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# Biological Properties of a Canine Distemper Virus Isolate Associated with Demyelinating Encephalomyelitis

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A canine distemper virus (CDV), designated R252, originally recovered from a dog with demyelinating encephalomyelitis was shown to reproduce this disease in gnotobiotic dogs with a high incidence as compared to other CDV strains, which produced an acute fatal infection. In this investigation, R252 was propagated for the first time in Vero cells and compared to two known strains of CDV, Snyder-Hill (SH) and Onderstepoort (Ond). The results of this study revealed that intracellular R252 accumulated more slowly than either SH or Ond. There was extensive destruction of Vero monolayers infected with either R252 or SH. Each virus induced the formation of intracytoplasmic and intranuclear inclusions. Ond infection resulted in minimal cytopathic changes and intracytoplasmic inclusions. Immunofluorescence studies indicated that the spread of R252 infection within the monolayers was intermediate between the rapidly spreading SH and slowly spreading Ond. R252-infected cells developed characteristic immunofluorescent cytoplasmic inclusions. Initially, each stained homogeneously and later appeared as a nonfluorescent body surrounded by a fluorescent ring. This characteristic pattern of fluorescence was observed only infrequently in the late stage of SH infection and was absent in Ond-infected cultures. Reciprocal neutralization studies indicated that the three strains are of one serotype. The findings suggest that R252-CDV has biological properties which differ from two other strains of CDV and which may have bearing upon the in vivo capability of this virus to produce demyelinating encephalomyelitis.

Canine distemper virus (CDV) produces a systemic infection in dogs which occasionally results in a subacute demyelinating encephalomyelitis. Several morphological characteristics of the CDV-induced encephalomyelitis are shared with two demyelinating diseases in man: subacute sclerosing panencephalitis (SSPE) and multiple sclerosis (16, 18). SSPE has been shown to be associated with measles virus (MV) infections (25). Both MV and CDV are classified as paramyxoviruses and have similar biochemical and biophysical properties (7). There is increasing evidence that multiple sclerosis may also be associated with a paramyxovirus infection (17, 26). Because of several morphological and etiological similarities among the three diseases, investigators in this laboratory have developed a model for investigating viral-induced demyelinating encephalomyelitis utilizing gnotobiotic dogs inoculated with an isolate of CDV designated R252 (20, 22, 23). The high incidence (45%) of demyelinating encephalomyelitis in the absence of acute respiratory disease has been the most significant effect of this isolate. Other investigators using different CDV strains reported a low incidence (2 to 12%) of demyelinating encephalomyelitis in conventional dogs (10, 13). In a comparative study, infection with only this virus and a specific isolate obtained from the Pasteur Institute of Paris resulted in demyelinating encephalomyelitis in gnotobiotic dogs, whereas infection with two other CDV strains resulted in acute fatal disease (23). It was of primary interest to determine whether the R252 strain had unique biological properties associated with this difference in in vivo behavior.

The objectives of this investigation were to isolate and propagate the R252 strain of CDV in a cell culture system and compare its in vitro biological properties with those of two known CDV strains.

## MATERIALS AND METHODS

**Viruses.** Three strains of CDV were compared in this investigation. The Onderstepoort strain (Ond) was furnished by M. J. G. Appel (New York State Veterinary College, Cornell University, Ithaca, N. Y.).

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This strain was originally isolated and passaged 208 times in chicken embryos and shown nonpathogenic for dogs (14). Prior to adaptation to Vero cells, Ond was passaged in chicken embryo cell cultures, ferret kidney, AV3, and HEp-2 cells (R. H. Bussell, University of Kansas, personal communication). The in vitro behavior of this strain has been studied extensively (4–6). The Snyder-Hill strain (SH) of CDV was obtained as a 10% suspension of infected canine spleen (Philips-Roxane, Inc., St. Joseph, Mo.). The virulence of this strain for dogs has been well documented (3, 13). The CDV strain designated as R252 was isolated in this laboratory by J. S. Shadduck.

**Cell culture.** African green monkey kidney (Vero) cell cultures were used at the 60th through 75th passage levels. Monolayer cultures were grown in Eagle minimal essential medium with Earle balanced salt solution, supplemented with 10% fetal calf serum, 0.5% of an 8.8% sodium bicarbonate solution, and an antibiotic mixture containing 100 U of crystalline penicillin G and 50 $\mu$g of dihydrostreptomycin per ml. For maintenance, fetal calf serum was decreased to 3% and sodium bicarbonate solution was increased to 1.25%. Infected and uninfected monolayers were determined to be free of *Mycoplasma* sp. by cultivation on artificial medium (19).

**Adaptation of CDV strains to Vero cells.** Vero-adapted Ond virus was subpassaged five times in Vero cell cultures before its in vitro properties were determined.

The SH-infected canine splenic suspension was diluted 1:10, and 0.5-ml volumes were inoculated onto Vero cell monolayers grown in 4-ounce (ca. 114 g) glass prescription bottles. The medium was changed twice a week. Cytopathic effects (CPE) were not observed during the initial 18-day incubation, and cells were trypsinized and reseeded at a split ratio of 1:2. CPE reached a maximum 11 days after subculturing. The cells were disrupted by three cycles of freeze-thawing, and the virus was passaged an additional five times in Vero cells.

