Aleutian Disease in Ferrets

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When 32 antibody-free ferrets were inoculated with the highly mink-virulent Utah-1 strain of Aleutian disease virus (ADV), most developed ADV antibody starting 15 days after infection, but the antibody titers were much lower than those seen in mink. Relatively small amounts of ADV were demonstrated in CRFK cell culture, using ferret spleen and lymph node homogenates only 4 to 10 days after experimental infection, but low-level viral persistence for 180 days was shown by mink inoculation. The ferrets inoculated with the Utah-1 strain of ADV did not develop elevated gamma globulin levels, but did have mild tissue lesions. Forty-two percent of a group of 214, approximately 1-year-old, recently pregnant, female ferrets were found to have antibody to ADV. An analysis of the serum proteins of the ferrets with ADV antibody showed that there was a significant, but mild, elevation of their serum gamma globulin. Serial ferret-to-ferret transmission of a ferret strain of ADV by inoculation of spleen homogenates was demonstrated, and some of these ferrets developed liver lesions. Mink inoculated with ferret ADV made antibody, but did not develop hypergammaglobulinemia or tissue lesions. Although both ferret and mink strains of ADV replicate and persist in the ferret, they fail to cause severe disease of the type usually seen in the closely related mink. Mink and ferret ADV strains appear to be biologically distinct.

Aleutian disease virus (ADV) may cause a severe or fatal persistent infection in mink. ADV is a xenotropic parvovirus which is temperature sensitive in its replication in vitro (4, 20) and which replicates relatively rapidly in vivo without the production of neutralizing antibodies (21). Mink that are homozygous for the Aleutian gene are the most severely affected and usually die from immune complex glomerulonephritis or arteritis within 5 months of infection (7, 11). Other genetic types of mink may develop progressive disease, or may have a persistent, but inapparent, infection, or may clear the virus and recover (1–3, 17). The pathogenesis of ADV infection in mink has been reviewed in detail (10, 22).

There have been several reports that other carnivores may have lesions of the type seen in Aleutian disease (12–16, 19). Mink (Mustela vison) and ferrets (Mustela furo) are carnivores and belong to the family Mustelidae (8). Many mustelids are ferocious and unsuitable as laboratory research subjects. Nearly all are wild animals, available in small numbers only by trapping. The ferret is obtainable as a ranch-raised animal, is easily handled, and lives well in a laboratory situation. Thus, it was chosen as a suitable family member for an ADV transmission study.

This study was initiated to determine if the Utah-1 strain of ADV, which is highly pathogenic for mink, was transmissible to ferrets and to examine viral replication, viral persistence, humoral antibody response, and the development of pathological lesions. We also observed that ferrets used for other studies had antibody to ADV and were persistently infected by an ADV strain which was not pathogenic in mink.


MATERIALS AND METHODS

Ferrets. Young, 4 to 6-month-old male ferrets, which were obtained from Marshall Research Animals, North Rose, N.Y., were used for the transmission experiments. The animals were immunized against canine distemper virus and were housed in stainless-steel rabbit cages. The ferrets were fed a diet of canned dog food, occasionally supplemented with fresh liver.

A group of 214 mature female ferrets from the same supplier which was used for respiratory syncytial virus studies was tested for the presence of naturally acquired antibody to ADV.

Mink. Two groups of 30 mink, each comprised of 15 violet and 15 black mink, were used to test the
pathogenicity of passaged and unpassaged ferret ADV. For comparison, 7 violet and 7 pastel mink were inoculated with the Utah-1 strain of mink ADV. All mink were free of ADV antibody before virus inoculation.

**Viruses.** The passage history, preparation, and in vitro titration of the Utah-1 strain of ADV used in this study was described previously (20, 21). Each ferret received 10⁵ focus-forming units (FFU) of cell culture-grown virus inoculated intraperitoneally.

The 5× serially passaged ferret ADV and the ADV from a naturally infected ferret were prepared by homogenizing a 10% (wt/vol) of spleen with phosphate-buffered saline (pH 7.0), removing particulates for 10 min at 400 × g, and using the supernatant as an inoculum. Each ferret or mink received 1 ml intraperitoneally.

