused a high incidence of demyelination as compared to parenterally inoculated dogs. In addition to demyelination, R252 CDV infection induced absolute lymphopenia which persisted for several weeks.

Two forms of demyelinating disease occurred following R252 CDV infection. Eleven dogs developed neurological signs several weeks after infection and rapidly became moribund. They had remained lymphopenic and were found to have thymic atrophy and generalized lymphoid depletion. Hematogenous mononuclear inflammatory cells were not found in the areas of demyelination in these dogs. Five of 24 dogs which survived the observation period with normal lymphocyte counts were found to have demyelination associated with hematogenous mononuclear inflammation. Two of these dogs had exhibited mild neurological signs.

A French strain of CDV (#720-2212-71) induced demyelination in 3 of 17 gnotobiotic dogs, while no demyelination occurred in 8 dogs inoculated with either Snyder-Hill or glial tissue culture-adapted (Lederle) CDV.
Table 1. Inoculation of gnotobiotic dogs with cerebellar suspensions prepared from naturally occurring cases of demyelinating distemper.

<table>
<thead>
<tr>
<th>Source of Virus</th>
<th>Dog No.</th>
<th>Route</th>
<th>Clinical Signs</th>
<th>Death (PID)</th>
<th>Demyelination</th>
<th>Perivascular Cuffing</th>
</tr>
</thead>
<tbody>
<tr>
<td>R252</td>
<td>R6101</td>
<td>IC</td>
<td>+</td>
<td>29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R6411</td>
<td>IC</td>
<td></td>
<td>+</td>
<td>70</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>R6412</td>
<td>IS</td>
<td>+</td>
<td>49</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>R6413</td>
<td>IC</td>
<td>+</td>
<td>44</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>R3977</td>
<td>R6135</td>
<td>IC</td>
<td>-</td>
<td>82</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R6136</td>
<td>IC</td>
<td>-</td>
<td>2</td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

a  IC = intracerebral; IS = intraspinal; 0.5 ml/dog
b  Persistent absolute lymphopenia; convulsions and pyrexia terminally
c  PID = post inoculation day
d  Treated with intravenous methotrexate 1 mg/kg on PID 21 and 24
e  Euthanized = clinically normal at euthanasia
f  Died secondary to IC injection
Table 2. Virulence of R252 CDV after one passage in gnotobiotic dogs.

<table>
<thead>
<tr>
<th>Age at Inoculation</th>
<th>Route</th>
<th>Dose</th>
<th>Nervous Signs</th>
<th>Death</th>
<th>Demyelination</th>
<th>Perivascular Cuffing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week</td>
<td>IC</td>
<td>0.1 ml</td>
<td>3/3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>4 weeks</td>
<td>IC</td>
<td>0.1 ml</td>
<td>1/4</td>
<td>1/4</td>
<td>2/4</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>IC</td>
<td>0.2 ml</td>
<td>2/4</td>
<td>2/4</td>
<td>2/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>--</td>
<td>3/5</td>
<td>2/5</td>
<td>3/5</td>
<td>1/5</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>--</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>8 weeks</td>
<td>IC</td>
<td>0.2 ml</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>0/2</td>
</tr>
<tr>
<td></td>
<td>IS</td>
<td>0.2 ml</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>0.2 ml</td>
<td>0/4</td>
<td>0/4</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>0.2 ml</td>
<td>1/3</td>
<td>1/3</td>
<td>1/3</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>IT</td>
<td>0.2 ml</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>IN</td>
<td>0.2 ml</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Contact</td>
<td>--</td>
<td>1/1</td>
<td>0/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>--</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Totals (excluding controls) 13/32 11/32 13/32 5/32
\textbf{a} IC = intracerebral, IS = intraspinal, IV = intravenous, ID = intradermal (footpads)

IT = intratracheal, IN = intranasal, contact = housed in isolator with inoculated dogs

\textbf{b} Inoculum = 10\% suspension of cerebellar tissue from dogs R6411, R6412, and R6413

\textbf{c} Surviving dogs were euthanatized 12 weeks post-inoculation

\textbf{d} Numerator = number affected, denominator = number studied

\textbf{e} Two received 0.2 ml 10\% normal dog cerebellum suspension IC
Table 3. Intraperitoneal inoculation of gnotobiotic dogs with lyophilized preparations of French isolate
(#720-2212-71) CDV.

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Dose of Virus</th>
<th>Treatment</th>
<th>Clinical Signs</th>
<th>Death (PID)</th>
<th>Demyelination</th>
</tr>
</thead>
<tbody>
<tr>
<td>R6132</td>
<td>0.5 ml</td>
<td>None</td>
<td>+</td>
<td>21</td>
<td>+</td>
</tr>
<tr>
<td>R6133</td>
<td>0.8 ml</td>
<td>None</td>
<td>+</td>
<td>30</td>
<td>+</td>
</tr>
<tr>
<td>S534</td>
<td>1.0 ml</td>
<td>None</td>
<td>±</td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>R6131</td>
<td>0.5 ml</td>
<td>MTX</td>
<td>+</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>S533</td>
<td>1.0 ml</td>
<td>CTX</td>
<td>±</td>
<td>14&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>S3923</td>
<td>2.0 ml</td>
<td>CTX</td>
<td>+</td>
<td>32</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> MTX = methotrexate; CTX = cyclophosphamide; see text for doses.

<sup>b</sup> + = Persistent absolute lymphopenia; convulsions with pyrexia terminally

± = Transient absolute lymphopenia early in the course

<sup>c</sup> PID = post inoculation day

<sup>d</sup> Euthanatized clinically normal at euthanasia
**Table 4. Effect of route of inoculation on virulence of R252 CDV after one passage in dogs**

<table>
<thead>
<tr>
<th>Route</th>
<th>Nervous Signs&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Death</th>
<th>Demyelination</th>
<th>Perivascular Cuffing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural (IC or IS)</td>
<td>4/14</td>
<td>4/14</td>
<td>6/14</td>
<td>2/14</td>
</tr>
<tr>
<td>Non-Neural (IV or ID)</td>
<td>1/7</td>
<td>1/7</td>
<td>2/7</td>
<td>1/7</td>
</tr>
<tr>
<td>Contact</td>
<td>4/6</td>
<td>2/6</td>
<td>4/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Controls</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
</tr>
</tbody>
</table>

<sup>a</sup> Inoculum = 10% suspension of cerebellar tissue from dogs R6411, R6412, and R6413. Dogs inoculated when 4 or 8 weeks of age.