For isolation and adaptation of R252, primary glial monolayers were prepared from the cerebral cortex of a CDV-R252-infected gnotobiotic dog. The dog had signs of chronic central nervous system disease (22). Methods for propagation of the glial monolayers were described previously (32). Nineteen days after seeding, degenerative changes had occurred. The cultures were trypsinized, and the contents of two bottles were combined and reseeded in 4-ounce bottles. These cultures were maintained for 12 days, during which foci of degenerating cells were observed. The cells where then disrupted by three cycles of freeze-thawing, and the suspension was inoculated onto Vero cell monolayers. After 7 days, CPE was noted. The virus was subpassaged five time in Vero cells.

**Antisera.** Canine-origin hyperimmune anti-CDV (COH) serum was produced in a gnotobiotic dog by repeated subcutaneous inoculations of an attenuated CDV (D-Vac, Bio-Ceutic Laboratories, Inc., St. Joseph, Mo.). Canine-origin anti-R252 serum was collected from two gnotobiotic dogs 72 days after intracerebral inoculation with a 10% cerebellar suspension of R252 (sera S2841 and S4082). Rabbit-origin hyperimmune sera against each of the three CDV strains (Rab-R252, Rab-SH, and Rab-Ond) were produced by three biweekly subcutaneous inoculations of 1 ml of clarified cell culture-origin virus ($10^{4.5}$ mean tissue culture infective doses [$TCID_{50}$] per ml) in an equal volume of Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). Two weeks after the final injection the serum was harvested.

**Infectivity assay.** Vero cells were seeded into 16-mm wells of plastic trays (Linbro Multi-Dish Disposo-Trays FB-24 TC, Linbro Chemical Co., Inc., New Haven, Conn.) at a concentration of 180,000 cells per well. Serial 10-fold dilutions of virus suspension were made, and 0.1-ml quantities of each were delivered to four monolayer cultures. Cultures were incubated at 37 C in 5% $CO_2$, and the medium was changed twice a week. Cultures were examined after 14 days for evidence of CPE. $TCID_{50}$ was calculated by the formula of Spearman-Kärber (12).

**Light microscopy.** Vero cells were grown in Leighton tube cultures and infected with one of the three CDV at a multiplicity of infection of 0.03. Three cover slips per virus were harvested at designated intervals (postinoculation day [PID] 1, 2, 3, 4, 5, 7, 10, and 14), fixed in Bouin solution, stained with hematoxylin and eosin, and examined.

**Growth curves.** Leighton tube cultures of Vero cells were infected with each of the CDV strains at a multiplicity of infection of 0.03. After incubation for 1 h at 37 C, the inoculum was removed, the monolayers were rinsed three times in sterile phosphate-buffered saline, and 1.5 ml of maintenance medium was added. Cultures were incubated at 37 C; at designated intervals (PID 0.5, 1, 2, 3, 4, 5, and 7) three cultures per virus were harvested, and the intracellular and extracellular growth curves were determined (30).

**Immunofluorescent test.** Vero cultures were grown in Leighton tubes and infected with the CDV strains under investigation at multiplicity of infection of 0.03. Three cultures inoculated with each of the viruses were examined by the indirect fluorescent antibody (FA) technique (31) at each of the designated intervals (PID 0.5, 1, 1.5, 2, 3, 4, 5, 7, 10, and 14). Sera employed in these tests were the COH serum and a fluorescein isothiocyanate-conjugated rabbit anti-canine globulin serum (21). Specificity was determined by performing the tests on uninfected monolayer cultures. In infected cultures, substitution of phosphate-buffered saline for COH serum and blocking the the reaction with unconjugated rabbit anti-canine globulin serum were utilized as controls.

The percentage of infected cells was estimated by dividing the number of fluorescent cells by the total number of cells (approximately 200 cells) observed in 10 high-power fields (×430) multiplied by 100.

**Neutralization tests.** Six sera (COH, S4082, S2841, Rab-Ond, Rab-SH, and Rab-R252) were tested for neutralizing capacity against each of the three CDV isolates. Each serum was heat inactivated (56 C, 30 min) and serially diluted in fourfold increments. Each dilution was mixed with an equal volume of test virus suspension containing 100 $TCID_{50}$/0.05 ml. The virus-serum mixtures were incubated at 37 C for 1 h. Volumes (0.1 ml) of each dilution were inoculated on

four monolayer cultures in plastic trays. Cultures were incubated at 37 C in 5% $CO_2$, and medium was changed twice a week. Cultures were examined for CPE after 14 days, and the 50% neutralization end point was calculated by the formula of Spearman-Kärber (12).

**Histochemistry.** Infected cells grown on coverslips were stained with either acridine orange (AO) or May Grunwald-Giemsa (MGG) stains (31) to evaluate the nucleic acid content of viral inclusions.

## RESULTS

**Adaptation of CDV strains to Vero cells.** No change was noted in the CPE produced by Ond during passage in Vero cells. In the adaptation of SH a focal CPE consisting of rounded, refractile cells and giant cells was noted 7 days after the cultures were split. Over the next 4 days the CPE progressed until the entire monolayer was affected. During subsequent passages, the time required for CPE to develop decreased to 3 days. When glial cell lysate containing R252 was inoculated onto Vero cell monolayers similar changes were observed.

**Cytopathology in tissue culture.** The CPE produced by R252 and SH were somewhat similar (Table 1). In R252-infected monolayers, small, dense eosinophilic cytoplasmic inclusion (CI) were seen on PID 2. They occurred in small foci of mononucleate cells and syncytia containing four to eight nuclei. Focal degenerative changes of vacuolated, rounded, or stellate cells were evident on PID 3. Eosinophilic nuclear inclusions (NI) occasionally were seen on PID 7. CPE progressed until on PID 10 the monolayer consisted of a sparse population of stellate cells and syncytia containing less than 10 nuclei. The joining of their elongated cytoplasmic processes gave the monolayer a weblike appearance (Fig. 1). Large CI were visible in most cells. The cell density decreased and the percentage of cells containing both CI and NI increased on PID 14.

