NINDB Monograph No. 2, Slow, Latent, and Temperate Virus Infections, pp. 279–285.

Some Observations on the Natural Occurrence of Aleutian Disease [1]

JOHN R. GORHAM,[2] ROBERT W. LEADER,[3] GEORGE A. PADGETT,[4] DIETER BURGER [5] and JAMES B. HENSON [4]

Washington State University, Pullman, Wash.

The epizootiology of Aleutian disease (AD) is an enigma to the mink rancher and the researcher alike. At the outset, we must confess that we do not know the primary means by which the disease is spread among ranch mink. It appears that both vertical and horizontal transmission occur. Furthermore, the durations of the prodromal stage and the infectious period are unknown.

A historical survey of AD sheds considerable light on the rapidity of its spread throughout the United States and foreign countries. Prior to 1940, in the early years of the mink industry, the ranches were small and relatively inefficient. Mink were trapped in the area surrounding the ranch or occasionally were brought from as far away as Alaska or Labrador. They were considered to be what is termed a "standard dark" or "wild type" mink. The problems associated with a proper diet and the devastating outbreaks of distemper and botulism were the ranchers' main concern. Thus, one cannot say for certain whether an insidious disease such as AD was present in these first ranch-raised dark mink. However, if it was present, it smoldered along unnoticed or perhaps was confused with some other malady.

The first ranchers, not realizing the possibility of commercially important mutations, promptly disposed of "off-colored" mink. In 1941, an astute rancher near Astoria, Oreg., saved for breeding a gun metal colored mink that was noticed in a litter of standard dark mink.

The color phase was called "Aleutian" after the Aleutian fox which has a similar pelt color. The gene, which was inherited as an autosomal recessive trait, was designated by the symbol "*a*". By crossing Aleutian mink with other mutant mink, several substrains were developed which were collectively called "blue mink." Since the pelts were desirable colors, the mink were extremely valuable and commanded high prices. There was a brisk sale of breeding stock and most of the industry geared itself for production of these mutants. In the early forties, Aleutian mink were sold to ranches in various parts of the country. Soon ranchers began reporting losses due to a condition seemingly confined to "blue mink."

Mink ranchers first noticed the condition when their "blue mink" lost weight, although there was usually no noticeable drop in food consumption. Faulty digestion was manifested by cereals and/or other undigested food stuffs in the feces. Dark tarry droppings were occasionally observed. Affected mink exhibited polydipsia. About 10 to 20 percent of those visibly sick bled at the mouth. Ragged ulcers at the gingival border, usually involving the teeth, bled when the mink were handled. Ranchers called them "bleeders." The mink were anemic as evidenced by pale mucous membranes and foot pads. They became progressively thinner until death intervened.

Hartsough (*1*) first observed the gross lesions of AD in 1946. These lesions were recorded in "blue mink" shipped to the Midwest. At first the disease was thought to be limited to the *aa* genotype inasmuch as

[1] Aided by the National Institutes of Health Research Grants AI–AM–06477, AI–06591, and I–K3–AM–28231, Training Grants TI–GM414, TI–GM691 Mic., and the Mink Farmers Research Foundation. Washington Agricultural Experiment Station, Scientific Paper No. 2752.

[2] Animal Disease and Parasite Research Division, USDA.

[3] Department of Veterinary Pathology. Present address Rockefeller University, New York, N.Y.

[4] Department of Veterinary Pathology.

[5] Department of Veterinary Microbiology.

AA and *Aa* mink raised on the same ranch appeared resistant. Some ranchers tried to cope with the malady by crossing back their Aleutian mink to *AA* stock and/or breeding *Aa* and *Aa*. While the *aa* progeny was somewhat stronger than their original Aleutian breeding stock, they were still more responsive to the disease than *Aa* or *AA* mink.

As knowledge of the malady increased, it became known as "kidney disease." Depending on the stage of the disease, the kidneys were enlarged and reddened, or swollen, pale and pitted. Other necropsy findings included initial enlargement of the spleen and lymph nodes, and occasional yellowish brown mottling of the liver.