<sup>b</sup> IC = intracerebral, IS = intraspinal, IV = intravenous, ID = intradermal (footpad), contact = housed in isolator with inoculated dogs.

<sup>c</sup> Signs of neurological disturbance; numerator = number affected, denominator = number studied.
CHAPTER II

EXPERIMENTAL CANINE DISTEMPER VIRUS-INDUCED LYMPHOID DEPLETION

Introduction

Canine distemper is a naturally occurring viral disease of dogs in which the virus infection is pantropic (1). Even though lymphoid depletion was a well recognized lesion of distemper (2,3) and viral antigens had been demonstrated in lymph nodes of infected ferrets by immunofluorescence (4), thymic atrophy was not reported as a lesion of distemper until studies were conducted in gnotobiotic dogs (5). Subsequently, thymic atrophy also was found in conventional dogs infected with canine distemper virus (6). The long term effects of CDV infection on lymphoid tissues, however, have not been reported.

During a recent investigation of the demyelinating activity of CDV in gnotobiotic dogs, infection with a new CDV isolate (R252) was demonstrated to cause absolute lymphopenia which persisted for several weeks (7). In addition, thymic atrophy and generalized lymphoid depletion were present in those dogs which were euthanatized due to central nervous system involvement 27 to 47 days after infection. The purpose of this investigation was to determine the hematological alterations and lymphoid lesions induced in gnotobiotic dogs by R252 CDV infection.
MATERIALS AND METHODS

The R252 CDV isolate originated from cerebellar tissue from a naturally occurring case of demyelinating distemper (7). The cerebellum from this dog was used to induce demyelination in 3 gnotobiotic dogs. Cerebellar tissue from these 3 dogs was then prepared as a 10% suspension, clarified by low-speed centrifugation, and subsequently stored as 1 ml aliquots in liquid nitrogen.

Thirty-four 4 or 8 week-old gnotobiotic dogs were used in this study. They were obtained by hysterectomy from unconditioned conventional bitches supplied by a local dealer. The methods described by Griesemer and Gibson (8) for raising gnotobiotic dogs in flexible plastic incubators were employed.

Inoculation of the dogs with 10% cerebellar suspension was as follows: 4 weeks old, intracerebral, 0.1 ml - 4 dogs, and 0.2 ml - 4 dogs; 8 weeks old, intracerebral, 0.2 ml - 2 dogs, intraspinal, 0.2 ml - 4 dogs, intravenous, 0.2 ml - 4 dogs, and intradermal (carpal footpad), 0.2 ml - 3 dogs (7). In addition, 1 eight-week-old dog and 5 four-week-old dogs were infected by contact exposure to their parenterally inoculated littermates. Seven littermate dogs were housed in separate isolators and served as uninfected controls. Two of these control dogs were inoculated intracerebrally with 0.2 ml of a 10% suspension of normal cerebellum obtained from a previous control dog. All inoculations were made while the dogs were sedated with Innovar (Pitman-Moore, Inc., Washington Crossing, New Jersey). Dogs receiving an 0.2 ml intracerebral dose were given 0.1 ml in each frontal lobe.
Blood for hemograms was collected weekly from each dog by jugular puncture with the dogs sedated with Innovar. At least two pre-inoculation blood samples were collected from each dog. Total cell counts were made with a Coulter counter. The differential distribution of leukocytes was determined by counting 100 cells on a smear of peripheral blood stained with Wright's stain.

Dogs were euthanatized when they became moribund or on post inoculation day (PID) 84 (approximately PID 77 for contact exposed dogs). Except for 8 dogs perfused for electron microscopy, dogs under Innovar sedation were exsanguinated by cardiac puncture preceding necropsy. Representative samples of all lymphoid tissues were fixed in 10% buffered formalin, sectioned at 6 μm, stained with hemotoxylin and eosin, and examined by light microscopy.

Eight dogs (6 inoculated and 2 controls) were deeply anesthetized with pentobarbital sodium and perfused with approximately 2 liters of 10% buffered formalin via the left ventricle of the heart. Portions of the thymus and the left prescapular lymph node were minced into 1 mm cubes for further fixation in 1.33% osmium tetroxide S-collidine buffer. The processed tissues were embedded in Epon 812. One micron thick sections for light microscopic study were stained with toluidine blue. Examination of thin sections stained with uranyl acetate and lead citrate was accomplished with a Phillips 200 electron microscope. The remainder of the thymus and left prescapular lymph node, and the spleen, tonsils, ileum (Peyer's patches), mandibular lymph nodes, and mesenteric lymph nodes were processed as described above for light microscopy.
Student's t-test was used for determination of the statistical significance of differences between the hematological parameters of infected and control groups.

RESULTS

Hematological Alterations

The means of the absolute lymphocyte, absolute neutrophil, absolute eosinophil, and total leukocyte counts are presented in figures 1, 2, 3, and 4, respectively. The 21 dogs inoculated with the R252 cerebellar suspension are considered together because results between groups inoculated by different routes, with different amounts, or at different ages were not significantly different. The contact exposed dogs probably became infected about 7 days after the inoculation of their littermates and are, therefore, grouped separately. Control counts were determined by grouping all weekly samples from the uninfected dogs.

The absolute lymphocyte counts were significantly decreased (P<.005) in the inoculated dogs for post-inoculation weeks (PIW) 1 through 7 and in the contact exposed dogs for PIW 2 through 8 (Fig. 1). The absolute neutrophil counts were not significantly decreased (P > .05) in either group, but a significant (P< .025) absolute neutrophilia occurred in the contact exposed group at PIW 6 (Fig. 2). The absolute eosinophil counts were not significantly affected (P > .05) in either group, but the counts after PIW 6 were elevated in comparison to the earlier values (Fig. 3). The total leukocyte levels reflected the decrease in lymphocytes with significantly lower (P< .005) levels present in the inoculated group at PIW 1 and 4 and in the contact exposed group at PIW 2, 3, and 4 (Fig. 4). Absolute monocyte counts, total erythrocyte counts, hemoglobin concen-
Gross Lesions

For purposes of presentation of the gross and microscopic lesions, the dogs are grouped into three categories. Group I - moribund with central nervous system (CNS) lesions, Group II - survived total observation period but had CNS lesions, and Group III - survived total observation period with no CNS lesions (9).