In SH-infected monolayers, degenerative changes and cells containing CI similar to those seen with R252 were noted on PID 2. During the next 5 days, syncytia became more numerous, CI became larger, and degenerative changes spread through the monolayer more rapidly than in comparable R252-infected cultures. The typical syncytium contained 20 to 30 nuclei (Fig. 2). Nuclear inclusions were first seen on PID 10, and by PID 14 the monolayers appeared similar to those infected with R252.

In Ond-infected monolayers, numerous syncytia were seen by PID 3; each contained 20 to 50 peripherally located nuclei. Often the central cytoplasm appeared vacuolated and degenerated (Fig. 3). The cell density of the monolayer decreased slightly between PID 4 and 5, and numerous foci of piled-up, degenerate, rounded or stellate cells were observed around acellular areas. On PID 7, CI occasionally were seen in small syncytia and in mononucleate cells. The number of cells containing inclusions increased over the next 7 days; however, cell density and the number of foci of degenerate cells remained constant. NI were not seen during the 14-day observation period.

**Growth curves.** Intracellular virus was detected in R252-infected cultures (Fig. 4) on PID 1; the titer increased gradually through PID 5, then remained constant through PID 7. Extracellular virus (Fig. 5) was detected on PID 2 and

TABLE 1. *Summary of some biological characteristics of canine distemper virus strains*

| Virus | Cytopathology | | | Fluorescent antibody | |
|---|---|---|---|---|---|
| | Destruction | Inclusion | Syncytia | Spread of infection | Pattern of immunofluorescence |
| R252 | Extensive | Cytoplasmic<br>PID 2<br>Nuclear<br>PID 7 | Small (10 or less nuclei)<br>Infrequent | Moderate<br>50% infected<br>PID 5 | Nonfluorescent cytoplasmic bodies<br>Fluorescent nuclear bodies |
| SH | Extensive | Cytoplasmic<br>PID 2<br>Nuclear<br>PID 10 | Large (20 to 30 nuclei)<br>Numerous | Rapid<br>100% infected<br>PID 5 | Diffuse cytoplasmic fluorescence<br>Fluorescent nuclear bodies |
| Ond | Minimal | Cytoplasmic<br>PID 7 | Large (20 to 50 nuclei)<br>Numerous | Slow<br>30% infected<br>PID 5 | Diffuse cytoplasmic fluorescence |

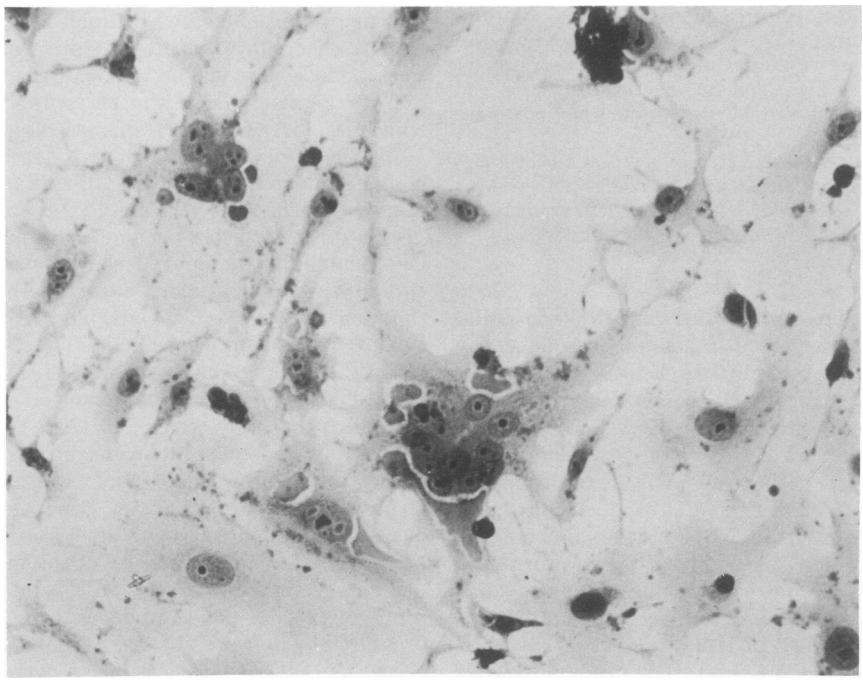

FIG. 1. *Small syncytia containing less than 10 nuclei and stellate cells in Vero cells 10 days after inoculation with R252-CDV. Hematoxylin and eosin stain. ×250.*

