Aleutian Disease (Plasmacytosis) of Mink
II. Responses of mink to formalin-treated diseased tissues and to subsequent challenge with virulent inoculum

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ABSTRACT

An experiment was carried out to examine the responses of Aleutian and standard dark types of mink to inoculations of formalin-treated suspensions of tissues of mink with experimentally transmitted plasmacytosis. Control groups of mink received similar injections of normal Aleutian mink tissues or diseased tissues without formalin treatment. A second experiment was conducted to test the formalinized diseased tissue suspension for immunogenic value. Groups of mink which received one, two, or three doses of "vaccine" were later challenged with virulent inocula. Additional groups of mink served as unvaccinated and environmental controls.

Treatment with 0.3% formalin with fine trituration and incubation at 37°C was effective in preventing the development of plasmacytosis in inoculated mink. These mink remained susceptible to subsequent challenge with untreated diseased tissue suspensions. No immunity was demonstrated in the vaccinated mink. Mink inoculated with normal mink tissues did not develop plasmacytosis, nor did uninoculated environmental controls.

Evidence from filtration experiments, suggesting that the transmissable agent of Aleutian disease is a virus, was the subject of the first paper of this series (1). Similar findings have subsequently been reported by others (2, 3). The production of Aleutian disease in mink by the inoculation of suspensions of formalin-treated tissues from diseased mink has also been reported (2, 4). The experiments described in this paper were devised to test the ability of a formalinized suspension of tissues from experimental cases of Aleutian disease to transmit the disease when inoculated into susceptible mink, or failing this, to induce a state of immunity in the inoculated animals to subsequent challenge with fully-virulent Aleutian disease tissue suspensions.

Natural and experimentally-induced occurrences of this disease in types of mink which are genetically unrelated to Aleutians has made the designation "Aleutian disease" somewhat of a misnomer. It is therefore proposed that "Plasmacytosis" be substituted as a more accurate descriptive term for this disease, characterized by proliferation of plasma cells throughout the body, especially in the lymph nodes, spleen, liver and kidneys (3, 5, 6).

Materials and Methods

All inocula used in these experiments were 15% suspensions of kidney, liver and spleen tissues from mink used in the second experimental passage of the plasmacytosis agent (1). Suspensions were prepared by finely grinding liver, spleen and kidney tissues in a colloid mill with cold 0.85% NaCl solution, with or without the addition of formalin to a concentration of 0.3%. Unformalinized suspensions were stored at -70°C until used.

The suspension prepared with formalin was incubated (immediately after grinding) for 24 hours at 37°C, then it was reground and incubated for a further 24 hours at 37°C. After this the formalinized suspension was stored at 4°C until used (19 days for experiment IV; 140 days for experiment V).

Liver, spleen and kidney tissues from normal unvaccinated Aleutian mink, ground in the same way, were not treated

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with formalin but were stored frozen at -70°C until used[1].

EXPERIMENT IV

Two Aleutian mink (A33 and A34) and four standard dark mink (SD35 to SD38, inclusive) were each inoculated intraperitoneally with 1.0 ml of untreated diseased tissue suspension from Aleutian mink. Five Aleutian mink (A31 and A51 to A54, inclusive) and five standard mink (SD32 and SD47 to SD50, inclusive) were each given intraperitoneal injections of 1.0 ml of the formalin-treated diseased tissue suspension. A third group of mink (5 Aleutians, A55 to A59 inclusive and 5 standards, SD60 to SD64, inclusive) were similarly inoculated with 1.0 ml quantities of the normal Aleutian mink tissue suspension.

Mink which died during the course of the experiment were examined for gross and histologic evidence of plasmacytosis. On the 113th day after inoculation all surviving mink in the group inoculated with the untreated diseased tissue suspension were killed for pathologic examination. One-half of the number of mink in each of the groups inoculated with formalinized diseased tissues and with the normal tissue suspension were also killed on day 113 post-inoculation. The remaining animals were challenged at this time with the fully-virulent suspension of diseased tissues by inoculation of 1.0 ml quantities by the intra-peritoneal route. These mink were held for a further 59 days for observation, then they were killed and examined for gross and histopathologic evidence of plasmacytosis.