Thymus was not demonstrable grossly in 5 of 7 dogs of Group I and in 2 of 5 dogs of Group II (Fig. 5). Abundant fat in the mediastinal tissue of these dogs made identification of the atrophic thymuses impossible. In contrast, 7 of 14 dogs in Group III had thymuses which were more than 2 standard deviations above the mean weight of the control thymuses (Fig. 5). The thymuses of all other dogs were of normal size. Use of thymus weight to body weight ratios did not appreciably change the relationships between the groups (Fig. 6).

Although the lymph nodes were of normal size, the cortex was not easily distinguished from medullary tissue in dogs of Group I. The lymphoid tissue in spleen, tonsil, and Peyer's patches was also difficult to demonstrate in these dogs. In Groups II, III and controls, however, the densely cellular, white cortical area of the lymph nodes was readily apparent as were the other lymphoid structures.

Histopathological and Ultrastructural Lesions

Thymus: In 3 dogs of Group I examination of several sections of anterior mediastinal tissue revealed no thymus. The lobules of the thymuses in the 4 other dogs in this group were smaller than normal (Fig. 7) and the medullary portion was either reduced considerably (Fig. 8) or...
absent. Necrosis of individual thymocytes was observed in one dog. In addition, one thymus had lobules which were only several cells in width and were composed predominantly of reticulum cells (Fig. 9). In the 3 thymuses with distinct medullary areas, Hassall's corpuscles were rare (Table 5) and those found were composed of lightly stained cells with small, round to slightly oval nuclei. Medullary cells in one dog of Group I contained eosinophilic intranuclear viral inclusions.

Atrophic thymus of the one dog of Group I selected for electron microscopic examination was composed of lymphocytes and reticulum cells. Many lymphocytes contained cytoplasmic accumulations of tubular strands of viral nucleoprotein (Fig. 10 and 11). Budding of viral particles from the plasma membrane and intranuclear viral inclusions were not observed. Degeneration and necrosis of individual cells was evidenced by loss of cytoplasmic organelles and increased electron density of nuclear material. In a few necrotic cells viral nucleoprotein was present in the cytoplasm (Fig. 12). No Hassall's corpuscles were found in samples selected for ultrastructural study.

The 5 dogs of Group II had thymic lobules similar to those found in control dogs. The medulla and cortex were approximately equal in area and Hassall's corpuscles were demonstrable with moderate frequency (Table 5).

Dogs of Group III had slightly larger thymic lobules than dogs of Group II or control dogs and the number of Hassall's corpuscles per lobule was increased (Table 5). The individual Hassall's corpuscles in dogs of this group also tended to be larger, containing hypertrophied epithelioid cells with abundant intracytoplasmic keratin granules and
large oval nuclei (Fig. 13). Germinal centers were prominent in the thymic medullas of 2 dogs of Group III (Fig. 14).

Lesions were not found in thymuses of uninfected control dogs (Fig. 7). Further, germinal centers were not observed in the thymuses of these dogs.

In 1 dog of Group II, 4 dogs of Group III, and 2 uninfected control dogs an extensive search of samples of thymus by electron microscopy failed to demonstrate structures suggestive of virus infection. A few necrotic cells were found in each case, but not to the extent that they were present in the dog of Group I. Examination of these tissues for CDV antigens by immunofluorescence has not been completed.

Lymph Nodes: The lymph nodes in dogs of Group I contained little cortical tissue and no germinal centers (Fig. 15). The medullary portions were abundantly populated with reticulum cells and macrophages. Many macrophages were distended with eosinophilic material. A few plasma cells were present in the medullary cords.

Due to the increased definition of cytoplasmic organelles available with electron microscopy more plasma cells were demonstrated in the lymph node of the dog of Group I than had been detected by light microscopy. The dense central nucleus and minimal amount of cytoplasm of the plasma cells were suggestive of lymphocytes light microscopically. Only a few macrophages contained intracytoplasmic viral nucleoprotein in the sections studied by electron microscopy. No budding of virus or intranuclear viral inclusions were found. Necrotic cells were rare.

The lymph nodes in dogs of Group II and III and uninfected dogs were similar. Both T- and B-dependent areas were densely cellular and numer-
ous germinal centers were present. The medullary cords were thickened by lymphocytes and plasma cells. Electron microscopic lesions were not observed.

Other Lymphoid Tissues: The splenic white pulp, tonsils, and Peyer's patches reflected the changes observed light microscopically in the lymph nodes of the respective groups. These structures were depleted of mature lymphoid elements and contained increased numbers of reticulum cells in dogs of Group I, while in dogs of Groups II and III they were densely populated with the expected cellular components.

Lymphoid lesions were not found in uninfected control dogs. A moderate number of germinal centers were present in all lymphoid structures.

DISCUSSION

In the present study, infection of gnotobiotic dogs with R252 CDV caused absolute lymphopenia without significantly affecting neutrophil, eosinophil, or monocyte counts. Lymphocyte counts returned to normal by 8 weeks after infection in dogs which survived the total observation period (Groups II and III), but the lymphocytes were persistently decreased in moribund dogs (Group I). Except for neurological signs which occurred in 10 dogs (9) R252 CDV-infected dogs remained asymptomatic even during the lymphopenic period.

Lymphopenia is considered characteristic of naturally occurring distemper (10), but the duration and severity of CDV-induced lymphopenia have not been adequately established under experimental conditions. Jacoby and Griesemer (11), demonstrated that elimination of endogenous corticosteroids by adrenalectomy did not affect the lymphopenia of acute
distemper in specific-pathogen-free dogs. Their investigation was limited to 8 days post-infection, however, because the adrenalectomized dogs became moribund. Previous studies in gnotobiotic dogs suggested that the lymphopenia of distemper lasts for only a few days, but the number of dogs evaluated after 12 days post-infection was small (5).

