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Comparative Pathogenicity of Four Strains of Aleutian Disease Virus for Pastel and Sapphire Mink

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Information was sought on the comparative pathogenicity of four North American strains (isolates) of Aleutian disease virus for royal pastel (a non-Aleutian genotype) and sapphire (an Aleutian genotype) mink. The four strains (Utah-1, Ontario [Canada], Montana, and Pullman [Washington]), all of mink origin, were inoculated intraperitoneally and intranasally in serial 10-fold dilutions. As indicated by the appearance of specific antibody (counterimmunoelectrophoresis test), all strains readily infected both color phases of mink, and all strains were equally pathogenic for sapphire mink. Not all strains, however, regularly caused Aleutian disease in pastel mink. Infection of pastel mink with the Utah-1 strain invariably led to fatal disease. Infection with the Ontario strain caused fatal disease nearly as often. The Pullman strain, by contrast, almost never caused disease in infected pastel mink. The pathogenicity of the Montana strain for this color phase was between these extremes. These findings emphasize the need to distinguish between infection and disease when mink are exposed to Aleutian disease virus. The distinction has important implications for understanding the natural history of Aleutian disease virus infection in ranch mink.

Aleutian disease (AD) virus, a nondefective parvovirus (5), causes a slowly progressive, fatal disease in some mink (*Mustela vison*) but only a subclinical infection of variable duration in others (15, 25). The response that supervenes is partly dependent on the genotype of mink. In general, those homozygous for the autosomal recessive Aleutian gene are more regularly and more severely affected by AD than are other genotypes. The strain of virus has a bearing on this, too. Yet the natural occurrence of strains (or isolates) of AD virus differing in their pathogenicity for mink has received little attention (11, 25). Such differences not only may account for the ostensibly discordant experimental results reported by persons using virus from diverse sources, but also may explain important epidemiological observations hitherto poorly understood.

To learn more about these differences, we studied four North American strains of AD virus, all of mink origin, in royal pastel (a non-Aleutian genotype) and sapphire (an Aleutian genotype) mink. Although all strains were equally pathogenic for sapphire mink, they varied greatly in their ability to cause typical progressive disease in pastel mink.

MATERIALS AND METHODS

Mink. Male and female mink of the royal pastel and sapphire color phases, 12 to 24 months old, were obtained locally from a single closed herd known to be free of AD for at least 15 years. They were housed individually in an outdoor isolation compound, fed a standard mink ration, and managed as described previously (10). None had been vaccinated for canine distemper, viral enteritis, or botulism.

Viruses. Four strains were used: Utah-1, Ontario (Canada), Montana, and Pullman (Washington). Suspensions of each were prepared with Eagle minimal essential medium containing heat-inactivated 10% fetal bovine serum and antibiotics (100 U of penicillin, 100 mg of streptomycin, and 0.25 mg of amphotericin B [Fungizone; E. R. Squibb & Sons, Princeton, N.J.] per 1 ml of medium).

The Utah-1 strain was obtained from David D. Porter, University of California, Los Angeles, as a 10% suspension of infected mink spleens representing the fourth serial passage in violet mink. It had been established from spleens of 30 naturally infected mink, mostly pastel (27). The suspension we received was passed serially through membrane filters (Millipore Corp., Bedford, Mass.) and inoculated into sapphire mink. A 10% suspension of spleens from two that became affected with AD after receiving the 100-nm filtrate (fifth passage) was inoculated into other sapphire mink. Spleens from two, collected 10 days after inoculation, were made into a 10% suspension (sixth passage) that was inoculated into five sapphire mink. A 10% suspension of their spleens, collected 9 days after inoculation, was our stock seventh passage virus. It was used in this study, except for the pastel mink inoculated intraperitoneally. They received fifth passage virus.