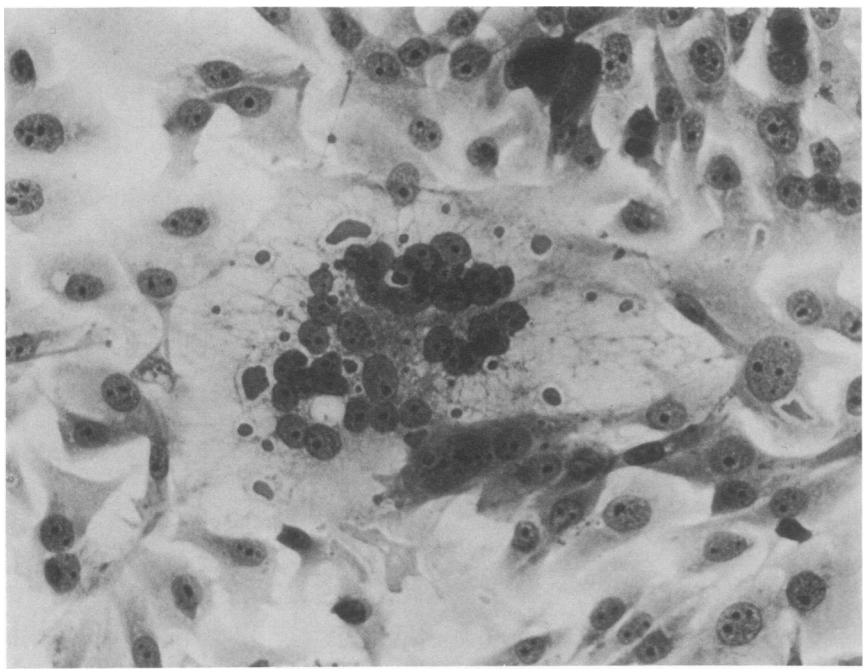

FIG. 2. *Large syncytium (33 nuclei) in Vero cells 3 days after inoculation with SH-CDV. Hematoxylin and eosin stain. ×315.*

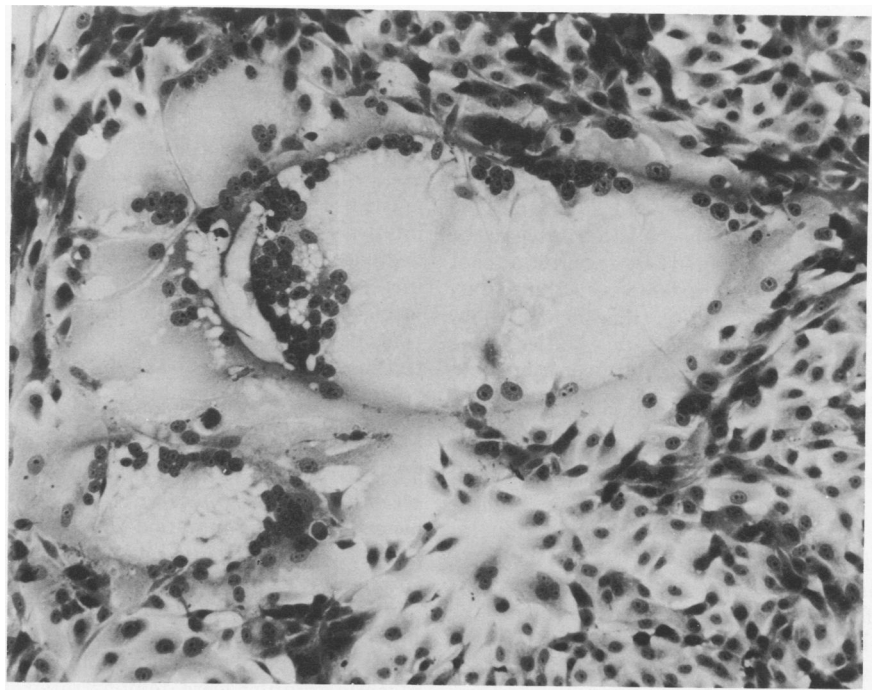

FIG. 3. *Large syncytium containing greater than 50 nuclei in Vero cells 3 days after inoculation with Ond-CDV. Hematoxylin and eosin stain. ×105.*

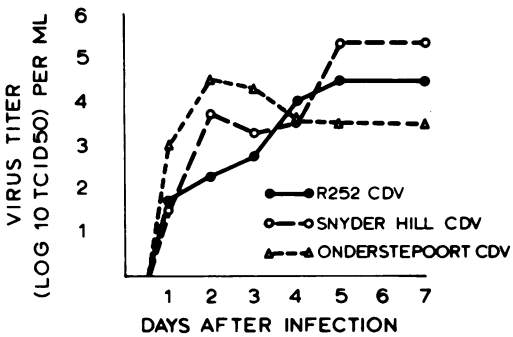


FIG. 4. *Intracellular virus growth curves for three CDV strains in Vero cells.*

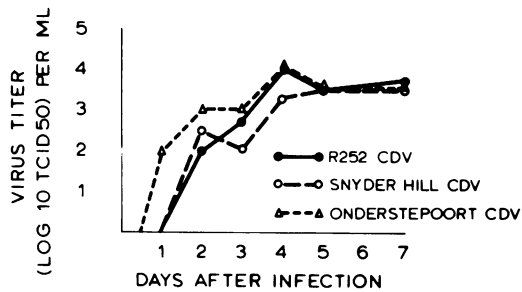


FIG. 5. *Extracellular virus growth curves for three CDV strains in Vero cells.*

increased gradually, reaching a maximal titer on PID 4.

Intracellular virus was detected in SH-infected cultures (Fig. 4) on PID 1. A sharp rise occurred in the titer on PID 2, and the maximal virus titer was measured on PID 5. Extracellular virus (Fig. 5) was detected on PID 2. The extracellular virus curve followed the same pattern as the intracellular curve and reached a maximum on PID 5. High titers of both intracellular and extracellular virus persisted through PID 7.

Both intracellular (Fig. 4) and extracellular virus (Fig. 5) could be detected in Ond-infected cells at PID 1. The maximal titer of intracellular virus was measured on PID 2; the titer declined, then remained constant, from PID 4 through 7. The maximal titer of extracellular virus was achieved on PID 4.