The Infectious and Contagious Nature

The losses from AD increased each year. Then a series of "vaccine incidents" occurred, which focused attention on the malady, suggesting that the disease might be of an infectious nature (*2*). Prior to the advent of chicken-embryo-origin attenuated distemper vaccines, it was common practice to prepare inactivated autogenous tissue vaccines. To make this vaccine, spleens were collected from distemper infected mink and ground with saline containing 0.3 to 0.5 percent formalin. After the mixture was incubated for a variable length of time—often dependent on how badly the vaccine was needed—it was injected. Following the use of these vaccines, widespread AD losses occurred. In 1949 one rancher lost 500 mink to AD after vaccination for distemper with such a vaccine. Floyd Marsh, a truly colorful mink raiser, said "they didn't make baskets big enough to pack out my dead mink."

A few years later a Connecticut mink rancher who had AD on his ranch decided to try one of these homemade vaccines against the disease. The result was reminiscent of the scrapie outbreaks which followed the use of louping-ill vaccine (*3*). Helmboldt and Jungherr (*4*) reported that nearly all of the inoculated mink were dead of AD after 6 months!

By 1950, it was apparent to many mink ranchers that AD was contagious. When mink from an affected ranch were brought to a "clean ranch," the disease appeared in the purchased breeding stock as well as in other mink on the ranch.. Such a prospective study was made in western Oregon in 1955 by our coworker, Dr. Keith Farrell. The rancher had many desirable mutations which, although he did not know it, were infected with AD. They were sold to several other ranches and new foci of disease appeared on these ranches. Overseas shipments by many U.S. ranchers of infected mink may have spread the disease to Scandinavia, England, Japan, Canada, West Germany, and elsewhere. However, the possibility exists that Aleutian disease was present but not recorded in these countries prior to the U.S. mink exports.

With the history of Aleutian disease in mind, we might speculate about the apparently sudden apearance of this "virus." Two alternatives appear possible: (1) the virus appeared about the same time and in the same area as the Aleutian gene mutation, or the virus might be associated with the Aleutian gene; (2) the virus was present in mink prior to selection of the Aleutian mutant but was not recognized until sufficient numbers of highly responsive *aa* mink became available in the ranch mink population. In considering the first possibility, it appears extremely unlikely that matching mutations would occur, not only at the same time, but in the same area.

The second proposal is not only more probable but is intellectually more acceptable. When the "blue mink" were shipped from the Oregon ranches, some were infected with AD while others were free of the disease. Infected animals established new foci of disease on clean ranches. On the other hand, when noninfected "blue mink" were sent to ranches where Aleutian disease was present in standard dark herds, the newly purchased "blue mink" served as sentinal animals by succumbing to AD.

There is no direct proof to support either of these possible explanations for the origin of the AD agent. However, if we could locate preserved tissues or tissue sections of standard dark mink with AD lesions in the late thirties, it would prove the latter possibility.

The Chediak-Higashi Syndrome

It was not until 1963 that a possible explanation for the remarkable genotype responsiveness of *aa* mink to AD virus was recorded. Leader, et al. (*5*) and Padgett, et al. (*6*) described a condition occurring in mink and cattle which resembled strikingly the Chediak-Higashi syndrome (CH–S) previously described in man(*6*).

In man, mink, and cattle, the syndrome appears to be caused by a simple recessive non-sex-linked gene. Among mink, the only animals affected with this condition are those which are homozygous recessive for the Aleutian gene "*a*." Thousands of mink have been examined, and we have never observed a mink with the *aa* genotype which does not have the CH–S.

TABLE I.—Losses From Naturally Occurring AD on One Ranch Comparing *aa* and *Aa* or *AA* Mink From December 1960 to December 1961

	Aleutian mink *aa*			Non-Aleutian mink *Aa* or *AA*		
	Total number of animals	Total death loss	Mortality rate (percent)	Total number of animals	Total death loss	Mortality rate (percent)
Adult females (animals carried over for breeding)	655	311	47. 5	740	44	5. 9
Adult male (animals carried over for breeding)	164	128	78	185	18	9. 7
Kittens (born during current year)	1, 800	162	9	2, 130	38	1. 8

In one study of CH–S in mink, Padgett, et al. (7) inoculated 40 *aa* and 40 *Aa* or *AA* mink with 10^4 ID_{50} of the AD virus. All animals developed Aleutian disease. Of those with the *aa* genotype, all but 4 were dead within four months after inoculation. In the same period, only 4 of the non-Aleutian (*Aa* or *AA*) animals were dead. After nine months, one-half of the latter group survived and 6 mink lived for more than a year. These findings and the results of similar work suggest that all mink are susceptible to AD, but early death occurs primarily in mink of the Aleutian genotype. Indeed, it was for this reason the name Aleutian disease was given to the condition. The difference in mortality rate between Aleutian and non-Aleutian mink in naturally occurring AD is illustrated in table I, and will be discussed under the section on mortality rate.