The Ontario strain was obtained from Lars Karstad,

University of Guelph, Guelph, Ontario, Canada, as a crude suspension of infected mink spleen, liver, and kidney. Its passage level was not designated, but by the time we received it many passages had been made in mink since it was established in 1961 (17). The suspension we received, labeled A94, was inoculated into two sapphire mink. A 10% suspension of their spleens, collected when the mink were sick with AD, was passed serially through Millipore membrane filters and inoculated into sapphire mink. A 10% suspension of spleens from three that contracted AD after receiving the 100-nm filtrate was prepared. This suspension, representing the second passage in mink at the Rocky Mountain Laboratories, was used in this study.

The Montana strain was established by us in 1973 from the spleen of an AD-affected sapphire kit obtained from a local herd composed mainly of non-Aleutian mink. Infection in this herd was confined to a small group of sapphire mink, presumably exposed to AD virus by contact with several infected blue iris (another Aleutian genotype) mink introduced into the herd about 2 years earlier. A 10% suspension of the single spleen was passed serially through Millipore membrane filters and inoculated into sapphire mink. Spleens from two that became affected with AD after receiving the 100-nm filtrate were made into a 10% suspension. This first passage virus was used in this study.

The Pullman strain was obtained from John R. Gorham, Washington State University, Pullman, Wash., as a crude 10% suspension of infected mink spleen representing the ninth serial passage in mink (12). Serial 10-fold dilutions of a 100-nm Millipore filtrate of the 10th-passage viral suspension (10) were inoculated into sapphire mink. Three months later, the spleen was collected from one sick mink that had received the 10^{-5} dilution. A 10% suspension of its spleen was inoculated into three sapphire mink. The 12th passage virus used in this study was a 10% suspension of their spleens collected 25 days after inoculation.

As calculated by the method of Spearman and Kärber (9), the log 50% sapphire mink intraperitoneal lethal dose (LD_{50}) of virus per 500 mg of tissue in each inoculum was: Utah-1, 4.8 (fifth passage) and 7.5 (seventh passage); Ontario, 5.2; Montana, 6.5; Pullman, 6.5.

Inoculation of mink. Two routes were used, intraperitoneal and intranasal, the latter to simulate a likely normal mode of exposure. (An earlier study demonstrated that mink readily become infected in this way.) In each case, the standard volume of inoculum was 0.5 ml. For intranasal inoculation, the mink were anesthetized with ether by a nose cone and then held vertically, head up, while 0.25 ml of the inoculum was instilled into each nostril from a 1-ml syringe fitted with a blunt 1-in. [2.54-cm] 20-gauge needle. For each route, serial 10-fold dilutions of the inocula were used, usually with three or four mink per dilution.

Evaluation of responses. Responses of the mink to each strain of virus were evaluated in terms of the appearance of specific antibody, rise in level of serum gamma globulin, and the occurrence of AD as judged clinically and at necropsy. Precipitating antibody was measured by counterimmunoelectrophoresis (CIEP) in serial fourfold dilutions of serum (4). The antigen used was prepared from livers of sapphire mink killed 10 days after they had been inoculated intraperitoneally with a high concentration of the Utah-1 strain of virus. With the inocula used, the antibody detected was assumed to have resulted only from replication of AD virus, however limited that may have been in some mink (28). Serum gamma globulin levels were determined by electrophoresis on cellulose acetate, and total serum proteins were determined by a clinical refractometer. Amounts of serum gamma globulin greater than 1.3 g/dl were considered elevated. Affected mink either died or were killed when death seemed imminent. For purposes of this study, the period that elapsed since inoculation was regarded as the duration of the disease.

RESULTS

The main comparative findings are summarized in Tables 1 and 2. Details of the responses to individual strains of virus are considered here.