**Immunofluorescence study.** There was an absence of nonspecific fluorescence in the three control systems (Table 1).

Viral antigen was detected in R252-infected Vero cells as fluorescent cytoplasmic granules on PID 0.5 (Fig. 6). On PID 1, 20% of the cells contained viral antigen and exhibited one of three patterns of immunofluorescence: discrete fluorescent cytoplasmic bodies, intensely

fluorescent nuclear membrane and perinuclear area, or the occasional cell with diffuse cytoplasmic fluorescence. On PID 1.5 35% of the cells fluoresced. Occasional small fluorescent granules were noted in the nucleus, and the centers of numerous cytoplasmic bodies stained less intensely, appearing ringlike. The intensity of fluorescence was maximal on PID 2. On PID 5 approximately 50% of the cells fluoresced; the majority of them contained large nonfluorescent round or oval cytoplasmic bodies surrounded by a thin ring of fluorescence (Fig. 7). Very few intensely staining cytoplasmic bodies were seen; however, discrete fluorescent nuclear bodies were present in approximately 50% of the infected cells. By PID 7, 70% of the cells contained viral antigen. The intensity of fluorescence decreased over the next week, and by PID 14 most cells contained large, fluorescent nuclear and unstained cytoplasmic inclusion bodies.

Monolayers infected with SH developed patterns of fluorescence similar to R252 during the first 24 h of infection. As with R252-infected monolayers, a granular nuclear fluorescence appeared on PID 1.5; however, intense diffuse cytoplasmic fluorescence was the predominant pattern; only an occasional cell contained discrete fluorescent cytoplasmic bodies. On PID 3 approximately 45% of the cells contained viral antigen, and the intensity of fluorescence appeared maximal. FA-positive cells usually were localized in foci of 8 to 20 cells, appeared rounded, had diffuse cytoplasmic fluorescence, and often contained small fluorescent nuclear bodies. Nearly 100% of the cells contained viral antigen on PID 5. Between PID 7 and 14 the intensity of cytoplasmic fluorescence markedly decreased, whereas fluorescent nuclear bodies increased in size and appeared to stain more intensely. On PID 10 and 14 large nonfluorescent cytoplasmic bodies were observed, similar to those in R252-infected cultures.

In Ond-infected monlayers, fluorescent granules were found in the cytoplasm of both mononucleate cells and small syncytia on PID 0.5. These granules, which were smaller than those observed in R252- and SH-infected cultures, occurred in approximately 20% of the cells. On PID 1, viral antigen was spread diffusely throughout the cytoplasm of large syncytia. This remained the predominant pattern of immunofluorescence through PID 7. Rarely, mononucleate cells containing fluorescent cytoplasmic granules were demonstrated. On PID 2 fluorescence was of maximal intensity. During the first week of observation the percentage of infected cells increased from 20 to 45%. On PID

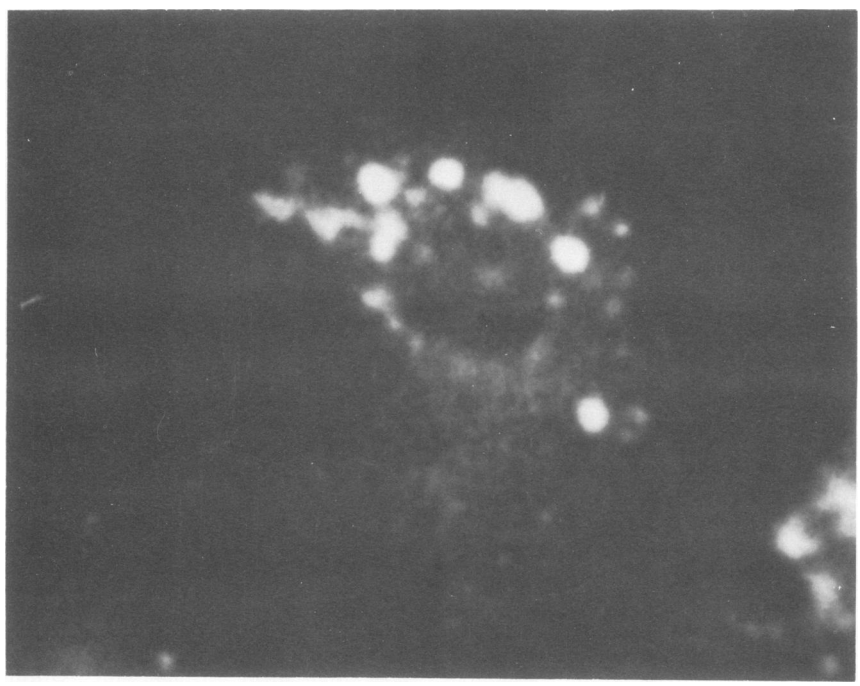

FIG. 6. *Fluorescent cytoplasmic granules in Vero cells 12 h after inoculation with R252-CDV; indirect immunofluorescence.* ×900.

