Persistent Newcastle Disease Virus Infection in Embryonic Chicken Tracheal Organ Cultures

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The persistent infection of embryonic chicken tracheal organ cultures with Newcastle disease virus (NDV) is described. Tracheal explants remained morphologically intact and were able to support the replication of NDV for 6 months. Peak titers of released virus occurred at 1 week postinfection, whereas maximal immunofluorescence was not observed until 30 days postinfection. The inoculum titer was not critical, and viral persistence resulted with either of two strains of NDV tested. Serum was not required in the medium for explant viability or to maintain the persistent infection. The presence of a contaminating virus morphologically resembling a leukovirus neither altered the course of infection nor affected the survivability of explants. Although interferon was not detected in the culture medium, persistently infected explants were resistant to heterologous viral challenge, and a similar resistant state could be induced in uninfected explants with exogenous interferon or ultraviolet light-inactivated NDV. No evidence was found to implicate antibody as a regulatory factor in the establishment or maintenance of persistence. The results from electron microscopy and immunofluorescence suggest the cells of the subepithelial connective tissue as the site of NDV persistence.

Tracheal organ cultures support the growth of a number of viruses and have been useful for viral studies in differentiated tissue (4, 8, 12, 17, 24). Persistent paramyxovirus infections in tracheal explants have been described, and these persistent infections have been accompanied by the local formation and secretion of either antibody (13, 14) or interferon (17, 20). Since viral persistence in certain cell cultures appears to depend upon the presence of either interferon or antibody in the medium (23), the presence of these two substances in persistently infected tracheal organ cultures raises the question of whether such substances are involved in the establishment and maintenance of the persistent infections.

In this report an organ culture system of embryonic chicken trachea persistently infected with Newcastle disease virus (NDV) is described. The course of infection was followed by viral assays, immunofluorescence, and electron microscopy. The effects of interferon pretreatment, titer of original inoculum, strain of NDV, and serum requirement on the establishment of viral persistence were studied. The roles of antibody and interferon as factors contributing to the maintenance of persistence were examined.

MATERIALS AND METHODS

Tracheal organ cultures. Fertile eggs from white Leghorn chickens, DeKalb strain 161 (Pan American Hatchery, Hammond, La.), or specific pathogen-free, COFAL-negative eggs from Leghorns of the Mt. Hope strain (Spafas, Inc., Norwich, Conn.) were incubated for 18 to 19 days at 37 C, and tracheal organ cultures were prepared according to the procedure described by Cherry and Taylor-Robinson (9). After excision and sectioning, each explant was placed at the bottom of a screw-cap culture tube (16 by 125 mm) in 1 ml of medium and incubated in a stationary vertical position for 24 to 48 h at 37 C before infection.

Ciliary activity was observed with a Leitz bottom-view microscope, with 60× or 100× total magnification. Uninfected explants continued to show actively beating cilia for at least a month in vitro, with an occasional explant retaining activity for as long as 2 months. In infected explants, complete ciliostasis routinely occurred by the 4th or 5th day postinfection. Any given explant was considered viable if infectious virus was present in the culture media, since electron microscopy routinely confirmed the presence of susceptible, viable cells within such explants. Among explants maintained in medium containing
serum, 98% of the uninfected explants and 90% of NDV<sub>PI</sub>-infected explants remained viable throughout the 6-month observation period. NDV<sub>PI</sub> is a small plaque mutant of the Herts strain of NDV (NDV<sub>H</sub>). Among explants maintained in serum-free medium, the viability of uninfected explants was similar, whereas the viability of infected explants ranged from 30 to 75%, depending upon the particular batch observed. Some seasonal effect was suggested in that the lower percentage was among those explants put into culture during the months of November and December of 2 successive years.

**Infection of organ cultures.** After preliminary incubation, the culture fluid was removed from the explants and replaced with 1 ml of medium containing a dilution of virus. After a 1-h adsorption at 37 C on a rotary shaker (50 to 60 rpm), the virus suspension was removed and fresh medium was added. The tubes were incubated at an incline of approximately 5° on a roller drum (0.20 rpm) at 37 C. The medium was changed every 4 to 8 days, and the culture fluids from like groups (minimum of five explants per group) were pooled and stored at 4 C until assayed for infectivity.