Utah-1 strain. The Utah-1 strain behaved

TABLE 1. Comparative pathogenicity of four AD virus strains for pastel and sapphire mink inoculated intraperitoneally[a]

Dilution of virus	Pastel mink								Sapphire mink							
	Utah-1		Ontario		Montana		Pullman		Utah-1		Ontario		Montana		Pullman	
	D[b]	A[c]	D	A	D	A	D	A	D	A	D	A	D	A	D	A
10^{-1}	4/4		3/3	3/3	3/3	0/3	0/3	3/3							3/3	3/3
10^{-2}	4/4		3/3	3/3	3/3	3/3	0/3	3/3							3/3	3/3
10^{-3}	4/4		3/3	3/3	0/3	3/3	0/3	3/3					3/3	3/3	3/3	3/3
10^{-4}	4/4		3/3	2/3[d]	1/3	3/3	0/3	3/3			3/3	3/3	3/3	3/3	3/3	3/3
10^{-5}	2/4	2/4	3/3	3/3	3/3	3/3	0/3	3/3	4/4	4/4	2/3	2/3	3/3	3/3	2/2	2/2
10^{-6}	1/4	1/4	0/3	1/3	1/3	1/3	0/3	1/3	4/4	4/4	0/3	0/3	3/3	3/3	3/3	3/3
10^{-7}	0/4	0/4	0/3	0/3	0/3	0/3	0/3	0/3	2/4	2/4	0/3	0/3	0/3	0/3	0/3	0/3
10^{-8}							0/3	0/3	2/4	2/4					0/3	0/3

[a] Number of mink positive/number inoculated.
[b] D, Disease ascertained at necropsy.
[c] A, Antibody (titer ≥ 1:64) determined by CIEP.
[d] Information on antibody was not available for one mink that died of the disease at 7 weeks.

TABLE 2. Comparative pathogenicity of four AD virus strains for pastel and sapphire mink inoculated intranasally[a]

Dilution of virus	Pastel mink								Sapphire mink							
	Utah-1		Ontario		Montana		Pullman		Utah-1		Ontario		Montana		Pullman	
	D[b]	A[c]	D	A	D	A	D	A	D	A	D	A	D	A	D	A
10^{-1}	3/3	3/3	3/3	3/3	1/3	3/3	0/3	3/3					3/3	3/3		
10^{-2}	3/3	3/3	3/3	3/3	2/3	3/3	0/3	3/3					2/3	3/3		
10^{-3}	3/3	3/3	2/2	3/3	0/3	3/3	0/3	3/3			3/3	3/3	3/3	3/3	3/3	3/3
10^{-4}	3/3	3/3	2/3	2/3	1/3	3/3	1/3	1/3	3/3	3/3	3/3	3/3	2/3	2/3	1/3	1/3
10^{-5}	3/3	3/3	2/3	2/3	1/3	3/3	0/3	1/3	1/3	1/3	3/3	3/3	3/3	3/3	2/3	2/3
10^{-6}	2/3	2/3	3/3	3/3	0/3	1/3	0/3	0/3	1/2	1/2	1/3	1/3	0/3	0/3	1/3	1/3
10^{-7}									0/3	0/3						

[a] Number of mink positive/number inoculated.
[b] D, Disease ascertained at necropsy.
[c] A, Antibody (titer ≥ 1:64) determined by CIEP.

much the same in pastel mink inoculated by either route (Tables 1 and 2). Information was not available on the presence of antibody in most pastel mink inoculated intraperitoneally, but antibody was detected 2 to 7 weeks after intranasal inoculation. Its terminal titer was ≧1:1,024 in all positive mink. In both inoculation groups, serum gamma globulin levels usually were elevated at 7 weeks. Terminally, they varied from 1.8 to 4.7 g/dl (mean, 3.1 g/dl) in mink inoculated intraperitoneally and from 2.0 to 6.4 g/dl (mean, 3.5 g/dl) in those inoculated intranasally. Mink that had hypergammaglobulinemia (and presumably antibody) after intraperitoneal inoculation succumbed to AD at 10 to 93 weeks (mean, 29 weeks). All mink of the intranasal group that had antibody succumbed to the disease 15 to 58 weeks (mean, 34 weeks) after inoculation (Table 3).