**Virus quantitation.** An immunofluorescence focus assay (20) with feline kidney cells (5) was used to determine the virus content of spleen and lymph node homogenates. The virus content was calculated per gram of tissue.

**Fluorescent antibody techniques.** The selection and preparation of normal and ADV antibody-containing mink globulins used to detect ADV antigen present in tissues has been previously described (21). Fluorescein labeling was carried out as for feline dehydrogenase virus studies (23).

Mink immunoglobulin (IgG, albumin, and ferret IgG) were prepared by ion-exchange chromatography. Mink complement was prepared by the method of Mardiney and Müller-Eberhard (18). Antisera were raised in New Zealand white rabbits, and the characterization, purification, and labeling were done as previously published (23). Strong cross-reactions were observed between mink and ferret proteins, using rabbit antiserum. (i) Indirect test for serum antibody: acetone-fixed ADV-infected CRFK cells grown as a monolayer on 15-mm glass cover slips were the antigen-containing targets. As a positive control, a pool of serum from infected mink was diluted 1:50, incubated on the cover slip, washed, and stained with fluorescein isothiocyanate (FITC)-labeled anti-mink IgG. Negative controls included infected cells stained with buffered saline followed by anti-mink and anti-ferret IgG.

(ii) Direct test for ADV antigens in tissues: this test was the same as previously published (21). FITC-labeled normal mink serum and uninfected ferret tissues were used as controls. The kidney, liver, and lymph node were cryostat sectioned at 6 μm, air dried, and acetone fixed for 10 min at room temperature. (iii) Evaluation of kidneys for immune complexes: cryostat sections were stained by using the direct method with FITC-labeled anti-IgG, anti-C3, and as a control, anti-albumin. FITC-labeled mink anti-ADV IgG was used to detect this antigen, and normal mink IgG was used as a control. The labeled antibody concentration used was 5 mg of protein per ml for each conjugate.

**Serum protein studies.** Separation and quantitation of serum proteins were done on cellulose acetate by using a Beckman Microzone System (Beckman Instruments, Inc.). A control human serum standard (Dade electrophoresis control, American Hospital Supply Corporation) was applied to each strip. A variation of ±10% of the stated values for the control was considered acceptable. The total serum protein was determined by using a refractometer. Mink were evaluated for the development of ADV antibody by counterelectrophoresis (6), rather than by indirect immunofluorescence.

**Experimental design.** Thirty-two young adult male ferrets which lacked ADV antibody and which had a serum gamma globulin under 16% were inoculated intraperitoneally with 1 ml containing 10⁵ FFU of the Utah-1 strain of mink ADV. This ADV stock is equally infectious in CRFK cell culture and in mink and causes severe disease in both Aleutian and non-Aleutian mink. Two animals were not inoculated and served as day-0 controls. Two animals per day were sacrificed on days 4, 6, 8, 10, 12, 15, and 21. Four animals were sacrificed on day 30, and 5 per day were sacrificed on days 60, 90, and 180. The day-180 ferrets were approximately 1 year 2 months of age at the end of the experiment.

At autopsy, the ferrets were exsanguinated by cardiac puncture under pentobarbital anesthesia. Spleen and mesenteric lymph nodes were removed by sterile technique, homogenized in buffered saline, (pH 7.0) and held at -40°C for infectivity titration. Sections of kidney, spleen, liver, heart, bladder, small intestine, and lymph nodes were preserved in 10% Lillie's buffered Formalin for routine histology. Except for the heart, matching 0.4-cm blocks of tissue were quick frozen in liquid nitrogen and stored at -70°C for immunofluorescence studies.

The sera from the day-0 through day-30 postinoculation ferrets were tested by indirect immunofluorescence for ADV antibody only at the time of autopsy. The longer-term animals were toe bled at monthly intervals, and serum was tested for rise in gamma globulins, total proteins, and viral antibody. Any serum found to be positive for ADV antibody at 1:10 was titrated by using fourfold dilutions until an end point was reached.