**Medium.** Eagle minimal essential medium (Grand Island Biological Co., Grand Island, N.Y.) containing 0.05 M N-2-hydroxyethylpiperazine-N'-2'-ethanesulfonic acid buffer (Calbiochem, San Diego, Calif.), 0.035% NaHCO<sub>3</sub>, penicillin (100 U/ml), and streptomycin (100 µg/ml) was used routinely for maintaining cultures and diluting inocula. The pH was adjusted to 7.1 with 2 N NaOH and, when called for, 4% calf or fetal calf serum was added after sterilization.

**Viruses.** A stock pool of NDV<sub>1</sub> was prepared in the allantoic cavities of 10- to 11-day-old embryonated eggs. The supernatant culture fluid from persistently infected L-cell cultures served as the stock pool of NDV<sub>1</sub> (22). Pools of NDV<sub>1</sub> and NDV<sub>PI</sub> for infection of explants were prepared by infecting chicken embryo monolayer (CEM) cultures with a 10<sup>-2</sup> dilution of the stock pool of NDV<sub>1</sub> or undiluted NDV<sub>PI</sub> stock pool. Supernatant culture fluids were harvested when cytopathic effect was maximal (24 to 48 h for NDV<sub>1</sub>, 72 to 96 h for NDV<sub>PI</sub>) by centrifugation at 12,000 × g for 20 min, and stored at 4 C. Pools of the Indiana strain of vesicular stomatitis virus (VSV) were prepared in L-cell monolayer cultures, and Sindbis virus pools were prepared in CEM cultures. Infectivity titers were determined by plaque assay in CEM cultures. NDV<sub>1</sub>, VSV, and Sindbis virus plaques were counted 48 and 72 h postinoculation; NDV<sub>PI</sub> plaques were counted after 72 and 96 h.

**Interferon.** Chicken interferon was prepared in 9-day-old embryonated eggs infected with the WS strain of influenza virus as previously described (25). Residual virus infectivity was inactivated by heating the harvested allantoic fluids at 56 C for 1 h. Supernatant culture fluids from infected and control explants were assayed for interferon at dilutions of 1:2 and higher by either plaque reduction (25) or single-cycle yield inhibition in CEM cultures, using VSV as the challenge virus. Before interferon testing, residual NDV<sub>1</sub> in the supernatant culture fluids was inactivated by dialysis at 4 C against a glycine buffer, pH 2.0, for 4 to 6 days, followed by dialysis against phosphate-buffered saline (PBS, pH 7.2; 0.01 M phosphate, 0.15 M saline) for 24 to 48 h. Control cultures were similarly treated. Single-cycle yield inhibition was determined as follows. Twofold serial dilutions of the culture fluid under test were incubated with CEM cultures (3 ml/culture) for 18 h at 37 C. After incubation, the cells were washed twice with medium and challenged with VSV at an input multiplicity of infection of 1. Following a 1-h adsorption, the monolayers were washed twice more, covered with 3 ml of fresh medium, and reincubated at 37 C. Fluids from the challenged cultures were harvested after 7 h and the VSV was assayed in CEM cultures. Controls consisted of CEM cultures incubated with medium alone or culture fluids from uninfected explants.

**Immunofluorescence antiserum.** Fluorescein-labeled horse anti-chicken globulin (Progressive Laboratories, Baltimore, Md.), at a 1:20 dilution in F.A. buffer (Difco Laboratories, Detroit, Mich.), pH 7.2, was used to detect immunoglobulin-containing cells in frozen tracheal sections. At this dilution the antiserum could detect immunoglobulin-containing cells in frozen sections of trachea or spleen from a 27-day-old chicken; the fluorescence could be blocked by pretreatment with goat anti-chicken globulin.

Anti-NDV serum was prepared by wing web stab immunization of young adult, specific pathogen-free chickens (Spafas, Inc., Norwich, Conn.) with the Roakin strain of live NDV vaccine (Salsbury Laboratories, Charles City, Iowa). The globulin portion was labeled (Sylvania Corp., Milltown, N.J.) with fluorescein isothiocyanate and used at a 1:15 dilution in F.A. buffer for direct staining of NDV in frozen sections. The conjugate stained NDV-infected tissues; such staining could be blocked by pretreatment with unlabeled anti-NDV serum but did not stain uninfected tissues or tissues infected with other viruses. Staining occurred in NDV-infected sections treated with post-immune serum and stained with conjugated horse anti-chicken globulin, but not in sections treated with the pre-immune serum.