Similarly, this strain behaved much the same in both groups of sapphire mink, though the higher dilutions of it caused AD more often after intraperitoneal than intranasal inoculation (Tables 1 and 2). In both groups, antibody was detected by 7 weeks; its terminal titer varied from 1:256 to ≧1:1,024. Serum gamma globulin levels were elevated at 7 weeks and terminally reached 1.8 to 7.4 g/dl (mean, 3.6 g/dl) in mink inoculated intraperitoneally and 2.6 to 4.7 g/dl (mean, 3.6 g/dl) in those inoculated intranasally. All mink with antibody succumbed to AD 7 to 17 weeks (mean, 13 weeks) after intraperitoneal inoculation and 10 to 44 weeks (mean, 19 weeks) after intranasal inoculation (Table 3).

As measured by the LD_{50} titer of virus in each of the four titration groups, all but one group having received the same inoculum, the extent of morbidity was greatest in the sapphire mink inoculated intraperitoneally (pastel mink intraperitoneal LD_{50}, 5.2; pastel mink intranasal LD_{50}, 6.2; sapphire mink intraperitoneal LD_{50}, 7.5; sapphire mink intranasal LD_{50}, 5.3).

Ontario strain. In pastel mink, the Ontario strain of virus behaved much like the Utah-1 strain, regardless of the route of inoculation (Tables 1 and 2). The pattern of morbidity in the intranasal titration, however, was not as regular as that with the Utah-1 strain. Antibody was detected 2 to 4 weeks after inoculation and soon reached a titer of ≧1:1,024 in all positive mink. In both groups, serum gamma globulin levels were elevated at 7 weeks; terminally they ranged from 2.6 to 5.5 g/dl (mean, 3.9 g/dl) in mink inoculated intraperitoneally and from 2.3 to 7.4 g/dl (mean, 4.0 g/dl) in those inoculated intranasally. Except for one in each group, all mink with antibody became affected with AD. The duration of disease was 7 to 164 weeks (mean, 43 weeks) after intraperitoneal inoculation and 18 to 92 weeks (mean, 43 weeks) after intranasal inoculation (Table 3). In two mink inoculated intraperitoneally with 10^{-2} and 10^{-3} dilutions of virus, the duration of disease was unusually long, 115 and 164 weeks, respectively.

The two mink with antibody that did not succumb to AD included one that had received the 10^{-6} dilution of virus intraperitoneally. Antibody was present at 3 weeks and soon reached a titer of ≧1:1,024, where it remained for at least 84 weeks. The serum gamma globulin level did

TABLE 3. Duration of disease in pastel and sapphire mink inoculated with four AD virus strains

Color phase	Route of inoculation	Duration of disease (wks) after inoculation with virus strain:			
		Utah-1	Ontario	Montana	Pullman
Pastel	Intraperitoneal	29	43	51	
	Intranasal	34	43	53	105[a]
Sapphire	Intraperitoneal	13	15	17	13
	Intranasal	19	13	16	16

[a] One mink.

not become elevated until 41 weeks after inoculation when it was 2.6 g/dl. It was still elevated (3.6 g/dl) nearly a year later, the last time the determination was made. Three and one-half years after inoculation, the mink died of a lymphoreticular proliferative disease, a condition that rarely affects pastel mink (14). The other exceptional mink had received the 10^{-3} dilution of virus intranasally. It had antibody at 4 weeks. At 7 weeks the antibody titer was ≧1:1,024 and the serum gamma globulin level was 2.3 g/dl. During the ensuing year, antibody remained at ≧1,024, and the serum gamma globulin level rose to 4.2 g/dl. Almost 5 years after inoculation, the mink died of lymphocytic lymphoma, a common neoplasm in older pastel mink (unpublished observation).