Paraffin-embedded tissue sections were sectioned at 4 μm, stained with hematoxylin and eosin, and evaluated for the presence of lesions of Aleutian disease. Thirteen ferrets which lacked ADV antibody were also sacrificed as a control for the presence of tissue lesions.

In addition, several ferrets arrived that had preexisting antibody to ADV and were excluded from the main experiment. A ferret with a high-antibody titer was selected to be used as a source of material for a rapid transfer experiment in hopes of producing a more severe Aleutian disease in the ferret. The splenic homogenate was passaged 5×, in groups of 2 or 3 antibody-free ferrets at 30-day intervals. The same homogenate was used to infect 15 normal violet (Aleutian) and 15 normal black (non-Aleutian) mink. A pool of unpassaged ferret ADV spleen homogenate was also used to infect 15 normal black and 15 normal violet mink.

**Statistical tests.** Analysis for significance used t tests and, where designated, X² or Wilcoxon tests.

**RESULTS**

**Infection of ferrets with mink ADV.** When ADV antibody-negative ferrets were inoculated with 10⁵ FFU of the highly pathogenic mink virulent ADV and were serially sacrificed for determination of viral replication, demonstrable amounts of virus were detected in the spleen and
lymph nodes starting at day 4 and continuing through day 10. The maximal amount of virus detected was $10^{4.3}$ FFU/g. From days 12 through 180 all cultures were negative for virus isolation (detection limit $> 10^{2.8}$ FFU/g), but 3 of 3 10% spleen suspensions from day-180 ferrets were infectious in mink.

The liver, spleen, and lymph nodes were selected as likely sites where AD antigen might be found. By day 4 small numbers of antigen-positive Kupffer cells were found in the liver. Spleen and lymph nodes were negative. At days 6, 8, and 10 only small numbers of hepatocytes and Kupffer cells contained antigen, which was mostly nuclear. From days 12 through 180 no antigen was found in the livers of the ferrets.

The development of ADV antibody and the serum protein analysis for these ferrets are summarized in Table 1. There was no significant rise in the mean % gamma globulin from days 0 through 180. ADV antibody first appeared in the serum of both ferrets sacrificed on day 15. Thereafter, all but three ferrets had antibody at autopsy, and individual titers ranged from 10 to 640. The highest geometric mean titer was 279 by day 150. Titers decreased slightly by day 180, the end of the experiment.

The ferrets inoculated with mink ADV had no glomerular or arterial lesions, a striking contrast to the prominent lesions seen in mink given this virus strain. A periportal lymphoid cell infiltration and stimulation of the lymphoid tissues were observed in some ferrets, and these observations are summarized in the third column of Table 2. Thirteen age-matched ADV antibody-free ferrets were autopsied for comparison. Six of the normal ferrets had periportal collections of lymphocytes and plasma cells in the liver, but only one had collections of lymphoid cells in the liver scored as ++ or more on an arbitrary 0 to +++ scale. Of the ferrets inoculated with mink ADV, 88% had such collections of cells in the liver, and 34% were scored as ++ or more. There was no correlation between the length of time after inoculation of virus and the occurrence of $\geq 2+$ peripheral lymphoid cell infiltration.

Abdominal lymph nodes and spleens were evaluated for the presence of lymphoid hyperplasia, suggestive of antigenic stimulation. A mild stimulation was noted in 31% of the control ferrets and 66% of the ADV infected ferrets, whereas a marked stimulation was seen in only 28% of the infected ferrets. These results are significantly different. Such stimulation was present in all ferrets from 4 through 21 days after infection and in 9 of 18 ferrets sacrificed at 30 days or later.