**Immunofluorescence staining.** Explants were harvested at various times after infection, rinsed in PBS, submerged in Tissue Tek OCT Compound (Ames Co., Elkhart, Ind) in small aluminum foil cups, and quickly frozen in a dry ice-isopentane bath. Serial 4-µm sections were cut on a cryostat maintained at −25 C, air-dried on albumin-glycerine-coated slides, fixed in acetone for 10 min, and stained for 20 min at room temperature. They were rinsed twice for 10 min each in F.A. buffer, mounted in buffered glycerine (pH 7.7), and examined on a Leitz fluorescence microscope with a BG 12 exciter filter and a blue absorbing barrier filter, with an Osram HBO 200-W mercury vapor bulb as the light source.

**Electron microscopy.** Whole tracheal explants, rinsed briefly in PBS, were fixed in cold 3% glutaraldehyde with cacodylate buffer, postfixed with 1% osmium tetroxide in phosphate buffer, dehydrated, treated with propylene oxide, and embedded in Maraglas epoxy resin (21). Thin sections were cut on a Porter-Blum MT 1 ultramicrotome with a diamond knife, placed on uncoated copper mesh grids, stained
with lead citrate alone or in combination with uranyl acetate, and examined in a Philips EM 300 electron microscope.

**Ultraviolet inactivation.** NDV₀, harvested from infected allantoic fluids, was diluted 1:10 in PBS and passed through a membrane filter (0.2 µm pore diameter, Millipore Corp.). Samples (5.0 ml) were placed in 120-mm petri dishes and rotated for 3 min at 10 cm from a Sylvania Germicidal G15T8 ultraviolet (UV) lamp, changing to a fresh petri dish after each 1-min interval. The preparation was tested for infectivity in CEM cultures. Infectivity titers of NDV₀ filtrates before inactivation were 10⁵ and 10⁴ plaque-forming units (PFU)/ml.

**Poly(IC).** Polyriboinosinic acid [poly(I)] and polyribocytidylic acid [poly(C)] (Miles Laboratories, Inc., Elkhart, Ind.) were prepared by dissolving in PBS to give a concentration of 1 mg/ml of each. Equal amounts of the poly(I) and poly(C) solutions were mixed and annealed for 10 min at 37 °C, cooled to room temperature, and diluted to 50 µg/ml in PBS containing 100 µg diethylaminoethyl-dextran/ml (Pharmacia, Uppsala, Sweden).

**Radial immunodiffusion.** Culture fluids (serum free) were harvested from groups of infected and uninfected explants at intervals from 24 h to 23 days after excision and concentrated 100- to 200-fold by vacuum dialysis. Such concentrations were tested for the presence of immunoglobulin (IgY), IgM, and IgA by radial immunodiffusion on 1% agarose (Marine Colloids, Inc., Rockland, Maine) plates with heavy-chain-specific goat anti-chicken IgY and IgM, or rabbit anti-IgA, as the developing antiseras. Details of the antiserum preparation and use in immunodiffusion have been previously described (15). Precipitin rings were measured to the nearest 0.1 mm with a Bausch and Lomb measuring magnifier and dark-field lighting. Immunoglobulin concentrations in the culture medium were determined by comparison of precipitin ring diameters with those of a standard curve prepared from twofold serial dilutions of pooled whole chicken sera with previously determined immunoglobulin concentrations.

**RESULTS**

**Establishment of persistent infection.** After infection with both NDV₀ and NDVₚᵢ, infectious virus was continuously recovered in the culture medium until the experiments were terminated, usually 5 or 6 months later. As shown in Fig. 1, which is typical of eight similar experiments, NDVₚᵢ titers rose during the first week, then declined for 2 weeks until they somewhat stabilized, usually within the range of 10⁴ to 10⁵ PFU/ml. Serum was not required in the medium either to maintain the persistent infection or to preserve explant viability, although viral titers were approximately 1 log lower when serum was omitted (Fig. 1).

At 1 week postinfection, higher titers of released virus occurred in those explants receiving a higher initial dosage, but thereafter no effect due to inoculum titer was seen (Fig. 1). Pretreatment of explants with 6 or 60 U of chicken interferon for 18 h before infection had no effect on the course of infection. Viral titers resulting from infection with NDV₀ were similar to those shown in Fig. 1 for NDVₚᵢ.