In sapphire mink inoculated by either route, this strain behaved the same as it did in pastel mink (Tables 1 and 2). Antibody was detected in most mink by 4 weeks after inoculation. Except for one mink of the 10^{-5} intranasal group, whose antibody titer was only 1:16 when it died of AD at 13 weeks, all positive mink had a terminal antibody titer of ≧1:1,024. Serum gamma globulin levels usually were elevated at 7 weeks. Terminally they varied from 2.3 to 7.7 g/dl (mean, 3.6 g/dl) in mink inoculated intraperitoneally and from 2.8 to 5.3 g/dl (mean, 3.6 g/dl) in those inoculated intranasally. AD supervened in all mink with antibody. The duration of disease was 8 to 24 weeks (mean, 15 weeks) after intraperitoneal inoculation and 8 to 26 weeks (mean, 13 weeks) after intranasal inoculation (Table 3).

As measured by the LD_{50} titer of virus in each of the four titration groups, the extent of morbidity was essentially the same in all (pastel mink intraperitoneal LD_{50}, 5.5; pastel mink intranasal LD_{50}, 5.5; sapphire mink intraperitoneal LD_{50}, 5.2; sapphire mink intranasal LD_{50}, 5.8).

Montana strain. In contrast to the findings with the Utah-1 and Ontario strains of virus, infection of pastel mink with the Montana strain resulted in an irregular pattern of morbidity (Tables 1 and 2). This was most notable after intranasal inoculation, when AD seldom supervened. Except in mink that had received the highest dilutions of virus, antibody was present in both groups at 4 weeks. A titer of ≧1:1,024 was soon reached in all positive mink; it was maintained at that level in most of them. Usually, serum gamma globulin levels, which differed little between the two groups, were elevated at 8 weeks in both. Terminal levels were 2.7 to 5.2 g/dl (mean, 3.6 g/dl) in mink inoculated intraperitoneally and 2.3 to 4.9 g/dl (mean, 3.6 g/dl) in those inoculated intranasally. Not all mink with antibody became affected with AD. In those that did, the duration of disease varied from 12 to 168 weeks (mean, 51 weeks) after intraperitoneal inoculation and from 9 to 142 weeks (mean, 53 weeks) after intranasal inoculation (Table 3). All but a few of the others with antibody had normal levels of serum gamma globulin.

Those with elevated levels, usually slight (<2.0 g/dl) and transient, died of diseases other than AD 3 to 7 years after inoculation. One mink that had been inoculated intranasally with the 10^{-5} dilution of virus had antibody at 4 weeks, which soon reached a titer of ≧1:1,024. Thirteen weeks after the inoculation, the serum gamma globulin level was 1.7 g/dl. During the next year it averaged 3.2 g/dl. When killed 84 weeks after inoculation, the mink had chronic interstitial nephritis but no certain histological evidence of AD.

In sapphire mink, by contrast, this strain behaved much like the Utah-1 and Ontario strains did in this color phase (Tables 1 and 2). This was so regardless of the route of inoculation, though the extent of the morbidity was greater after intraperitoneal inoculation (LD_{50} titer, 6.5) than after intranasal inoculation (LD_{50} titer, 4.8). Antibody was detected at 4 weeks and soon reached a titer of ≧1:1,024 in most mink, where it remained throughout the course of the disease. In both groups, serum gamma globulin levels were elevated in nearly all mink at 11 weeks. Terminal levels varied from 3.4 to 6.3 g/dl (mean, 4.7 g/dl) in mink inoculated intraperitoneally and from 2.4 to 5.2 g/dl (mean, 3.2 g/dl) in those inoculated intranasally. All but one mink with antibody became affected with AD. The duration of disease varied from 8 to 39 weeks (mean, 17 weeks) in the intraperitoneal group and from 8 to 37 weeks (mean, 16 weeks) in the intranasal group (Table 3).

The only mink with antibody that did not succumb to AD had been inoculated intranasally with the 10^{-2} dilution of virus. Antibody was present at 4 weeks and reached a titer of ≧1:1,024 at 21 weeks, where it remained for at least the next year. At 7 weeks, the serum gamma globulin level was 1.2 g/dl. For the next 2 years, it varied between 1.0 and 1.7 g/dl. The mink died of pleural empyema 164 weeks after inoculation; it did not have histological changes of AD.