Traces of a small amount of ferret IgG and C3 were found in the glomeruli of eight day-90 and day-180 ferrets in a mesangial location. Several of the un inoculated ADV antibody-negative fer-

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**TABLE 1. The development of antibody to ADV, and serum gamma globulin levels in ferrets inoculated with the Utah-1 strain of ADV**

<table>
<thead>
<tr>
<th>Days post-inoculation</th>
<th>No. positive/no. tested</th>
<th>Antibody titer (Geometric mean)</th>
<th>Gamma globulin % of serum protein (Geometric mean)</th>
<th>Gamma globulin (g/dl) (Geometric mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prebled</td>
<td>0/34</td>
<td>0</td>
<td>8.2</td>
<td>0.65</td>
</tr>
<tr>
<td>0 through 12</td>
<td>0/12</td>
<td>0</td>
<td>9.1</td>
<td>0.50</td>
</tr>
<tr>
<td>15 through 30</td>
<td>19/22</td>
<td>18</td>
<td>7.8</td>
<td>0.54</td>
</tr>
<tr>
<td>60</td>
<td>14/14</td>
<td>80</td>
<td>9.9</td>
<td>0.69</td>
</tr>
<tr>
<td>90</td>
<td>10/10</td>
<td>92</td>
<td>6.8</td>
<td>0.48</td>
</tr>
<tr>
<td>120</td>
<td>5/5</td>
<td>160</td>
<td>7.2</td>
<td>0.61</td>
</tr>
<tr>
<td>150</td>
<td>5/5</td>
<td>279</td>
<td>7.2</td>
<td>0.53</td>
</tr>
<tr>
<td>180</td>
<td>4/4</td>
<td>226</td>
<td>6.4</td>
<td>0.42</td>
</tr>
</tbody>
</table>

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**TABLE 2. Lesions observed in control ferrets and in those inoculated with mink (Utah-1) or ferret strains of ADV**

<table>
<thead>
<tr>
<th>Finding</th>
<th>Control ferrets (No inoculation)</th>
<th>Animals inoculated with mink ADV</th>
<th>Animals inoculated with ferret ADV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periportal lymphoid cell infiltration</td>
<td>6/13 (46%)</td>
<td>28/32 (88%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12/12 (100%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Periportal lymphoid cell infiltration $\geq 2+$</td>
<td>1/13 (8%)</td>
<td>11/32 (34%)</td>
<td>6/12 (50%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stimulation of lymphoid tissue</td>
<td>4/13 (31%)</td>
<td>21/32 (66%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/12 (75%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stimulation of lymphoid tissue $\geq 2+$</td>
<td>0/13 (0%)</td>
<td>9/32 (28%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7/12 (58%)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> The presence and severity of tissue lesions were scored on an arbitrary 0 to 3+ scale.

<sup>b</sup> Significantly different from uninoculated ferrets ($P < 0.05$), using a 2 by 2 $X^2$ analysis.
Ferrets also had a similar localization of these proteins, suggesting that such complexes might be unrelated to the virus inoculation.

**Natural occurrence of ADV in ferrets.** An ongoing respiratory syncytial virus study provided individual sera from 214 adult female ferrets, which were tested for antibody to the Utah-1 mink strain of ADV. When these ferrets were tested by immunofluorescence at a dilution of 1:10, 90 (42%) had ADV antibody. A representative number of 28 antibody-free and 42 antibody-positive ferrets was tested for the serum gamma globulin level, and the results are shown in Fig. 1. The mean gamma globulin level for the antibody-free ferrets was 0.49 ± 0.17 g/dl and was 1.06 ± 0.71 g/dl for the antibody-positive ferrets. A Wilcoxon rank sum test showed that the gamma globulin was significantly higher in ferrets with ADV antibody than in those without antibody (P < 0.0005).

**Infection of ferrets with ferret ADV.** Twelve antibody-free ferrets were inoculated with 5× passaged ferret ADV. These ferrets were bled at days 63 and 120 and were sacrificed and necropsied at day 182. The serum protein and histological findings in these animals are summarized in Table 3. The serum gamma globulin rose significantly (P < 0.001) by 63 days after infection and, with some individual fluctuations, remained significantly elevated thereafter. At 182 days, when the ferrets were sacrificed, all had ADV antibody at titers ranging from 1:10 to 1:10,240 (geometric mean 1:640). Most of the ferrets with the highest ADV antibody titers also had the greatest increases in serum gamma globulin levels and the most tissue lesions. The tissue lesions consisted of perportal lymphoid cell collections in all ferrets in this group and stimulation of lymphoid cells in the spleen and abdominal lymph nodes in 9 of 12 of the animals. The tissue lesions are listed for each ferret (Table 3) and are summarized in the fourth column of Table 2. Although the tissue lesions in ferrets which received ferret ADV were significantly (P < 0.05) more frequent and severe than those seen in uninoculated ferrets, they were not present significantly more often than those seen in ferrets infected with mink ADV. An example of the lymphoid tissue stimulation is shown in Fig. 2.