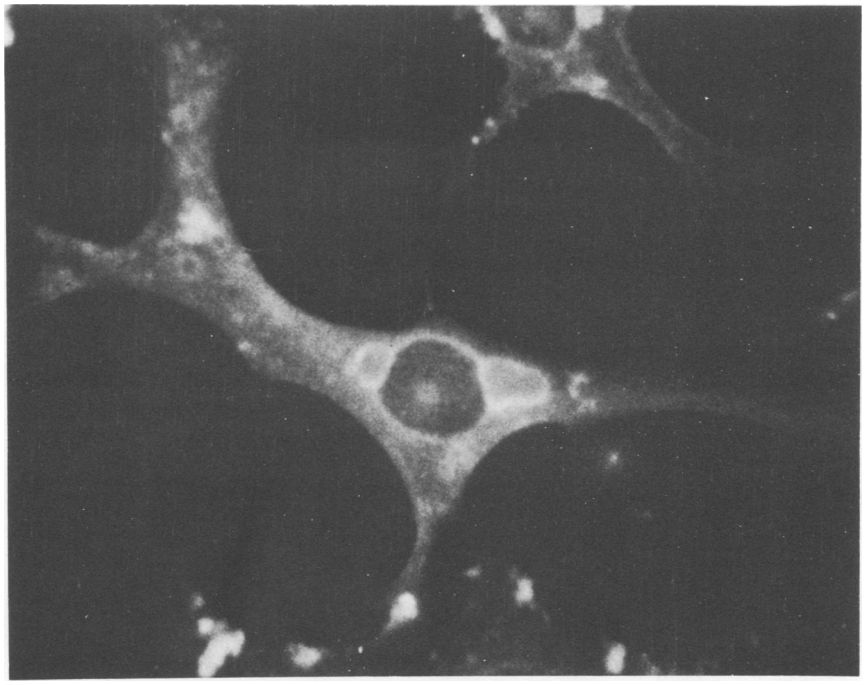

FIG. 7. *Nonfluorescent cytoplasmic body in Vero cells 5 days after inoculation with R252-CDV; indirect immunofluorescence. ×750.*

7 occasional cells were seen which contained small fluorescent nuclear granules. The intensity of staining decreased and the number of antigen-containing cells declined to 20% on PID 10 and 14. At this time two patterns of immunofluorescence were detected in syncytia; the first was diffuse cytoplasmic fluorescence of low intensity whereas the other was one of large fluorescent cytoplasmic bodies. Nuclear fluorescence was not observed. Foci of piled-up degenerative cells did not contain viral antigen.

No difference in the pattern of fluorescence was noted when canine anti-R252 serum (S4082) was substituted for COH as the primary reagent in the FA test.

**Neutralization.** No significant differences were detected when the neutralizing capacity of the six sera were tested against each of the three viruses (Table 2).

**Histochemistry.** Uninfected Vero cell cultures conformed to previously published patterns when stained with either AO or MGG stains (31). Both cytoplasmic and nuclear inclusions in virus-infected cells fluoresced pale green when stained with AO. Early inclusions stained similar to late inclusions. Inclusions stained variably with MGG. Infrequently the inclusions were pale purple in color, but usually they were not stained.

TABLE 2. *Neutralization of canine distemper virus isolates by sera of rabbit and canine origin*

| Virus | Median neutralizing antibody titers[a] | | | | | |
|---|---|---|---|---|---|---|
| | Rabbit antiserum to canine distemper viruses | | | Canine antiserum to canine distemper virus | | |
| | R252 | SH | Ond | Hyperimmune[b] | Convalescent anti-R252 | |
| | | | | | S4082 | S2841 |
| R252 | *3.15* | 2.7 | 0.9 | 3.3 | 2.7 | 1.65 |
| SH | 2.4 | *2.7* | 0.9 | 2.7 | 2.1 | 1.65 |
| Ond | 2.7 | 2.4 | *1.5* | 3.3 | 2.1 | 2.1 |

[a] Expressed as negative $\log_{10}$.

[b] D-Vac (Bio-Ceutic Laboratories, Inc., St. Joseph, Mo.).

## DISCUSSION

CDV-R252 virus, originally recovered from the cerebral cortex of a dog with demyelinating encephalomyelitis (23), was adapted to grow in Vero cell cultures. Various in vitro properties of R252 have been compared with the SH and Ond strains of CDV. Similarities exist: Vero-adapted stocks of all three strains were cytopathogenic,

inducing cellular necrosis, syncytium formation, and inclusion bodies within infected cultures. Reciprocal serum-neutralization tests determined that the three strains belong to a common serotype.

Comparisons of R252 with SH and Ond strains of CDV indicate that it more closely resembles SH in its in vitro properties than Ond. Both R252 and SH caused extensive lytic changes, whereas the highly cell culture-adapted Ond produced minimal destruction. Both R252 and SH strains induced cytoplasmic inclusion bodies by PID 2, followed by intranuclear inclusion body formation. Only cytoplasmic inclusions were detected in Ond-infected Vero cell cultures.

The general pattern of the growth curves for R252 and SH appeared similar to that reported by Shisido et al. (30) for the Vero-adapted Lederle strain of CDV. The growth curve for Ond is similar to that observed by Bussell and Karzon (4) for Ond in chicken embryo cell cultures. Comparison of the growth curves of R252, SH, and Ond indicates that early intracellular virus titers were greater in SH- and Ond-infected cultures than in those infected with R252. However, there was little difference in the infectivity titers of extracellular virus. It was observed by FA that the spread of infection in Vero cell cultures was more rapid with SH than R252.

The cytoplasmic pattern of immunofluorescence characterized by discrete fluorescent cytoplasmic bodies progressing to nonfluorescent bodies circumscribed by a ring of fluorescence seen in R252- and SH-infected cultures has not been described previously. Yamanouchi et al. (34) compared Vero-adapted Lederle strain CDV to MV and Rinderpest virus. Their immunofluorescence studies covered only a 3-day period and may not have continued long enough to observe this phenomenon. The CI observed by light microscopy at PID 2 corresponded to cytoplasmic bodies which were beginning to lose their fluorescence; however, no morphological change occurred in CI over the 14-day observation period that paralleled the observed changes in immunofluorescence. Albeit viral antigenic determinants could not be detected by FA, the nonfluorescent bodies corresponded ultrastructurally to aggregates of filamentous structures morphologically compatible with CDV nucleocapsids (A. W. Confer et al., submitted for publication). Using Shorr stain, Coffin and Liu (9) observed both faint gray-blue- and red-staining CI in transitional epithelium from a CDV-infected dog. Only the faint gray-blue inclusions exhibited fluorescence. It has been shown that inclusion bodies persist longer than immunofluorescence in tissues of CDV-infected dogs (27). Interpretation of these phenomena requires further investigation.