Pullman strain. The Pullman strain caused infection in pastel mink as readily as did the other three strains, but unlike them it caused almost no disease (Tables 1 and 2). In both groups of pastel mink, antibody was detected 2 to 4 weeks after inoculation, but maximum titers seldom exceeded 1:256, especially in the intraperitoneal group. And serum gamma globulin levels never became elevated (they varied from 0.5 to 1.3 g/dl) in that group. In only four mink with antibody, all inoculated intranasally, was it

elevated (1.7 to 2.6 g/dl at 11 weeks). One succumbed to AD at 105 weeks (Table 3). It was the only pastel mink to do so. Its serum gamma globulin level varied from 1.4 to 3.7 g/dl (mean, 2.8 g/dl). From 11 weeks on, its antibody titer was ≧1:1,024. In the other three mink, the mean levels of serum gamma globulin during the first 76 weeks after inoculation were 1.4, 1.6, and 1.8 g/dl. Accompanying antibody titers in two mink were ≧1:1,024; in the third mink, the titer was 1:64. All three mink died of diseases other than AD 4 to 5.5 years after inoculation.

By contrast, this strain was much more pathogenic for sapphire mink, though the pattern of morbidity in the intranasal titration (LD_{50} titer, 4.8) was much less regular than that for intraperitoneal titration (LD_{50} titer, 6.5) (Tables 1 and 2). In most mink, antibody was present at 2 to 7 weeks and nearly always reached a titer of ≧1:1,024 terminally. Serum gamma globulin levels in both groups usually were elevated at 4 to 7 weeks. Terminal levels varied from 1.3 to 5.8 g/dl (mean, 3.6 g/dl) in mink inoculated intraperitoneally and from 1.1 to 3.5 g/dl (mean, 2.7 g/dl) in those inoculated intranasally. All mink with antibody succumbed to AD 7 to 24 weeks (mean, 13 weeks) after intraperitoneal inoculation and 7 to 35 weeks (mean, 17 weeks) after intranasal inoculation (Table 3).

All strains. Although detailed clinicopathological evaluations were not made, no consistent differences in the disease caused by each strain of virus were recognized. This was reflected in the similarity of the mean terminal levels of serum gamma globulin with all strains. And as found in another study with the Pullman strain (10), the severity of the disease bore little relation to the concentration of virus in the inoculum, regardless of strain. The usual difference in the tempo of the disease process between the two color phases, however, occurred with all strains (Table 3 and Fig. 1). Even so, a few pastel mink inoculated with the Utah-1, Ontario, or Montana strain succumbed to AD as quickly as most sapphire mink generally do. On the other hand, the evolution of the disease was unusually protracted (in our experience) in a few pastel mink inoculated with the Ontario, Montana, or Pullman strain.

With all strains, none of the mink that did not have antibody, whether or not they had slightly elevated levels of serum gamma globulin, became affected with AD during the period (up to 8 years in some mink) they were observed after inoculation.

DISCUSSION

Results of this limited study emphasize the need to distinguish between infection and disease when mink are exposed to AD virus. Thus,