The cause of the lymphopenia in CDV infected dogs is most probably related to the ability of CDV to replicate in and destroy lymphoid tissues. Electron microscopy was used in the present study to demonstrate accumulations of viral nucleoprotein strands in most of the thymic lymphocytes of a dog which had become moribund from R252 CDV infection 41 days after contact exposure. Similar observations have been reported by Tajima, et al. (12) from their electron microscopic study of lymph nodes of mink acutely infected with CDV and moribund 8 to 11 days after inoculation. Immunofluorescence also has been used to identify CDV in the lymph nodes of experimentally infected ferrets (4) and in the lymph nodes and thymuses of experimentally infected dogs (6). Examination of tissues of R252 CDV infected dogs by immunofluorescence has not been completed. The influence of the gnotobiotic environment on the prolongation of R252 CDV-induced lymphopenia is not known, but in preliminary studies with 4 caesarian-derived, colostrum-deprived dogs raised in a conventional environment, R252 CDV infection caused lymphopenia of only 2 weeks duration (13).

The severe thymic atrophy found in 7 dogs infected with R252 CDV in conjunction with the electron microscopic demonstration of virus suggests that some dogs infected with R252 CDV may be unable to regenerate thymus after severe depletion or that if regeneration occurs it does so slowly.
Whether this dysfunction is due to an absence of stem cells, a deficiency of some lymphopoetic thymic factor(s), or a persistent destruction of maturing lymphocytes due to CDV infection remains to be determined.

The mechanism of thymus atrophy and regeneration has been extensively studied in relation to radiation damage. High but sub-lethal doses of whole-body x-irradiation cause necrosis of cortical lymphocytes with subsequent involution of the thymic lobules (14). Regeneration of the thymuses in these animals is biphasic and requires several weeks for completion (15-17).

In contrast, the thymic medullary areas were more extensively damaged than the cortices in dogs debilitated by R252 CDV infection. Intranuclear viral inclusions were observed occasionally in medullary remnants of these thymuses. More pronounced destruction of medullary cells and a larger number of cells with intranuclear viral inclusions were found in the thymuses of 3 pups infected with R252 CDV when one week-old. These pups died approximately 3 1/2 weeks after infection and the thymuses were of normal size (13). Loss of medullary tissue may decrease the thymus's potential for regeneration because the medulla is relatively radioresistant and regeneration has been demonstrated in irradiated animals (15-17).

Severe malnutrition has been reported as a cause of thymic atrophy in children (18), but the dogs in the present study continued to eat and gain weight normally. In addition, abundant adipose tissue was found in all dogs at necropsy. Corticosteroids are also known to adversely affect lymphoid tissues, but as pointed out above, adrenalectomy has been shown to have no influence on CDV-induced lymphopenia (11). Moreover, no
morphologic indication of adrenal hyperplasia was detected (13).

Measles and rinderpest viruses, both closely related to CDV are immunodepressive during the acute phase of infection (19,20). Vaccination with live attenuated measles virus also transiently depresses the skin test response in patients positively sensitized to tuberculin (21-23). Hematological studies of children with measles and of children vaccinated with live attenuated measles virus have shown that transient lymphopenia results from measles virus infection (24,25). Recently, White and Boyd (26) reported that children dying from measles virus infection had atrophic thymuses in which the cortico-medullary demarcation was absent. Aggregation of thymocytes and formation of syncytia from thymocytes were observed in one patient who died 4 days after the onset of clinical signs. In addition, thymuses from "post-measles" patients, who died of unrelated causes, were atrophic and had significantly fewer Hassall's corpuscles per high power field of medulla (26). Hematological data on these patients was not presented.

The significance of the marked reduction of Hassall's corpuscles in the atrophic thymuses on the lymphopenia induced by R252 CDV infection is not understood, because a definite function has not been demonstrated for these structures. Likewise, the larger size of thymuses and the increase of Hassall's corpuscles in dogs of Group III and the presence of germinal follicles in the thymuses of 2 of these dogs can not be explained at present. The extent to which R252 CDV infection affects the two arms of the immune system, i.e. thymic (T-cell) and bone marrow (B-cell), and its influence on immune responsiveness to other non-viral antigens, is presently being investigated in this laboratory.
R252 CDV-infected dogs of Group I had demyelinating lesions of the central nervous system which were not accompanied by hematogenous inflammation, while dogs of Group II had no lymphoid lesions but had CNS demyelination and nonsuppurative encephalitis (9). The similarity of these CNS lesions to human demyelinating diseases has been discussed separately (9). Although the relation between the lymphoid lesions and demyelination of the central nervous system in R252 CDV infected dogs remains to be elucidated, the gnotobiotic dog infected with R252 CDV provides an excellent model for the study of virus-induced alterations of the lymphoid and central nervous systems.
Summary

Thirty-four gnotobiotic dogs were used to study the effects of R252 canine distemper virus (CDV) infection on the lymphoid system. Twenty-one dogs were inoculated parenterally and 6 dogs were infected by contact exposure to inoculated littermates. Seven littermate dogs were maintained as uninfected controls.

The means of the absolute lymphocyte counts of dogs infected with R252 CDV were significantly decreased for 7 weeks after infection. The lymphocyte counts failed to return to normal in 8 dogs which became moribund with neurological signs and were euthanatized 27 to 47 days after infection (Group I). The 19 dogs which survived the 12 week observation period had normal lymphocyte counts by 8 weeks after infection. Five of these dogs (Group II) were subsequently found to have demyelination of the central nervous system (CNS) while no CNS lesions were demonstrated in the remaining 14 infected dogs (Group III).