A contaminating virus, morphologically resembling a leukovirus, was present in certain of the explants derived from Pan American eggs. The leukovirus agent was about 100 nm in diameter and was readily distinguishable from NDV. The agent was usually found in groups and was located in and about all of the cell types in the tracheas including the chondrocytes. When certain of the experiments were repeated in leukosis-free explants, no differences in explant longevity or in the course of persistent NDV infection could be detected.

Three to four weeks after the initiation of infection, a change occurred in plaque morphology of the virus recovered from explants infected with NDVₚᵢ, but not with NDV₀. The newly emerging plaques, at first present along with typical NDVₚᵢ plaques, were later in developing in CEM cultures, had diffuse margins, and were difficult to see by reflected light. When this agent was isolated from a persistently infected explant 139 days postinfection, plaque-purified in CEM cultures, and used to initiate a persistent infection in tracheal explants, the pattern of released viral titers was indistinguishable from that seen with NDVₚᵢ- or NDV₀-initiated infections.

No evidence was found to implicate antibody as a necessary factor in the establishment or maintenance of the persistent infection. Mater-
nal antibody was not required to establish persistence, and the course of infection was similar in explants derived from immunized or nonimmunized flocks. Immunoglobulin-containing cells were not detected by direct immunofluorescence in frozen sections from either infected or control explants harvested at intervals from the time of excision through 93 days in culture. IgM and IgA were not detected in the culture medium from either infected or control explants. However, immunoglobulins of the IgY class were present in concentrations ranging from 0.08 to 0.16 μg/ml in the initially harvested culture medium, either 24 or 48 h after excision, and thus only before infection. Once the medium had been renewed IgY was no longer detected.

Interferon activity was not detected in the culture medium of persistently infected explants at any time throughout the course of infection or in culture medium harvested from uninfected explants 18 h after treatment with either of two interferon inducers, poly(IC) (50 μg/explant) or UV-inactivated NDV (1 ml/explant).

However, persistently infected explants were resistant to challenge with either of two interferon-sensitive viruses, VSV or Sindbis virus. The resistance to VSV developed gradually after infection with NDV, and continued to increase for the 14 days of the experiment (Fig. 2). A transient resistance to VSV which could be induced in uninfected tracheal explants by a 1-h treatment with UV-inactivated NDV (1 ml/explant) was demonstrated when the explants were challenged immediately after treatment but not when the explants were returned to fresh medium and challenged 3 or 7 days later (Table 1). A viral-resistant state could also be induced in uninfected tracheal explants by exogenous interferon. An 18-h pretreatment with chicken interferon (60 U/ml) resulted in a VSV yield reduction of 98% (Table 2). In explants pretreated and maintained in the presence of interferon after challenge, VSV replication was inhibited 100%.

**Immunofluorescence.** Direct immunofluorescent staining of viral antigen in frozen sections from tracheal explants was first detected at 48 h postinfection, when one to three small foci per tracheal ring were seen in the extracartilaginous connective tissue. A minor amount of fine granular staining was occasionally observed at the luminal surface as early as

<table>
<thead>
<tr>
<th>Days after treatment</th>
<th>Log$_{10}$ drop from controls</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.05</td>
</tr>
<tr>
<td>3</td>
<td>1.09</td>
</tr>
<tr>
<td>7</td>
<td>-0.07</td>
</tr>
<tr>
<td>14</td>
<td>-0.02</td>
</tr>
<tr>
<td>21</td>
<td>0.09</td>
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</tbody>
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$^a$ VSV challenge (1.4 x 10$^8$ PFU/explant) was harvested 20 h after inoculation.

**Table 2. Replication of VSV in tracheal explants treated with interferon**

<table>
<thead>
<tr>
<th>Culture</th>
<th>Cell culture assayed in</th>
<th>Yield of VSV (PFU/ml)</th>
<th>Yield reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>CEM</td>
<td>1.8 x 10$^3$</td>
<td>97.6</td>
</tr>
<tr>
<td>Pretreated with interferon$^a$</td>
<td>CEM</td>
<td>4.3 x 10$^3$</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>L cells</td>
<td>3.9 x 10$^3$</td>
<td>100</td>
</tr>
<tr>
<td>Maintained in interferon$^b$</td>
<td>L cells</td>
<td>2.8</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Groups of uninfected explants were incubated for 18 h with chicken interferon (60 U/ml), washed in PBS, and challenged with VSV (2 x 10$^4$ PFU/explant). After a 1-h adsorption at 37°C, the challenge virus was removed and the cultures were washed in PBS and returned to fresh medium. The VSV was harvested 48 h after inoculation and assayed for infectivity in CEM cultures. The VSV yield from explants treated with medium alone served as the control.