The histochemical determinations are consistent with the findings of others (11, 13) that the large CI produced by CDV do not stain with MGG or AO. The pale-green fluorescence of inclusions was not diminished by treatment with nucleases and was judged to be nonspecific. The purple coloration of occasional inclusion in MGG also was considered to be artifactual. Further investigation into the type and site(s) of viral nucleic acid localization in CDV-infected cells is indicated.

In vitro studies comparing MV strains and SSPE isolates have indicated differences in biological behavior (1, 2, 15, 24). SSPE isolates display decreased reactivity with MV-neutralizing antibody (28). It has been suggested that SSPE strains are antigenic variants which arise in the host under selective pressures. In our investigation, cross-neutralization studies demonstrated no significant differences in the 50% neutralization end point titers of each of six sera when reacted with each CDV strain. In such serological comparisons, an eightfold difference in titer is considered as significant (8). Other serological tests may detect minor antigenic differences. A difference in patterns of immunofluorescence for MV strains and SSPE isolates was demonstrated when anti-SSPE or early anti-measles convalescent serum in the indirect FA test (24). These observations have been interpreted to suggest that some early viral component which normally disappears in measles infection persists in SSPE. No difference in the pattern of immunofluorescence was detected when serum from a dog with R252-induced subacute demyelinating encephalomyelitis (S4082) was substituted for COH anti-CDV serum in the indirect FA test; however, the fluorescent staining pattern of convalescent serum obtained from a dog without demyelination should be examined.

SSPE isolates may represent MV that has been altered antigenically by prolonged residence in the central nervous system, and SSPE sera reflects either altered antigenicity or persistence of early viral components. Additional investigation of immunological and virological parameters of CDV is necessary before it can be determined if similar changes occur in CDV isolates cultured from dogs with chronic central nervous system disease.

R252 has been shown to produce a different disease in gnotobiotic dogs than that produced using SH (23). Whereas prolonged lymphopenia

in the absence of overt disease or subacute demyelinating encephalomyelitis are characteristically produced following intracerebral inoculation of R252, intracerebral inoculation of gnotobiotic dogs with SH results in an acute disease that usually is fatal within 10 days. The rapid progress of this lethal infection suggests that rapid viral proliferation and spread observed in cell culture also is an in vivo characteristic of SH. A more slowly progressive infection was characteristic of R252 infection in Vero cell cultures, a pattern which appears to be analogous to that seen in vivo. Although it may be speculative to draw in vitro and in vivo analogies, we feel R252 has biological properties related to rate of replication which allow it to establish itself in an in vivo or in vitro host as a "persistent" infection without overwhelming the host to cause an acutely fatal disease. In vivo interaction of the host, virus, and immune system probably results in demyelination (20). Because demyelinating disease has been produced experimentally with other CDV strains, the possibility exists that within different populations of CDV various proportions of R252-like viruses normally arise. Where the percentage of such viruses is great, the incidence of demyelinating encephalomyelitis would be great. Reculard and Guillon (29) reported that CDV isolates differ in their pathogenicity for ferrets. Some isolates exhibit prolonged incubation, a more frequent production of NI, and a low neutralization index.

Further investigation is necessary concerning the interaction of CDV and host as well as the biological properties of other CDV isolates. Further in vivo and in vitro investigations utilizing R252 are in progress in this laboratory.