The thymuses of dogs of Group I were atrophic and their lymph nodes were depleted of lymphoid cells. In addition, the number of Hassall's corpuscles per thymic lobule was decreased in dogs of Group I. Viral nucleoprotein was demonstrated in thymus and lymph node cells by electron microscopy. No lymphoid lesions were found in the dogs of Group II. Some of the thymuses of dogs of Group III were significantly larger than those of uninfected controls, and they contained increased numbers of Hassall's corpuscles per thymic lobule. Further, germinal centers were found in two thymuses of dogs of Group III.
Figure 1. Means of absolute lymphocyte counts of gnotobiotic dogs infected with R252 CDV. Stippled area represents mean absolute lymphocyte count ± S.E. of all samples from uninfected controls. Numbers below abscissa - contact exposed dogs/parenterally inoculated dogs.
Figure 2. Means of absolute neutrophil counts of gnotobiotic dogs infected with R252 CDV. Stippled area represents mean absolute neutrophil count ± S.E. of all samples from uninfected controls. Numbers below abscissa - contact exposed dogs/parenterally inoculated dogs.
Neutrophils per mm³ x 10³

- Controls
- Parenterally inoculated
- Contact exposed

Mean ± Standard error

Weeks post inoculation

1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
2 0 2 4 6 8 10 12
6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6
17 20 21 21 21 21 21 21 19 17 16 16 16 16 16 16 16 16 16 16 16 16
Figure 3. Means of absolute eosinophil counts of gnotobiotic dogs infected with R252 CDV. Stippled area represents mean absolute eosinophil count ± S.E. of all samples from uninfected controls. Numbers below abscissa - contact exposed dogs/parenterally inoculated dogs.
CONTROLS
PARENTERALLY INOCULATED
CONTACT EXPOSED

EOSINOPHILS PER mm$^3 \times 10^2$

WEEKS POST INOCULATION

MEAN ± STANDARD ERROR
Figure 4. Means of total leukocyte counts of gnotobiotic dogs infected with R252 CDV. Stippled area represents mean total leukocyte count ± S.E. of all samples from uninfected controls. Numbers below abscissa = contact exposed dogs/parenterally inoculated dogs.
Figure 5. Thymus weights of gnotobiotic dogs infected with R252 CDV.

See text for detailed description of groups.
THYMUS WEIGHT (GRAMS)

GROUP I  MORIBUND, NEURAL LESIONS
GROUP II  SURVIVED, NEURAL LESIONS
GROUP III  SURVIVED, NO NEURAL LESIONS
Figure 6. Thymus weight/body weight ratios of gnotobiotic dogs infected with R252. See text for detailed description of groups.
BODY WEIGHT (GRAMS/100 GRAMS)

GROUP I MORIBUND, NEURAL LESIONS
GROUP II SURVIVED, NEURAL LESIONS
GROUP III SURVIVED, NO NEURAL LESIONS

% BODY WEIGHT (GRAMS/100 GRAMS)
Figure 7. Thymus from uninfected control dog. Note near equal amounts of medullary (M) and cortical (C) regions. H & E X 85.
Figure 8. Thymus of dog of Group I. Medulla (M) is markedly reduced. H & E X 70.
Figure 9. Thymus of dog of Group I. Severely atrophic thymic lobule is composed principally of reticulum cells, but lymphocytes are present, especially at the periphery of the lobule. Increase in interlobular space is due to shrinkage of the lobules. H & E X 185.
Figure 10. Viral nucleoprotein (V) in the cytoplasm of thymocytes of dog of Group I. Note the absence of degeneration in infected cells. X 6500.
Figure 11. Viral nucleoprotein (V) in the cytoplasm of a thymocyte of dog of Group I. The dark spots represent cross-sections of the tubular strands of viral nucleoprotein. Note absence of viral budding. X 20,940.
Figure 12. Necrotic cell in thymus of dog of Group I. The cytoplasm of their degenerating phagocytic cell contains viral nucleoprotein (V) in addition to phagocytized debris. X 7710.
Table 5. Hassall's corpuscles per thymic lobule in gnotobiotic dogs infected with R252 CDV.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Dogs</th>
<th>Hassall's Corpuscles/Thymic Lobule</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>$0.25 \pm 0.04^c$</td>
</tr>
<tr>
<td>II</td>
<td>4</td>
<td>$1.95 \pm 0.12^c$</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>$1.90 \pm 0.10^c$</td>
</tr>
<tr>
<td>Controls</td>
<td>5</td>
<td>$1.40 \pm 0.09$</td>
</tr>
</tbody>
</table>

a See text for description of groups

b Mean $\pm$ S.E.; 30 lobules counted for each dog

c Significantly different ($P < .005$)
Figure 13. Thymus of dog of Group III. Well-developed hypertrophied Hassall's corpuscle is composed of large epithelioid cells. Keratin granules (K) are present in the cytoplasm. H & E X 1333.
Figure 14. Thymus of dog of Group III. Germinal center in medulla.

H & E X 470.
Figure 15. Pre-scapular lymph node of dog of Group 1. Depletion of lymphoid cells with concomitant reticulum cell hyperplasia. Multinucleated giant cells (arrows) are present in the cortex (C). Medullary cords (M) are hypocellular. H & E X 310.
CHAPTER III

EXPERIMENTAL CANINE DISTEMPER VIRUS-
INDUCED Demyelination

Introduction

Complete elucidation of the pathogenesis of the demyelination which occurs in association with subacute measles virus (MV) infection of man, i.e., subacute sclerosing panencephalitis (SSPE), and possibly other demyelinating diseases such as multiple sclerosis, will be dependent upon experimental reproduction of the lesion by infection of non-human hosts. Several investigators have, in fact, induced neural lesions in experimental animals including dogs, by infecting them with measles-like viruses isolated from human patients with SSPE (5,11,13,14,19,20,24), but induction of demyelination by infection with these viruses has not been reported. The current understanding of the role of viral infections in the induction of central nervous system demyelination has recently been presented by Weiner, et al. (25). The potential usefulness of canine distemper as an animal model for demyelinating disease was emphasized (25).

Canine distemper is a naturally occurring viral disease of dogs. The causative canine distemper virus (CDV) is a paramyxovirus that is related biophysically, biochemically, and antigenically to MV (4). Demyelination often occurs in dogs with the nervous form of this disease and the light microscopic features of this lesion have been well charac-
terized (10,12). In addition, Wisniewski, Raine, and Kay (27), who in-
vestigated the ultrastructure of distemper-associated demyelination,
concluded that the demyelination in naturally occurring canine distemper
was similar to that in acute multiple sclerosis and probably other in-
flammatory demyelinating diseases.

Canine distemper is well suited as a model for virus-induced demye-
linating diseases in man. Reports, however, indicate that demyelination
occurs rather infrequently following experimental CDV infection of dogs
(1,6,7,8). We have succeeded recently in inducing demyelination in 13
of 32 gnotobiotic dogs by infecting them by various routes with a CDV
isolate obtained from a naturally occurring case of distemper with demye-
1ination (16). The percentage of demyelination was further increased by
the use of selected routes of infection such as intracerebral inoculation
or by contact exposure. The purpose of this investigation was to deter-
mine the neurological disturbances and characterize the histological and
ultrastructural lesions of the central nervous systems in gnotobiotic
dogs experimentally infected by various routes with R252 CDV.