The idea that the genetic constitution of the host has a profound effect on the severity of disease caused by a particular virus is certainly not new. One strain of potato X virus kills one variety of potato, causes a mosaic in another and is completely asymptomatic in a third. All three varieties of potato are capable of transmitting the virus.

Seasonal and Age Incidence

The yearly loss pattern is related (1) to the age of the mink, and (2) to seasonal stress factors. To understand this pattern, a brief explanation of the yearly ranch operation is necessary (fig. 1). Mink are bred in March, generally whelp in May and the young kits are weaned in June or July. In late July and August, the litters are split to prevent fighting and subsequent pelt damage. At this time, the young mink (kits) are put into individual cages which vary somewhat in size on different ranches—generally about 12 x 18 x 24 inches. The cages are made of ½-inch galvanized wire mesh and usually contain a wooden nest box. They are placed about 2 inches apart and 18 inches off the ground. Both kits and adults are pelted in November or December at about 6 or 7 months of age or are held as breeding stock for the following year. Occasionally, productive healthy adult females are held for a second breeding season.

In adult-breeder female mink of any genotype, the first loss peak usually occurs in May during whelping (fig. 2). Losses occur in the early fall in both kittens and adults when they are subject to the stress of molting or during the first cold weather or sudden temperature changes. For example, in a herd of 10,000 mink where there is a fair amount of AD infection, 40 to 50 mink may succumb with an abrupt change in the weather. Failure of an automatic water-

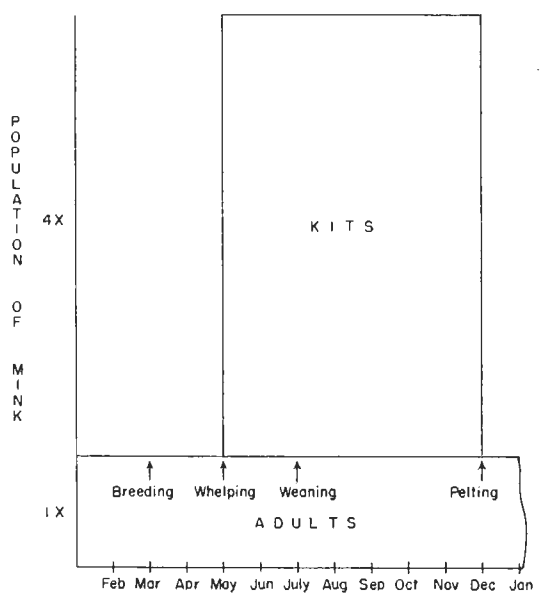

FIGURE 1.—The relative population of mink on a ranch through 1 year.

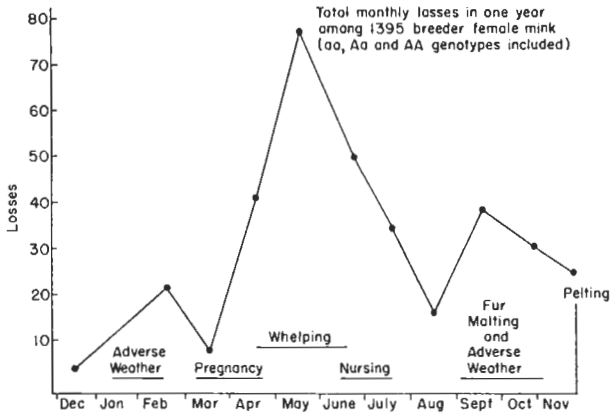

FIGURE 2

ing system in freezing weather will also result in a sharp loss peak due to the stress on the mink.

While adult losses occur usually in chronically infected animals in the terminal stages of the disease, fall losses following a short rapid course are observed in primarily *aa* animals. Losses also occur among *Aa* and *AA* kits, but it is rare to find heavy losses in these groups. *Aa* and *AA* infected animals frequently survive to pelting showing no sign of the disease.