EXPERIMENT V

An experiment was carried out to test the formalin-treated diseased tissue suspension for possible immunogenic value. Eight Aleutian mink and six standard mink were inoculated with 1, 2 or 3 doses of 1.0 ml of the formalin-treated tissue suspension, administered subcutaneously, as follows: On day 1 of the experiment all mink (Aleutians numbered A93 to A100 and standards SD101 to SD106, inclusive) were given 1.0 ml of "vaccine" subcutaneously. On day 11, with the exception of A93, A94, SD101 and SD102, the mink were given second 1.0 ml subcutaneous inoculations of vaccine. On day 25 mink A97 to A100 and SD105 and SD106 were given third 1.0 ml doses of vaccine. (This schedule of vaccination is presented in Table II).

On the 87th day of the experiment all mink were challenged with 1.0 ml doses of untreated diseased tissue suspension inoculated intraperitoneally. Eight unvaccinated control mink, four Aleutians and four standards, also were inoculated at this time with the same dose of challenge inoculum. Six mink, four standards (SD143 to SD 146) and two Aleutians (A147 and A148) held in adjacent cages and fed the same ration, were cared for by the same attendant as were the inoculated animals. These were regarded as unvaccinated and "unchallenged" environmental controls. Mink which died and all survivors, killed on days 184 to 205 of the experiment, were examined for gross and microscopic evidence of plasmacytosis.

Observations

EXPERIMENT IV

Aleutian mink A33 was found dead on the 44th day after inoculation with the untreated diseased tissue suspension. Although there were pronounced lesions of plasmacytosis, the precipitating cause of death was found to be a hemolytic *Escherichia coli* septicemia. Aleutian mink A34 and standard dark mink SD36 were killed when found moribund on days 65 and 81 post-inoculation, respectively. Both animals were found to have the enlarged spleens and mottled kidneys and livers characteristic of plasmacytosis, as well as marked plasmacyte proliferation in these organs, demonstrated histologically.

Of the 14 mink killed for examination on the 113th day after inoculation, only the animals inoculated with the untreated diseased tissue suspension possessed lesions of plasmacytosis. The mink in the groups inoculated with normal Aleutian mink tissues and formalinized diseased tissues were found to have no gross or histologic signs of disease, except in those cases where "vaccinated" animals were later challenged with the virulent inoculum. These data are summarized in Table I.

EXPERIMENT V

Seven days after receiving the third dose of formalin-treated diseased tissue suspension, Aleutian mink A97 was found

[1]Grinding of tissues and treatment with formalin was performed with the assistance of personnel of the Connaught Medical Research Laboratories, Toronto, Canada.

dead. Gross examination of liver, lymph nodes and spleen revealed nodular granuloma-like lesions, suggesting tuberculosis. Histologic examination identified the lesions as granulomas; however, acid-fast organisms could not be demonstrated in sections stained by the method of Ziehl-Neelsen.

One hundred and seventy-nine days after start of the experiment, mink A138 was found moribund and was killed for pathologic examination. A diagnosis of plasmacytosis was made.

On the 183rd day of the experiment mink SD103 was found dead. Examination of the carcass was not made because of advanced postmortem decomposition. Mink A136, killed next day, the 184th day of the experiment, was found to have typical lesions of plasmacytosis. Mink A100 was found on the 186th day to have a severe cervical abscess and was killed at this time for pathologic examination. Lesions characteristic of plasmacytosis were found. On day 199, mink A96 was found moribund and killed. A diagnosis of plasmacytosis was made.

All survivors were killed for examination on the 205th day of the experiment. The histologic diagnoses made are summarized in Table II.

Discussion

It is at once apparent from the results of experiment IV, presented in Table I, that the untreated diseased tissue suspension was fully virulent and capable of inducing plasmacytosis in both Aleutian and standard dark mink when inoculated in 1.0 ml amounts by the intraperitoneal route. Tissues from normal Aleutian mink, on the other hand, were incapable of causing this response when given in the same way.