MATERIALS AND METHODS

The origin of R252 CDV was reported previously (16). Briefly, cere-
bellar tissue from a spontaneous case of distemper with demyelination
(R252) was used to induce demyelination in 3 gnotobiotic dogs. Cerebellar
tissue from these 3 dogs was then prepared as a 10% suspension, clarified
by low-speed centrifugation, and subsequently stored as 1 ml aliquots in
liquid nitrogen.

Thirty-six 4 or 8 week-old gnotobiotic dogs were used in this study.
They were obtained by hysterectomy from unconditioned conventional bitches
supplied by a local dealer. The methods described by Griesemer and Gibson (9) for raising gnotobiotic dogs in flexible plastic isolators were employed.

Inoculation of the dogs with 10% cerebellar suspension was as follows: 4 weeks old, intracerebral, 0.1 ml - 4 dogs, and 0.2 ml - 4 dogs; 8 weeks old, intracerebral, 0.2 ml - 2 dogs, intraspinal, 0.2 ml - 4 dogs, intravenous, 0.2 ml - 4 dogs, intradermal (carpal footpad), 0.2 ml - 3 dogs, intranasal, 0.2 ml - 1 dog, and intratracheal, 0.2 ml - 1 dog (16). In addition, 1 eight-week-old dog and 5 four-week-old dogs were infected by contact exposure to their parenterally inoculated littermates. Seven littermate dogs were housed in separate isolators and served as uninfected controls. Two of these uninfected control dogs were inoculated intracerebrally with 0.2 ml of a 10% suspension of uninfected cerebellum obtained from a gnotobiotic dog. All inoculations were made under Innovar sedation (Parke-Davis, Inc., Washington Crossing, New Jersey). Dogs receiving a 0.2 ml intracerebral dose were given 0.1 ml in each frontal lobe.

The dogs were examined daily for neurological signs and rectal temperatures were taken twice daily in the morning and late afternoon. Dogs were killed when they became moribund or after a 12 week observation period. Except for 8 dogs perfused for electron microscopy, all animals were sedated with Innovar and exsanguinated by cardiac puncture preceding necropsy. The brains and spinal cords were removed, fixed in 10% buffered formalin, section at 6 u, stained with hematoxylin and eosin, and examined by light microscopy. In addition, selected sections were stained with Luxol-fast-blue-PAS (15).
The 8 dogs (6 inoculated and 2 controls) selected for electron microscopic study were deeply anesthetized with pentobarbital sodium and perfused with approximately 20 liters of 10% buffered formalin via the left ventricle of the heart. Portions of the left and right anterior and middle cerebellar peduncles and the optic nerves at the chiasma were minced into 1 mm cubes for further fixation in osmium tetroxide. The processed tissues were embedded in Epon 812. One micron thick sections for light microscopic study were stained with toluidine blue. Examination of thin sections stained with uranyl acetate and lead citrate was made with a Phillips 200 electron microscope. The remainder of the brain and the spinal cord of these dogs was processed as described above for light microscopic examination.

RESULTS

Neurological Signs

Signs indicative of nervous dysfunction were observed in 10 of the 29 dogs starting 25 to 50 days after infection. In 8 dogs (2 contact exposed and 6 parenterally inoculated) twitching of muscles, most commonly those of the face, progressed to generalized convulsions within 24 hours. The convulsions became more severe and more frequent and the dogs were moribund within 2-5 days after onset of nervous signs. These dogs would often eat or drink when not convulsing. Rectal temperatures were invariably elevated 2-4 degrees above normal during this phase of the disease. As previously reported, lymphocyte counts in dogs which became moribund were significantly decreased from one week after infection until euthanasia (17).
Two contact exposed dogs developed less severe nervous signs 32 and 55 days post-infection. The first dog seemed to have difficulty remaining awake and its eyelids only opened to a half-open position. This condition lasted for 28 days. The second dog had motor incoordination affecting all 4 limbs, which gradually worsened but did not cause serious disability. These dogs remained eager to eat and drink and fever was not associated with the nervous signs. Both were lymphopenic for 7 weeks after infection (17).

**Gross Lesions**

The arachnoid of the spinal cord and the leptomeninges of the basilar portion of the brain were severely thickened in one dog (Group II). In the remaining dogs no gross lesions of the brain or spinal cord were observed. Macroscopic lesions of the thymuses of some dogs have been reported elsewhere (17).

**Histopathological and Ultrastructural Lesions**

For clarity of presentation of the light and electron microscopic lesions of the central nervous system (CNS) the dogs infected with R252 CDV by various routes (16) are grouped as follows (Table 6): 1. moribund with nervous signs and CNS demyelination, II. survival for total observation period with or without nervous signs but with CNS demyelination, and III. survival for total observation period with no CNS signs or lesions. No lesions were found in uninoculated control dogs or control dogs inoculated intracerebrally with normal brain.

**Group I.** The principal brain lesion in the 8 dogs of this group consisted of multifocal areas of demyelination in the medulla and cere-
bellum. Demyelination occurred most frequently adjacent to meningeal surfaces and about the fourth ventricle, but it was also found occasionally within the deeper white matter of the cerebellum. A striking feature of the brains of these dogs was the absence of lymphocyte and plasma cell infiltration, a usual component of naturally occurring distemper encephalitis (10,12).

Areas of demyelination were characterized by disruption of normal white matter architecture. This was due not only to the breakdown of myelin but also to a marked proliferation of astrocytes (Fig. 16). The reactive nature of the astrocytes participating in this process was indicated by the increased size and vesiculation of their nuclei. Many astrocytic nuclei were swollen and contained eosinophilic viral inclusions (Fig. 17), as did some oligodendroglial nuclei. The extent of demyelination was better demonstrated with Luxol-fast-blue staining, especially in areas with only moderate astrocytosis (Fig. 18).

Spongiosis was inconsistently associated with demyelination and complete loss of tissue was rare. In the more destructive lesions, gitter cells could be identified by periodic acid-Schiff staining. Diffuse necrosis was seldom a part of demyelination, but a few foci of gray matter necrosis were observed. The granular layer was most severely affected (Fig. 19). Gitter cells were occasionally involved in these malacic gray matter lesions.