Transmission

Vertical Transmission

The familial occurrence of deaths from AD was first recognized by ranchers who raised some of the early Aleutian and sapphire mink. Ranchers reported various patterns of AD deaths (fig. 3) but, in general, when the female was infected, it appeared more likely that her litter would contain infected kittens. If we assume that the kits are susceptible in the period prior to weaning and separation, it appears that if a dam is infected, there would be sufficient direct contact with her young in the confinement of a nest box to effect transmission. When the iodine agglutination test (I.A.T.) (*8*) became available, it seemed profitable to study the prevalence of the disease within families at pelting.

Henson, et al. (*9*), studied the problem using the I.A.T. and confirmed the ranchers' belief of the importance of the affected dam. Thirty-one families of mink were tested on a ranch having a large number of AD deaths. Thirty-two of 71 kits (45 percent) from 14 positive dams were positive to the I.A.T. Conversely, 15 kits of 78 (19 percent) from 17 negative dams were positive. Thus, vertical transmission from the dam to part of her litter must be considered.

Horizontal Transmission

Current ranch studies conducted at this laboratory (*7*) have revealed that horizontal transmission between families also occurs. Before and after separation, 145 families of mink with a total of 680 kits were examined at monthly intervals using the I.A.T. In this study, it was shown repeatedly that animals found to be negative by the I.A.T. for several months, which were housed in cages next to positive animals, converted to positive. In addition, on several occasions, the infection was traced from cage to cage, as far as four cages (64 inches) away from the original source of infection. While there may be as yet unrecognized explanations for this phenomenon, it does suggest horizontal transmission of Aleutian disease between families.

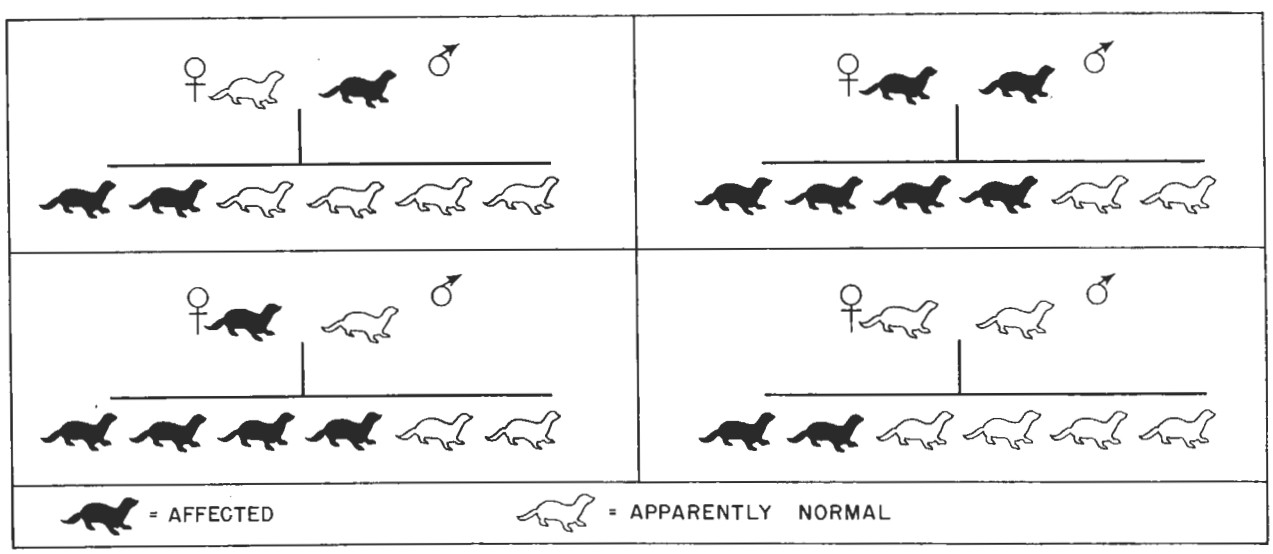

FIGURE 3.—The distribution of naturally occurring Aleutian disease among selected families.