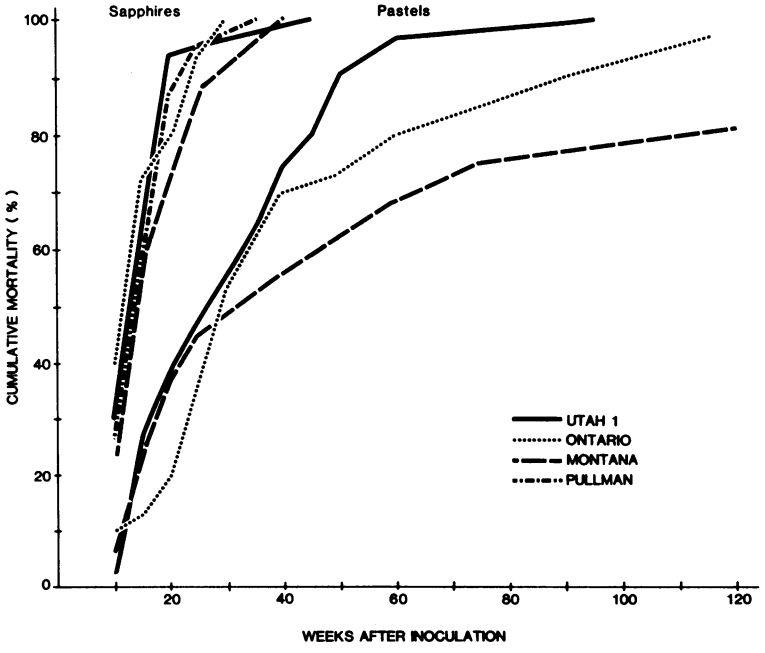

FIG. 1. Contrasting patterns of mortality in pastel and sapphire mink inoculated either intraperitoneally or intranasally with four AD virus strains. Because the patterns for the two routes of inoculation were similar, the data are combined in this figure. Not included is the single pastel mink that succumbed to infection with the Pullman strain 105 weeks after inoculation.

although all four strains readily infected both sapphire and pastel mink as indicated by the appearance of specific antibody, not all regularly caused disease in pastel mink, slight elevations in serum gamma globulin levels notwithstanding. Infection with the Utah-1 strain invariably led to fatal disease in pastel mink, a finding somewhat at variance with that reported by others (20) who used a different preparation of this strain. Infection with the Ontario strain resulted in disease nearly as often. At the other extreme, disease almost never supervened in pastel mink infected with the Pullman strain, much as reported previously (4). Nevertheless, this strain will cause disease in pastel mink that receive large amounts of it, but the pattern of morbidity in a titration is always irregular (10). The pathogenicity of the Montana strain was between these extremes. Although much more pathogenic than the Pullman strain, it was far less uniformly so than the Utah-1 and Ontario strains, especially after intranasal inoculation.

By contrast, all four strains were equally pathogenic for sapphire mink. With the exception of one mink inoculated intranasally with the Montana strain, infection invariably resulted in fatal disease, regardless of the route of inoculation. In our experience with many hundreds of sapphire mink that became infected after intraperitoneal inoculation with the Pullman strain, only six failed to become affected with the disease (unpublished observation). Most likely this occurred because these mink represented a few sapphire mink extremely resistant to disease in a large and otherwise extremely susceptible population. In view of the genetic heterogeneity of ranch mink (other than those genes affecting mainly coat color) (16), such a response is expected. Supposedly, all known mink strains of AD virus in North America are equally pathogenic for sapphire mink and other color phases homozygous for the Aleutian gene (11). Only an atypical mink strain in the Netherlands has been reported to be much less pathogenic for such mink (11). On the other hand, ferret strains of AD virus do not cause typical progressive disease in mink of either Aleutian or non-Aleutian genotype, even though they cause infection in both (23, 29). What effect, if any, the passage history of each strain of virus we used had on our results is not known.

When considered in terms of host response, the differences in pathogenicity of the four strains indicate susceptibility to disease is independent of susceptibility to infection (22), a distinction not always made clear in discussions on the pathogenesis and genetic aspects of AD. Whether, as was suggested previously (15), some genotypes of mink are resistant to infection with AD virus is not known. Nor have specific host attributes been identified that allow infection to result in overt disease in some mink and not in others. Certainly susceptibility or resistance of pastel mink to AD does not have a simple genetic explanation (15, 20). And despite the profound constitutional differences between pastel and sapphire mink attributable to the pleiotropic effects of the Aleutian gene in sapphire mink (18, 19, 24), the basis for the generally greater susceptibility of sapphire mink to AD is not understood (21).