Whereas many normal ferrets have some lym-

![Graph](image-url) FIG. 1. The distribution of serum gamma globulin in normal ferrets (open bars) and those naturally infected with ADV (solid bars). The infected ferrets have a significantly greater (P < 0.0005) serum gamma globulin than the normal ferrets do.

### TABLE 3. The development of antibody to ADV and serum gamma globulin levels in ferrets inoculated with 5× passaged ferret ADV

<table>
<thead>
<tr>
<th>Ferret</th>
<th>Antibody titer</th>
<th>Gamma globulin (g/dl) Day 0</th>
<th>Gamma globulin (g/dl) Day 3</th>
<th>Gamma globulin (g/dl) Day 120</th>
<th>Antibody titer</th>
<th>Gamma globulin (g/dl) Day 182</th>
<th>Lymphoid stimulation</th>
<th>Liver lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>0.62</td>
<td>0.55</td>
<td>160</td>
<td>0</td>
<td>0.50</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>1.97</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0.97</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>1.20</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>1.20</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0.34</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.34</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>1.26</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>1.26</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>0.45</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.45</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>12</td>
<td>0.84</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>0.84</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>22</td>
<td>1.84</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>1.84</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>0.57</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.57</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>0.32</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.32</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>10.56</td>
<td>0.74</td>
<td></td>
<td></td>
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</table>

**a** Scores on an arbitrary 0 to +++ scale.
phoid cells in the portal areas of the liver (Table 2), a large number of lymphoid cells in this location is uncommon. As noted previously, ferrets inoculated with mink ADV have an increased incidence of portal lymphoid cell infiltrates. Ferrets which received the passaged ferret ADV frequently (50%) had a marked degree of portal lymphoid cell infiltration. In these animals, the lymphoid cell infiltrates were dense and frequently showed germinal center formation (Fig. 3), which at times distorted the lumen of the bile ducts. No similar germinal center formation was noted in the 45 ferrets that were uninoculated or inoculated with mink ADV. No proliferation of bile ducts was seen in any of the groups of ferrets.

**Infection of mink with ferret ADV.** The serum gamma globulin of mink inoculated with ferret ADV is shown in Fig. 4. For a control, a group of mink was inoculated with the mink virulent Utah-1 strain of ADV. All of the mink became infected and developed ADV antibody detected by the counterelectrophoresis assay. Mink ADV caused an elevation of the percentage of serum gamma globulin from 13.0 ± 1.9% on day 0 to 25.8 ± 5.5% on day 30 and 31.6 ± 10.2% on day 60, a significant ($P < 0.001$) and continued increase. In contrast, mink given either the unpassaged or passaged ferret ADV had a slight increase in gamma globulin, which just reached statistical significance ($P < 0.05$) at day 43, but was indistinguishable from pre-inoculation levels at day 82 ($P > 0.10$ and $> 0.50$). No difference was found in the percentage of gamma globulin between violet and black mink given unpassaged ferret ADV. At day 82, passaged ferret ADV caused a slightly higher gamma globulin of 15.1 ± 4.9% in black mink compared with 12.1 ± 1.9% for violet mink ($P < 0.05$). At autopsy, all mink which received the Utah-1 ADV had renal lesions typical of Aleutian disease, whereas only 2 of 60 of the mink which received ferret ADV had renal lesions consistent with Aleutian disease. Unfortunately, we did not obtain liver for histological study from any of the mink which received ferret ADV.