Infectious Materials and Natural Transmission

The first solid evidence of a natural transmission mechanism was reported by Kenyon, et al. (*10*). In this study, urine was found to contain the virus. We confirmed this work and found virus in the feces as well (*11*). However, we could not exclude fecal contamination of urine or contamination of feces. In any event, it appears that the virus is present in the feces and/or urine. In addition to feces and urine, our study revealed that blood, serum, saliva, and bone marrow contained the agent, and of the materials tested, only colostral milk was apparently free. However, since only four animals were used in testing the last fluid, a larger trial involving more animals is needed before valid conclusions can be drawn.

The results of our efforts to learn of the natural routes or transmission are shown in table II. In this experiment, mink were also infected by oral and aerosol routes. It would appear further that infected spleen was more infective than feces; however, no attempt was made to quantitate the virus in the inocula.

The lack of an in vitro system for the detection of virus is a serious detriment to research on Aleutian disease. At present all tests for the presence of virus must be done in susceptible mink, which is an expensive procedure.

Infectious Period

The infectious period of AD appears to be of long duration. Kenyon (*10*) demonstrated the virus in the urine of an advanced case of AD. We have scanty evidence that virus was demonstrated in the serum of a mink 2 years after experimental infection. In this study, one of two test mink was infected. Presently, a trial which will offer more useful data is in progress.

TABLE II.—Material From Advanced Cases of Aleutian Disease Tested for the Presence of Virus by the Inoculation of Susceptible Mink

Source of Inoculum	Results [1]
Whole blood	5/5
Undiluted serum	15/15
Urine	6/9
Colostral milk	0/4
Feces	5/5
Saliva	5/5
Bone marrow	9/9
Normal mink control [2] (no exposure)	0/55

[1] Number of mink infected/number exposed.

[2] Includes 5 mink which were given 1 ml. inoculations of normal mink serum and 15 mink receiving 1 ml. of nutrient broth.

Case Fatality and Mortality Rates

If one considers AD in the context of ranch operations, it is impossible to calculate a case fatality rate, since the population of mink on all ranches is decimated at pelting time in November and December. Naturally, ranchers select for pelting those animals that are sick, unthrifty or have poor pelt quality and color. This accounts for low December and January losses when examining seasonal death loss patterns. They keep for breeding animals that are apparently healthy and of good size and color. Pelting precludes the calculation of the case fatality rate. It is commonly accepted that AD infected mink invariably succumb. This may well be valid when considering *aa* mink. On the other hand, there is now limited evidence that *Aa* or *AA* mink may be infected with Aleutian disease and recover if a rise plateauing, and subsequent fall to normal limits in serum gamma globulin is a valid criterion.

The mortality rate is dependent on whether *Aa, AA* or *aa* mink are involved. One of our test ranches, having a population composed almost entirely of "blue mink," had an increasing annual death loss until 1961 when it reached 700 deaths among 5,000 mink—a mortality rate of 14 percent. We compared mortality rates of naturally occurring AD in *Aa, AA* and *aa* mink on another test ranch in 1960 (table I). The table reveals the mortality rate to be 5- to 8-fold higher among *aa* mink than *Aa* or *AA* mink. The chronicity of the disease is indicated by the marked difference in mortality rate between kits and adults regardless of genotype.

Control

In 1961, Henson and his coworkers reported that AD affected mink exhibit a hypergammaglobulinemia (*12*). In 1962, the same workers adapted Mállen's iodine test(*13*) as a useful ranch procedure for the detection of subclinically infected mink(*8*). In our hands, the I.A.T. becomes positive when the gamma globulin is greater than 2 grams per 100 ml. of serum, and the A/G ratio = 1. Depending on the stage of the disease, the test has an accuracy of 75 to 95 percent. At this writing (May 1965), millions of mink have been tested in all mink-raising areas of the world.