Ultrastructural evaluation of lesions of demyelination of one dog of Group 1, identified first in thick sections, confirmed the infiltration of macrophages and the presence of small pieces of debris. A few myelin lamellae were disrupted, but the cause was not obvious, i.e., association
between macrophages and myelin breaks could not be made in the area samples. Lymphocytes or plasma cells were not seen. Intracytoplasmic paracrystalline arrays often associated with CDV infection (22,27) were found only in endothelial cells. Viral inclusions were not observed.

**Group II.** In contrast to Group I, inflammatory infiltrates composed of lymphocytes and plasma cells were found in the brains of the 5 dogs of Group II. Diffuse nonsuppurative leptomeningitis was found in the meninges covering the medulla, cerebellum, and optic nerves and tracts of 4 dogs. Perivascular accumulations of lymphocytes and plasma cells and demyelination were also observed in these regions. The non-suppurative encephalitis was most apparent in areas of demyelination, but some non-demyelinated white matter was also infiltrated by inflammatory cells and some areas of demyelination were free of inflammation. In some areas brain tissue was almost totally replaced by inflammatory cells. However, the perivascular pattern of the inflammation was maintained (Fig. 20). Except for the optic involvement, the distribution of demyelination within the brains of Group II dogs was similar to that in Group I dogs with the submeningeal and periventricular regions being preferentially affected. Astrocytic, intranuclear viral inclusions were an inconsistent finding and were less numerous than in dogs of Group I (Fig. 21).

Foci of demyelination and inflammation were found throughout the length of the spinal cord of 1 dog, while nonsuppurative leptomeningitis was the only spinal lesion in 3 animals. The meninges covering the cervical cord were most severely affected in all 4 dogs. Demyelination of the optic tracts and nerves varied in severity from discrete foci to near total destruction with little stainable myelin remaining (Fig. 22).
Spongiosis was prominent in the latter type lesion while nonsuppurative inflammation occurred in both. Additionally, 2 dogs had mild nonsuppurative retinitis. Intraneural viral inclusions were difficult to demonstrate within optic tract or nerve lesions.

The fifth dog in Group II had demyelination and nonsuppurative inflammation limited to the anterior medullary vellum. It was markedly thickened due to the inflammation (Fig. 23). A few astrocytes with intraneural viral inclusions were observed.

Demyelination was observed electron microscopically in all 5 areas of brain sampled from one dog of Group II. As demonstrated light microscopically, lymphocytes and plasma cells were abundant and were present around small blood vessels (Fig. 24). Macrophages were often in close apposition to myelinated axons and sometimes axons devoid of myelin were entirely enclosed by macrophages (Fig. 25). Other axons had their myelin lamellae disrupted by macrophage processes (Fig. 26). Although present in the lesions, lymphocytes and plasma cells did not appear to be involved morphologically in the demyelination of axons. Intracytoplasmic accumulations of CDV nucleoprotein in the form of tubular strands were frequently observed in astrocytes (Fig. 27), but intraneural viral inclusions were rare (Fig. 27). This type of lesion is similar to that referred to by Wisniewski, et al. (27) as acute demyelination.

A few areas were found where several axons were completely demyelinated. Edema greatly increased the amount of interaxonal space in these areas (Fig. 28). Evidence of remyelination was indicated in this chronic type of lesion (27) by the finding of uncompacted myelin lamellae around some axons (Fig. 28 and 29).
Group III and Controls. Microscopic lesions were not found in the brains or spinal cords of dogs in Group III or in uninfected control dogs.

DISCUSSION

The present report represents the first description of multiple cases of demyelination induced by canine distemper virus (CDV) infection of dogs under experimental conditions. Central nervous system (CNS) demyelination was induced in 13 of 29 gnotobiotic dogs infected with R252 CDV when 4 or 8 weeks old. Two forms of demyelinating disease occurred, but these were independent of route of infection (16). Eight dogs (Group I) developed severe neurologic signs within 7 weeks after infection and rapidly became moribund. Multifocal areas of demyelination in the cerebellum and medulla unaccompanied by hematogenous inflammatory cells were found in these dogs. Astrocytosis was often a feature of this type of lesion and viral inclusions in astrocytic nuclei were numerous. In contrast, 5 other dogs (Group II), which had either mild or no neurologic signs, were found to have demyelination along with mononuclear inflammation of the CNS when examined 12 weeks after infection. Intranuclear viral inclusions were an inconsistent finding in areas of demyelination in dogs of Group II. Demyelinated lesions were demonstrated in the cerebellum, medulla, optic nerves and tracts, and spinal cord of dogs of Group II. The distribution of demyelinating lesions in our experimentally infected dogs corresponds to that reported for naturally occurring distemper (10,12).

In most cases of naturally occurring demyelinating distemper hematogenous mononuclear inflammatory cells accumulate in the meninges and perivascular spaces of the CNS (10,12). These infiltrates are especially
dense in areas of demyelination (10, 12). Jubb and Kennedy (12) postulate that this perivascular response is secondary to demyelination and should be considered a late event in the pathogenesis of the lesion. The results of the present study support their statements. Severity of demyelination was directly related to the length of the prepatent period in dogs which became moribund (Group I) and only affected areas of the CNS of dogs which survived the total observation period (Group II) contained obvious hematogenous inflammatory cells.

Wisniewski (26) recently discussed the possibility that demyelination in multiple sclerosis is initially of a non-inflammatory type and that any inflammation is indeed secondary. Our findings suggest that this may also be true for distemper and although the question of whether the inflammatory process precedes or follows demyelination is still not resolved, our ability to induce CNS demyelination experimentally with R252 CDV provides us with the opportunity to answer this question in future investigations.

The electron microscopic appearance of experimental R252 CDV-induced demyelination was similar to the demyelination described by Wisniewski, et al., in the spinal cords of dogs with naturally occurring distemper (27). Applying their criteria (27), both acute and chronic types of demyelinating lesions were demonstrated in our experimental cases, but areas comparable to the destructive lesions were not observed in the dogs selected by us for electron microscopic examination. The presence of CDV at the ultrastructural level in experimental lesions was comparable to the findings of Wisniewski, et al. (27).
Wisniewski, et al. (27) were unable to determine the actual time of infection in their dogs with naturally occurring distemper and were forced to rely solely on the onset of clinical signs as the indicator of disease. The fact that the 5 dogs of Group II of our study had either minimal or no signs of nervous dysfunction up to 12 weeks after infection suggests that the lesions reported by Wisniewski, et al. (26) could have resulted from an even longer disease process than was clinically apparent. The effect of viral dose on such lesions is, however, not known. Bornstein (2) has discussed the frequent lack of correlation between neurological signs and severity of demyelination in patients with multiple sclerosis.