**DISCUSSION**

When the highly mink-virulent Utah-1 strain of ADV is inoculated into antibody-free ferrets, small amounts (10$^{4.3}$ FFU/g or less) of virus were demonstrable in cell cultures inoculated with spleen and lymph node homogenates 4 to 10 days after infection. However, virus was found to persist at a low level in the spleen for 180 days. ADV antibody was shown in 19 of 22 ferrets inoculated for 15 to 180 days and the titers were low when compared with mink inocu-
lated with the same virus. Some lymphoid tissue stimulation and periportal lymphoid cell infiltration were observed in these ferrets. These ferrets failed to develop hypergammaglobulinemia. Ohshima et al. (19) inoculated 28 ferrets with the weakly pathogenic Pullman strain of mink ADV; 11 developed ADV antibody, whereas only 2 developed hypergammaglobulinemia. These workers did not mention lesions in these ferrets. Kenyon and co-workers (14, 15) inoculated ferrets with a highly pathogenic strain of mink ADV and observed that the virus persisted for as long as 136 days at low titer, but that the ferrets did not develop hypergammaglobulinemia. This study found more severe periportal lymphoid cell infiltration than we noted. It is thus clear that three mink ADV strains may infect ferrets, persist, induce ADV antibody, and produce mild to moderate lesions. However, the amount of virus present in ferret tissue is quite small, and no evidence of immune complex disease of the type seen in mink (10) was found.

Our study demonstrated ADV antibody in 42% of a group of uninoculated ferrets, and a mild, but highly significant, degree of hypergammaglobulinemia was present in these same animals. We also serially passaged a ferret ADV strain in vivo and found that this strain caused the development of ADV antibody in all inoculated normal ferrets and hypergammaglobulinemia and tissue lesions in most ferrets. The ferret ADV strain was essentially nonpathogenic in mink, although it caused the development of ADV antibody in all inoculated mink. No biologically significant difference in the response of Aleutian (violet) and non-Aleutian (black) mink to ferret ADV infection was noted. Ohshima et al. (19) inoculated 12 ferrets and 15 Aleutian mink with a spleen suspension from a ferret with spontaneous Aleutian disease and noted that 5 of 15 mink died of Aleutian disease and 4 of 12 ferrets became hypergammaglobulinemic within 6 months. The results of mink inoculation of ferret spleen (19) are atypical for a mink ADV strain even of very low virulence. Kenyon and co-workers (12, 16) observed lymphoproliferative lesions, hypergammaglobulinemia, and monoclonal gamma globulins in ferrets that were not experimentally infected with ADV. These ferrets may have been infected with ferret ADV, but the lesions described were more severe than those seen by us and by Ohshima et al. (19) in ferret ADV infection and included vasculitis and thymic hyperplasia, which we failed to find in our animals.

It is clear that the Utah-1 strain of mink ADV and the ferret strain of ADV described in this report are biologically different and fairly spe-

FIG. 3. The portal triad of a ferret (animal 7 in Table 4) experimentally infected with ferret ADV for 182 days. A dense lymphoid cell aggregate around the bile duct is present with characteristics of a lymphoid follicle. Hematoxylin and eosin, ×340.
The habitat of mink and natural infection of mink has been related to commercial and presently clear. The range of viruses of the ADV group are not presently clear. ADV is not immunologically related to other paroviruses (20). Aleutian disease was recognized in mink only after animals with the recessive Aleutian coat color mutation were raised in large numbers. The intensive close-quartered arrangement of mink and ferrets on commercial ranches is ideal for continued horizontal transmission of ADV, and vertical infection of mink has also been shown (9). The natural habitat of mink and skunks overlaps, with both utilizing areas near stream banks. The ferret ADV described in this report coexists with its host in a far more benign relationship than does the mink ADV. We therefore believe that the more virulent mink ADV may be a mutant of the ferret ADV that was introduced into mink. The high frequency of ADV antibody in skunks suggests that isolation of the virus from skunks should be attempted and that such an isolate should be tested for virulence in the species of origin as well as in other mustelids.

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