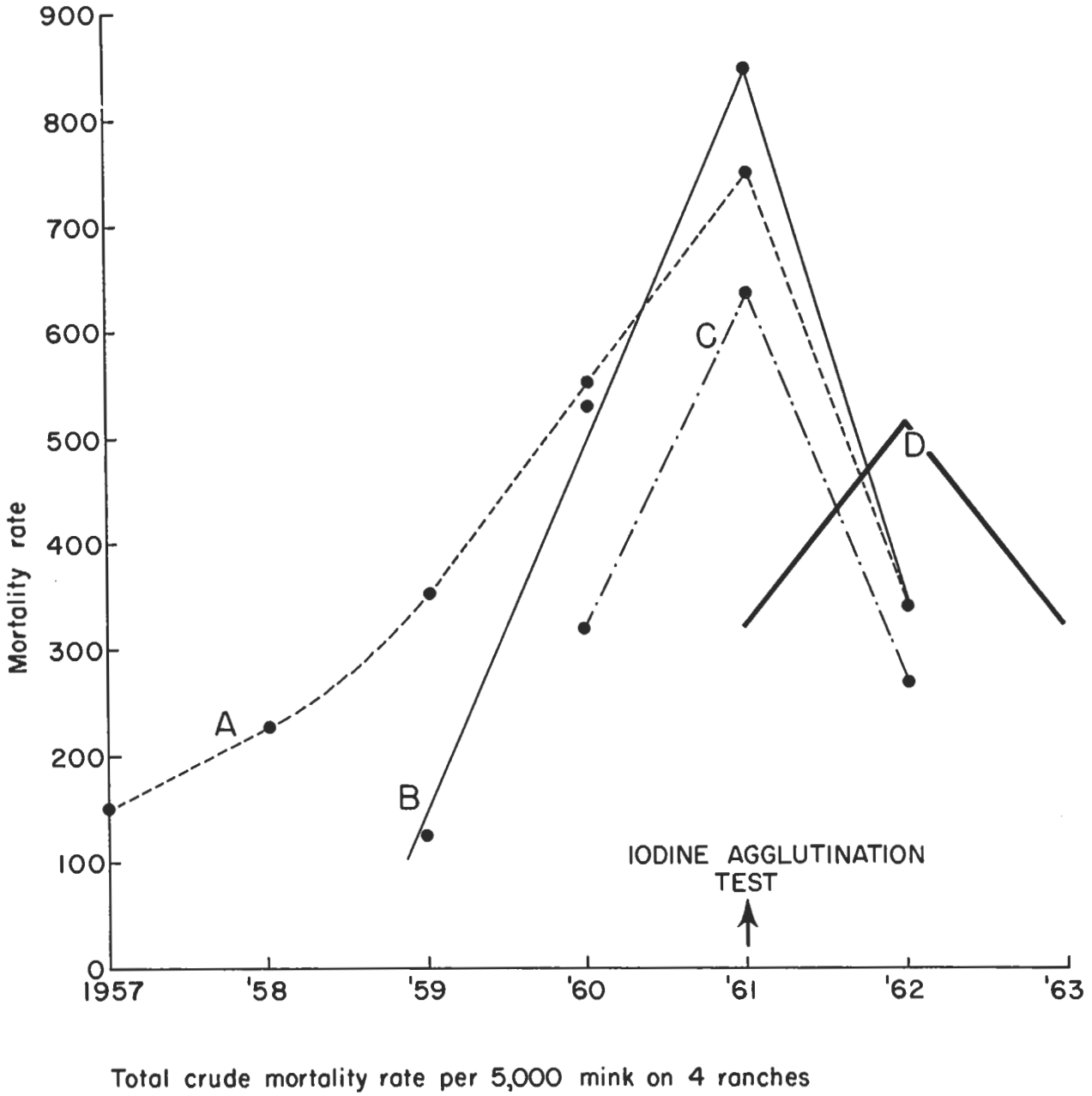

FIGURE 4

In figure 4, we plotted the total crude mortality, adjusted to 5,000 mink on 5 different western Washington ranches located within 25 miles of each other. On ranches A, B, and C, annual losses of increasing severity occurred through 1961. In November and December 1961, the iodine test was conducted on all mink on these ranches held over for breeding. Only mink which were I.A.T. negative were kept.

The mink on ranch D were first tested in November and December of the following year (1962). Therefore, we felt that we could consider ranch D an untested control. The value of the test is documented since the losses on ranch D were ascending during 1962 while the others were dropping. Furthermore, the losses declined in 1963 on ranch D, the year following the initial test.

The relationship of AD to sterility was shown by Olsoni and Kangas (*14*). They found that of 1,116 sterile females from 32 farms, 496 or 44.4 percent were I.A.T. positive. I.A.T. positive reactions varied on

individual ranches from 8.3 percent to 87.5 percent. The relationship between the I.A.T. results and the number of kittens per female is shown in table III.