The relationship between demyelination and the severe lymphoid depletion which occurs in response to R252 CDV infection has not been determined (17). Although the effect of CDV infection on immune responsiveness remains to be investigated, two closely related paramyxoviruses, measles and rinderpest, are known to be immunodepressive (3,18,21,23). Establishment of CDV infection in the CNS may be enhanced by lymphoid depletion.

The absence of remissions and relapses during the course of demyelinating diseases of animals has been given as a disadvantage for their use as models for human demyelinating diseases (25). In the present study, one dog exhibited signs of neurologic dysfunction for a period of 4 weeks (post-infection days 32-60) and then remained asymptomatic until it was euthanatized 24 days later. Because of the extreme amount of CNS demyelination and inflammation present in the brain and spinal cord of this dog at the time of euthanasia it is reasonable to assume that the
dog would have ultimately suffered additional nervous impairment. Studies of longer duration will be needed to determine if such dogs do in fact demonstrate exacerbations and remissions, similar to those which occur during the course of multiple sclerosis.

Many aspects of the pathogenesis of demyelinating diseases remain to be explained, but their elucidation will be hastened through the availability of a reliable experimental model system such as the one presented here.
Summary

Thirty-six 4 or 8 week-old gnotobiotic dogs were used to study induction of central nervous system demyelination by R252 canine distemper virus (CDV) infection. Twenty-one dogs were inoculated parenterally, 6 dogs were infected by contact exposure to inoculated littermates, and 2 dogs were infected by direct inoculation of the upper respiratory tract. Seven littermate dogs were maintained as uninfected controls.

Demyelination was induced in 13 of 29 infected dogs. Eight dogs became moribund 27 to 47 days after infection and were euthanatized (Group I). Multifocal areas of demyelination in the cerebellums and medullas of these 8 dogs were not associated with hematogenous inflammation.

In 5 dogs which survived the 12 week observation period (Group II) demyelination of the brain and spinal cord was associated with hematogenous inflammation, principally lymphocytes and plasma cells. Demyelinated axons and axons undergoing remyelination were demonstrated by electron microscopy in samples of cerebellum and optic tract. In addition, viral nucleoprotein was present in the cytoplasm and nuclei of astrocytes.

Lesions of the central nervous system were not found in 16 infected dogs (Group III) or in the 7 uninfected control dogs.
Figure 16. Cerebellum of dog of Group I. Demyelination (right side of field) associated with astrocytosis (center of field). Perivascular lymphocytic infiltration is absent, but some reactive pericytes are present. H & E X 85.
Figure 17. Intranuclear viral inclusions (arrows) in astrocytes of cerebellar folia of dog of Group I. Palor and vacuolation of the white matter are indicative of demyelination. Small dark nuclei (top and bottom of field) are those of granular layer neurons. H & E X 450.
Figure 18. Demyelination and moderate astrocytosis in cerebellum of dog of Group I. Palor (D) is due to loss of myelin, i.e. Luxol-fast-blue staining. Luxol-fast-blue-PAS X 250.
Figure 19. Cerebellum of dog of Group I. Focal gray matter necrosis.

The granular layer is most severely affected. H & E X 290.
Figure 20. Demyelination and nonsuppurative inflammation in the medulla of dog of Group II. Effacement of the medulla has resulted from the intense inflammation below the meningeal surface (arrow). The dark rings of cells are perivascular lymphocytic plasmacytic cuffs. H & E X 190.
Figure 21. Intranuclear viral inclusions (arrows) in astrocytes near perivascular cuff in cerebellum of dog of Group II. H & E X 420.
Figure 22. Demyelination of optic chiasma of dog of Group II. The extensive spongiosis is readily apparent. Perivascular cuffs of mononuclear inflammatory cells are present. Nonsuppurative meningoitis (M). H & E X 48.
Figure 23. Demyelination and nonsuppurative inflammation of anterior medullary vellum of dog of Group II. The molecular layer of vermis of the cerebellum is not affected (top of field). H & E X 170.
Figure 24. Plasma cell (P) near small venule (L) in cerebellum of dog of Group II. Viral nucleoprotein (V) in astrocytes and their processes. X 9000.
Figure 25. Demyelinated axon (A) surrounded by macrophage (M) in cerebellum of dog of Group II. X 29500.
Figure 26. Demyelinated axon (D), macrophage (M), and edema (E) in cerebellum of dog of Group II. X 16875.
Figure 27. Intracytoplasmic (C) and intranuclear (N) viral nucleoprotein astrocyte in cerebellum of dog of Group II. X 12190.
Figure 28. Demyelinating (D) and remyelinating (R) axons and edema (E) in cerebellum of dog of Group II. X 9000.
Figure 29. Remyelination of axon (A) in cerebellum of dog of Group II.

Note that the myelin lamellae are not compacted around the axon. X 31875.
Table 6. Effect of R252-CDV infection on the central nervous system (CNS) of gnotobiotic dogs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Moribund&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Nervous Signs</th>
<th>Demyelination in CNS</th>
<th>Hematogenous Inflammation in CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>27-47</td>
<td>8/8</td>
<td>8/8</td>
<td>0/8</td>
</tr>
<tr>
<td>II</td>
<td>N.A.</td>
<td>2/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>III</td>
<td>N.A.</td>
<td>0/16</td>
<td>0/16</td>
<td>0/16</td>
</tr>
<tr>
<td>Controls</td>
<td>N.A.</td>
<td>0/7</td>
<td>0/7</td>
<td>0/7</td>
</tr>
</tbody>
</table>

<sup>a</sup> Days post infection - Contact exposed dogs considered to be infected 7 days after parenterally inoculated littermates - days adjusted accordingly.

<sup>b</sup> N.A. - not applicable, dogs survived total infection observation period.


CHAPTER II