$^b$ These cultures were pretreated with interferon and challenged with VSV as described above. However, after challenge with VSV they were returned to fresh medium containing chicken interferon (60 U/ml). The VSV was harvested 48 h after inoculation and assayed for infectivity in L cells to eliminate the effect of chicken interferon.

Fig. 2. Development of resistance to VSV in persistently infected chicken embryo tracheal organ cultures. Tracheal explants were infected with NDV$_{pi}$ (5 x 10$^4$ PFU/explant) and challenged at intervals after infection with VSV (2 x 10$^5$ PFU/explant). The VSV was harvested 48 h after inoculation and assayed for infectivity in CEM cultures. Controls consisted of the VSV yield from uninfected tracheal explants.
48 to 72 h after infection, but marked staining of the luminal surface was not seen until 20 days postinfection (Fig. 3). When immunofluorescence observations were correlated with the findings from light and electron microscopy, it was our impression that staining of the mucosal epithelium was minimal, and the luminal surface staining seen later in the course of infection may have been located in the superficial connective tissue cells of the lamina propria rather than in the surface epithelium.

With progression of time, staining of both the lamina propria and extracartilaginous connective tissue increased, and maximal fluorescence was seen by day 30. Little change occurred in the staining pattern thereafter. This late peak in immunofluorescence contrasts with the 7-day peak in titers of released virus. Muscle and cartilage cells did not stain at any time, although they were autofluorescent.

**Electron microscopy.** Viral replication in explants infected with NDV<sub>pi</sub> was initially detected by electron microscopy at 7 days postinfection. Replication was seen in macrophages and fibroblast-like cells located in the connective tissue region just exterior to the tracheal cartilage. Round and filamentous budding forms, characterized by the presence of a triple-layered membrane and by the presence of nucleocapsid beneath the membrane, were found at the plasma membrane and in intracytoplasmic locations in infected cells (Fig. 4 and 5). The dimensions of the rounded and filamentous buds and of the extracellular virions were variable. In general, the virion diameter was 120 to 170 nm for the rounded forms and 150 to 170 nm for the filamentous forms, whereas the diameter of viral nucleocapsid was 11 to 15 nm. By 14 days postinfection, budding NDV and mature virions could also be found in and adjacent to many macrophages and fibroblast-like cells, which together with a rare lymphocyte comprised the cell population of the lamina propria of the embryonic explants. Morphological evidence of NDV replication was confined to the fibroblast-like cells and the macrophages in both the lamina propria and extracartilaginous connective tissue through the 139 days that infected explants were examined.

No evidence of viral replication was detected in the epithelial cells, in the chondrocytes of the hyaline cartilage, nor in the scattered skeletal muscle elements which were present at the extreme outer edge of the explants. It should be noted that the presence or absence of serum in the medium of these explants had no effect on the ultrastructural aspects of NDV replication.

![Figure 3](http://iai.asm.org) Immuno-fluorescent staining of NDV<sub>pi</sub> in frozen section from a tracheal explant 20 days after infection. ×100.

![Figure 4](http://iai.asm.org) Connective tissue cell from explant at 7 days after infection with NDV<sub>pi</sub>. Note budding virions in intracytoplasmic locations and at plasma membrane. ×29,000. Insert, extracellular NDV. ×39,000.
Rather, the principal effect of the serum supplementation was one of prolongation of viability of the infected explants.

During the course of examining the explants infected with NDV, numerous fibroblast-like cells (occasionally infected) and several epithelial cells were noted to contain an intranuclear structure. The structure was composed of compact parallel arrays of fibrils (Fig. 6) of varying lengths which measured 8 to 10 nm in diameter. A similar structure, composed of fewer 7- to 9-nm fibrils arranged in a rather loose array, was rarely encountered in control explants where they were found only in fibroblast-like cells.