## ACKNOWLEDGMENT

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## LITERATURE CITED

1. Albrecht, P., and H. P. Schumacher. 1972. Markers for measles virus. I. Physical properties. Arch. Gesamte Virusforsch. **36**:23-35.
2. Albrecht, P., and H. P. Schumacher. 1972. Markers for measles virus. II. Tissue culture properties. Arch. Gesamte Virusforsch. **36**:296-310.
3. Appel, M. 1969. Pathogenesis of canine distemper. Am. J. Vet. Res. **30**:1167-1182.
4. Bussell, R. H., and D. T. Karzon. 1962. Canine distemper virus in chick embryo cell culture. Virology **18**:589-600.
5. Bussell, R. H., and D. T. Karzon. 1965. Canine distemper virus in ferret, dog, and bovine kidney cell cultures. Arch. Gesamte Virusforsch. **17**:163-182.
6. Bussell, B. H., and D. T. Karzon. 1965. Canine distemper virus in primary and continuous cell lines of human and monkey origin. Arch. Gesamte Virusforsch. **17**:183-202.
7. Bussell, R. H., and D. T. Karzon. 1968. Measles-canine distemper-rinderpest group, p. 313-336. *In* J. E. Prier (ed.), Basic medical virology. Williams and Wilkins Co., Baltimore.
8. Casals, J. 1967. Immunological techniques for animal viruses, p. 113-198. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, Vol. III. Academic Press Inc., New York.
9. Coffin, D. L., and C. Liu. 1957. Studies on canine distemper infection by means of fluorescein-labeled antibody. II. The pathology and diagnosis of the naturally occurring disease in dogs and the antigenic nature of the inclusion body. Virology **3**:132-145.
10. Cornwell, H. J. C., R. S. F. Campbell, J. T. Vantis, and W. Penny. 1965. Studies in experimental canine distemper I; clinico-pathological findings. J. Comp. Pathol. **75**:19-34.
11. Cornwell, H. J. C., H. M. Laird, N. G. Wright, and H. Thompson. 1971. Ultrastructural features of canine distemper virus infection in a dog kidney cell line. J. Gen. Virol. **12**:281-292.
12. Finney. D. J. 1952. Statistical methods in biological assay. Hafner Publishing Co., New York.
13. Gillespie, J. H., and C. G. Rickard. 1956. Encephalitis in dogs produced by distemper virus. Am. J. Vet. Res. **17**:103-108.
14. Haig, D. A. 1948. Preliminary note on the cultivation of Green's distemperoid virus in fertile hen eggs. Onderstepoort J. Vet. Sci. Anim. Ind. **23**:149-155.
15. Hamilton, R., L. Barbosa, and M. Dubois. 1973. Subacute sclerosing panencephalitis measles virus: study of biological markers. J. Virol. **12**:632-642.
16. Innes, J. R. M. 1968. Subacute diffuse sclerosing encephalitis in the dog. Neurology **18**:161.
17. Iwasaki, Y., H. Koprowski, D. Müller, V. ter Meulen, and Y. M. Käckell. Morphogenesis and structure of a virus from brain tissue from two cases of multiple sclerosis. Lab. Invest. **28**:494-500.
18. Johnson, R. T., and L. P. Weiner. 1972. The role of viral infections in demyelinating diseases, p. 245-262. *In* F. Wolfgram, G. W. Ellison, J. G. Stevens, and J. M. Andrews (ed.), Multiple sclerosis. Immunology, virology, and ultrastructure. Academic Press Inc., New York.
19. Kenny, G. E., and M. E. Pollock. 1963. Mammalian cells contaminated with pleuropneumonia-like organisms. J. Infect. Dis. **112**:7-16.
20. Koestner, A., B. McCullough, G. S. Krakowka, J. F. Long, and R. G. Olsen. 1974. Canine distemper. A virus-induced demyelinating encephalomyelitis, p. 86-101. *In* W. Zeman, E. H. Lennette, and J. G. Brunson (ed.), Slow virus diseases. Williams and Wilkins Co., Baltimore.
21. Krakowka, S., B. McCullough, A. Koestner, and R. G. Olsen. 1973. Myelin-specific autoantibodies associated with central nervous system demyelination in canine distemper virus infection. Infect. Immun. **8**:819-827.
22. McCullough, B., S. Krakowka, and A. Koestner, 1974. Experimental canine distemper virus-induced demyelination. Lab. Invest. **31**:216-222.
23. McCullough, B., S. Krakowka, A. Koestner, and J. Shadduck. 1974. Demyelinating activity of canine distemper virus isolates in gnotobiotic dogs. J. Infect. Dis. **130**:343-350.
24. Meulen, V. ter, M. Katz, Y. M. Käckell, G. Barbanti-Brodano, H. Koprowski, and E. H. Lennette. 1972. Subacute sclerosing panencephalitis. In vitro characterization of viruses isolated from brain cells in culture. J. Infect. Dis. **126**:11-17.
25. Meulen, V. ter, M. Katz, and D. Müller. 1972. Subacute sclerosing panencephalitis. A review. Curr. Top. Microbiol. Immunol. **57**:1-38.
26. Meulen, V. ter, H. Koprowski, Y. Iwasaki, M. Kächell,

and D. Müller. 1972. Fusion of cultured multiple sclerosis brain cells with indicator cells. The presence of nucleocapsids and virions and isolation of parainfluenza type virus. Lancet **2**:1-5.

27. Mickwitz, C. U. V., and H. D. Schröder. 1968. Histologische, fluoreszenz-serologische, und histochische untersuchungen zum verhatten der einschlusskörperchën bei der staupe des hundes. Zentralbl. Veterinaermed. Reihe B **15**:453.
28. Payne, F., and J. V. Baublis. 1973. Decreased reactivity of SSPE strains of measles virus with antibody. J. Infect. Dis. **127**:505-511.
29. Reculard, P., and J. C. Guillon. 1972. Etude experimentale de quelques souches du virus de la maladie de Carré du chien. Identification et définition des souches varientes. Ann. Inst. Pasteur **123**:477-487.
30. Shishido, A., K. Yamanouchi, M. Hikita, T. Sato, A. Fukuda, and F. Kobune. 1967. Development of a cell culture system susceptible to measles, canine distemper and rinderpest viruses. Arch. Gesamte Virusforsch. **22**:364-380.
31. Spendlove, R. S. 1967. Microscopic techniques, p. 475-520. *In* K. Maramorosch and H. Koprowski (ed.), Methods in virology, Vol. III. Academic Press Inc., New York.
32. Storts, R. W., A. Koestner, and R. A. Dennis. 1968. The effects of canine distemper virus on explant tissue cultures of canine cerebellum. Acta Neuropathol. **11**:1-14.
33. Toyoshima, K., S. Hata, M. Takahashi, T. Miki, and Y. Okuna. 1960. Virological studies in measles virus. III. Morphological changes and virus growth in FL cultures. Biken J. **3**:241-248.
34. Yamanouchi, K., F. Kobune, A. Fukuda, M. Hayami, and A. Shishido. 1970. Comparative immunofluorescent studies on measles, canine distemper, and rinderpest viruses. Arch. Gesamte Virusforsch. **29**:90-100.