At the present time, the iodine agglutionation test is the only measure which has proven effective in the control of AD. However, the I.A.T. has disadvantages. There is a period of about 3 weeks after infection before the test becomes positive, and ranchers cannot afford to kill infected animals other than when their pelt is prime.

TABLE III.—Relationship Between I.A.T. Reaction and Number of Kittens of Female Mink

Blood reaction I.A.T.	Number females	Total number kittens	Kittens/female
—	150	614	4.1
+?	15	51	3.4
+	53	166	3.1
2+	40	132	3.3
3+	32	46	1.8
4+	2		
	292	1,009	3.5

These are the results of iodine agglutination tests reported by Drs. Olsoni and Kangas of the State Veterinary Medical Institute, Helsinki, Finland.

Future

Before we can fully understand the natural history of AD, we should (1) confirm horizontal transmission circuits and investigate whether kits can be infected in utero or neonatally; (2) determine the duration of the infectious period; (3) determine if one or more strains of the AD agent exist; (4) study the range of host species involved.

One of the major difficulties which confronts investigators is detection of the AD agent. Presently, the only indicator host is the mink. Before real progress can be made, the agent must be adapted to a sensitive cell line or other convenient laboratory animal. Hopefully, an in vitro system for the detection of antibody will be found—if antibody occurs in this malady. Such a test would provide relevant information on the antigenic constitution and behavior of the agent in animals.

Aleutian disease will remain an enigma until we can determine the means by which the agent persists in nature from generation to generation of mink. Clearly, it will be some time before this disease is fully understood.

REFERENCES

(1) HARTSOUGH, G. R. Personal communication, 1946.

(2) HARTSOUGH, G. R., and GORHAM, J. R. Aleutian disease in mink. National Fur News, *28*, 10–11, 1956.

(3) GORDON, W. S. Scrapie Panel, United States Livestock Sanitary Association, Proceedings Sixty-Third Annual Meeting, 286–294, 1960.

(4) HELMBOLDT, C. F., and JUNGHERR, E. L. The pathology of Aleutian disease in mink. Am. J. Vet. Res., *19*, 212–222, 1958.

(5) LEADER, R. W., PADGETT, G. A., and GORHAM, J. R. Studies of abnormal leukocyte bodies in mink. Blood, *22*, 477–484, 1963.

(6) PADGETT, G. A., LEADER, R. W., GORHAM, J. R., and O'MARY, C. C. The familial occurrence of the Chediak-Higashi syndrome in mink and cattle. Genetics, *49*, 505–512, 1964.

(7) PADGETT, G. A. Unpublished data, 1965.

(8) HENSON, J. B., LEADER, R. W., and GORHAM, J. R. A field test for Aleutian disease—preliminary report. National Fur News, *34*, 8–9, 1962.

(9) HENSON, J. B., GORHAM, J.R., and LEADER, R. W. The familial occurrence of hypergammaglobulinemia in mink. Tex. Rep. on Exp. Biol. & Med., *21*, 37–42, 1963.

(10) KENYON, A. J., HELMBOLDT, C. F., and NIELSON, S. W. Experimental transmission of Aleutian disease with urine. Am. J. Vet. Res., *24*, 1066–1067, 1963.

(11) GORHAM, J. R., LEADER, R. W., and HENSON, J. B. The experimental transmission of a virus causing hypergammaglobulinemia of mink: sources and modes of infection. J. Inf. Dis., *114*, 341–345, 1964.

(12) HENSON, J. B., LEADER, R. W., and GORHAM, J. R. Hypergammaglobulinemia in mink. Proc. Soc. Exp. Biol. & Med., *197*, 919, 1961.

(13) MALLÉN, M. S., AGALDE, E. L., BALCAZAR, M. R., BOLIVAR, J. I., and MEYRAN, S. Precipitation of abnormal serums by Lugol's solution. Am. J. Clin. Path., *20*, 39–47, 1950.

(14) OLSONI, J., and KANGAS, J. Cited by Kull, K. E. Aleutian disease and its control. Våra Pälsdjur, *11*, 231–232, 1963.

785-990—66——20