Apart from its application in methods used to titrate AD virus (median infective dose versus median lethal dose) (20), this distinction between infection and disease has important epidemiological implications: it must be considered in evaluating results of serological tests used to control or eradicate AD in commercial herds (6). As a sensitive, highly specific, and practical test for AD virus antibody, the CIEP test is now used widely in such herds. Great disparity has been found between results obtained with it and those obtained with the iodine agglutination test (13), hitherto commonly used to identify AD-affected mink by detecting hypergammaglobulinemia, the best single laboratory diagnostic feature of the disease. In the absence of information on the level of serum gamma globulin, the significance of antibody as an indicator of disease is obviously influenced by several variables: the genotype (Aleutian or non-Aleutian) of the mink; the stage of the infection and titer of antibody, at least in non-Aleutian mink; and the virus strain causing the infection. Of these, the virus strain is now recognized as having an important bearing on the epidemiological significance of AD virus antibody in non-Aleutian mink, as our observations illustrate. In our experience, with the rare exceptions mentioned, antibody in sapphire mink has always heralded fatal disease, regardless of the strain of infecting virus.

AD virus may persist for a variable period in the blood (perhaps at least a month or more) and in various tissues (no doubt much longer) of some infected non-Aleutian mink that never succumb to the disease (1, 2). Such mink may be a source of virus transmitted horizontally to nearby susceptible mink or vertically to kits in utero (3). The extent of this threat and its epidemiological importance in a commercial herd, however, can only be surmised until more is known about the duration of subclinical infection in non-Aleutian mink. The relation of the virus strain to such persistent infection without disease is not clear. In one study, viremia was not detected in subclinically infected pastel mink 120 days after they had been inoculated intraperitoneally with the Utah-1 strain (20). Whether virus was still present in other tissues was not

determined. In another study of subclinically infected non-Aleutian mink, viremia subsided in a few by 28 days after intraperitoneal inoculation with the Pullman strain (G. A. Padgett, Fed. Proc. **28**:685, 1969). But again the persistence of virus elsewhere in the body was not mentioned. We have found small amounts of virus to persist for many months, mainly in the mesenteric lymph node, in a few pastel mink inoculated intraperitoneally with the Pullman strain, but prolonged persistence has been the exception (10). The occurrence of many inapparently infected pastel mink in some commercial herds indicates that strains of AD virus causing infection in them were not highly pathogenic for non-Aleutian mink (1–3). Most strains responsible for natural infections are probably like that.

Although the strain differences in virulence have not been explained, they may be reflected in other attributes of AD virus. For example, some evidence indicates the temporal pattern of virus replication in each main genotype of mink may vary with the strain. Thus, the virulent Utah-1 (27) and Ontario (7) strains replicate early to high titer in both genotypes. The Pullman strain, on the other hand, replicates more slowly and reaches a higher titer in sapphire mink than in pastel mink (10). To what extent such differences have any bearing on the infectiousness or transmissibility of AD virus under natural conditions remains to be demonstrated. Then too, whether these differences in virulence bear any relation to the ease with which the several strains can be established in cell culture systems is not clear. The Utah-1 strain readily replicates in one such system, the Ontario and Pullman strains do not (26). The differences do have a practical bearing on the usefulness of these strains in the preparation of antigen (mostly from liver) for the CIEP test. The more virulent strains that replicate to high titer early, such as the Utah-1 and Ontario strains, yield satisfactory antigen 10 days after mink are inoculated with them (4, 7), the Pullman strain does not, most likely because of low concentrations of virus in the liver at that time (unpublished observation).

Despite differences in their biological behavior, strains of AD virus are not known to differ antigenically. Antibody to each one we studied was detectable by the CIEP test in which partially purified virus of the Utah-1 strain was used as antigen (4). Other tests for antibody likewise do not appear to distinguish immunogenically distinct strains of AD virus (8).

So far, too few strains (or isolates) of AD virus have been studied to know how greatly they differ from one another. Our limited comparison of the four strains attests to the variation that may be found, at least in terms of their pathogenicity for non-Aleutian mink. A more complete examination of these and other strains would provide a better understanding of the biological behavior of AD virus and of the relation this has to the natural history of infection in ranch mink.

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