In general, the course of infection in tracheal explants infected with NDV was paralleled that seen with NDV\textsubscript{pi}. The following differences, however, were uniformly present. Viral replication could be found in connective tissue cells in both the extracartilaginous connective tissue and the lamina propria at 7 days postinfection. Similar to the findings with NDV\textsubscript{pi}, however, replication was detected only in fibroblast-like cells and macrophages of these regions during the 101 days that infected explants were examined. Infection with NDV\textsubscript{e} was, in general, more devastating to the individual explant than was NDV\textsubscript{pi}. This was primarily evidenced by an increase in cellular debris and disrupted cells in which nucleoprotein-like material was often apparent. Similarly, there was a concomitant decrease in viable macrophages and fibroblast-like cells of the NDV\textsubscript{e}-infected explants as seen with NDV\textsubscript{pi}.
compared with those infected for similar periods with NDV$_{pi}$.

**DISCUSSION**

Chicken embryo tracheal organ cultures are potentially valuable as an in vitro system for the study of persistent NDV infection, and perhaps other respiratory viruses as well. Viral persistence was the normal outcome of infection with NDV and required no special manipulations of the system. Unlike monolayer cultures, tracheal explants are structurally similar to the natural in vivo host target organ, and remain intact while supporting the replication of NDV for longer than 6 months. Blaskovic et al. have reported a spontaneous degeneration of similarly prepared embryonic chick tracheal organ cultures after 15 to 20 days of in vitro maintenance and tentatively ascribed the degeneration to the presence of a passenger virus (5). Although certain of our cultures contained a morphologically similar agent, degeneration did not occur in those explants during the 6-month observation period.

No evidence was found in this study to implicate antibody as a regulatory factor in the establishment or maintenance of the persistent infection. There was no requirement for antibody in the medium or for maternally derived antibody. The IgY which was present after excision was most likely maternally derived immunoglobulin. This conclusion is supported by the absence of immunoglobulin-containing cells in the explants and the presence of only a rare lymphocyte. These latter findings may reflect an immunological incompetence of the embryonic chick trachea.

Although released interferon was not detected in the culture medium, the resistance of persistently infected explants to heterologous viral challenge suggests that an interferon-like mechanism may be operating. This viral resistance was apparent by the third day postinfection; yet cell damage was not apparent by electron microscopy until 7 days postinfection, suggesting that a loss of susceptible cells was insufficient to account entirely for the magnitude of resistance seen within the first week. This resistance may have been due to small amounts of interferon, perhaps intracellular, present but not detectable by the assay system. In certain cell cultures a viral resistant state, seemingly interferon-mediated, can occur before, or in the absence of, detectable amounts of released interferon (2, 11). A similar phenomenon of resistance without detectable interferon may be relevant to the present experiments. The tracheal explants were clearly able to respond to exogenous interferon, and the transience of the UV-NDV-induced resistance is compatible with an interferon-mediated resistance.

The minimal epithelial staining seen with immunofluorescence and the absence of infected epithelial cells by electron microscopy were surprising since other investigators have described NDV in the epithelium (3, 12, 14). The early and uniform ciliostasis observed in NDV$_{pi}$-infected explants implies an involvement of the virus with the epithelium. Bang and Foard noted a 100-fold decrease in the number of NDV-infected cells from the epithelium of chick tracheal explants within the first 5 h after infection and attributed this to a sloughing of infected cells from the mucosa (1). Reed and Boyd suggested that a rapid detachment of rhinovirus-infected cells occurred in calf tracheal epithelium (18). Possibly the present experiments represent a similar situation and individual epithelial cells became detached soon after infection. Such a loss of infected cells would explain the apparent lack of epithelial participation in the persistent infection.

The nuclear fibrils observed by electron microscopy in infected explants bear some morphological resemblance to the "smooth filaments" described in the nuclei of cells infected with SSPE agents (16). These fibrils, although occasionally seen early in the infection, became common 14 to 21 days postinfection. Although it is tempting to speculate upon the significance of the intranuclear fibrils in the cells of infected explants, considerable caution is warranted by at least two factors. First, although of a slightly different array, an almost identical intranuclear structure was present in certain of the cells in control explants. Second, remarkably similar intranuclear structures have been reported in a wide variety of "normal" cells in several species (6, 10, 19).

In the intact bird, infection with NDV is not always self-limiting (7), and a carrier state may result when the virus establishes itself within the host. Our observations with immunofluorescence and electron microscopy suggest that serious consideration should be given to the subepithelial connective tissue as the possible site of NDV persistence in the intact bird. Multiple mechanisms such as interferon, or a delay in maturation and release, may be operating simultaneously or sequentially to preserve a delicate balance between viral replication and cell survival.

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LITERATURE CITED