It is apparent also that the virulent tissue suspension, after treatment with formalin, was no longer able to induce plasmacytosis in mink: nor was it able to afford protection to mink "vaccinated" and later challenged with the untreated diseased tis-

TABLE I — Results Obtained in a Third Serial Transmission of Plasmacytosis with Untreated and with Formalin-Treated Tissue Suspensions (Experiment IV)

Mink	Type	Inoculum	Death	Histologic Diagnosis
		Diseased		
A33	Aleutian	Tissue suspension	D44*	Plasmacytosis
A34	Aleutian	" "	K65	"
SD35	Standard Dark	" "	K113	"
SD36	" "	" "	K81	"
SD37	" "	" "	K113	"
SD38	" "	" "	K113	"
		Diseased tissue		
A31	Aleutian	Formalinized suspension	K113	Normal
A51	"	" "	CH113, K172	Plasmacytosis
A52	"	" "	K113	Normal
A53	"	" "	K113	"
A54	"	" "	K113	"
SD32	Standard Dark	" "	K113	"
SD47	" "	" "	K113	"
SD48	" "	" "	CH113, K172	Plasmacytosis
SD49	" "	" "	CH113, D168	Not examined
SD50	" "	" "	CH113, K172	Plasmacytosis
A55	Aleutian	Normal Tissue Susp.	K113	Normal
A56	"	" " "	CH113, D171	Plasmacytosis
A57	"	" " "	CH113, K172	"
A58	"	" " "	K113	Normal
A59	"	" " "	CH113, K172	Plasmacytosis
SD60	Standard Dark	" " "	CH113, K172*	Plasmacytosis
SD61	" "	" " "	CH113, K172	"
SD62	" "	" " "	K113	Normal
SD63	" "	" " "	K113	"
SD64	" "	" " "	K113	"

*Numerals in this column indicate the day postinoculation on which the animal died (D) was challenged (CH) with virulent suspension or was killed (K) for examination.

sue suspension, when the formalinized vaccine was given by the intraperitoneal route.

It was reasoned, however, that slower absorption and better immune response might result from formalinized suspensions inoculated subcutaneously. The results of experiment V, however, as summarized in Table II, indicate that as many as three spaced doses of the vaccine, inoculated subcutaneously, were unable to stimulate the development of demonstrable immunity in mink later challenged with a virulent inoculum. It can be argued, however, that the type of challenge given bears no resemblance to natural means of exposure, and being much more severe than any exposure to virulent materials that might occur under natural ranch conditions, it is an unfair test of the vaccine. This may be true. The fact remains, however, that a really effective vaccine should show at least some protection under the most severe challenge. It is our intention, therefore, to discard the method of direct formalinization of tissues as a means of attempted vaccine preparation in favor of other methods. Perhaps the preparation of a living attenuated vaccine is possible, by adaptation of the agent to a host other than the mink; or failing this, attempts may be made to obtain a useful preparation by inactivation of the agent by means other than formalin treatment.

Failure of our formalin-treated suspension of tissues from cases of plasmacytosis to induce the disease on inoculation into susceptible mink is at variance with the observations of Henson and co-workers (2, 4). This apparent disagreement may be explained on the basis of differences in time and temperature of incubation with formalin and in trituration of tissues before and during formalin treatment (2). Henson *et al* (2) found that suspensions of diseased tissues treated with 0.3% formalin would cause hypergammaglobulinemia (characteristic of Aleutian disease) in mink inoculated after the formalinized material had

TABLE II — Results of an Experiment to Examine a Formalin-Treated Diseased Tissue Suspension for Possible Immunogenic Effects in Mink

Mink	Type	Days of Vaccination*	Day of Challenge	Day of Death	Histologic Diagnosis
A93	Aleutian	1	87	K205	Plasmacytosis
A94	"	1	87	K205	"
SD101	Std. Dark	1	87	K205	"
SD102	" "	1	87	K205	"
A95	Aleutian	1, 11	87	K205	"
A96	"	1, 11	87	K199	"
SD103	Std. Dark	1, 11	87	D183	Not examined
SD104	" "	1, 11	87	K205	Plasmacytosis
A97	Aleutian	1, 11, 25	none	D32	Granulomatosis
A98	"	1, 11, 25	87	K205	Plasmacytosis
A99	"	1, 11, 25	87	K205	"
A100	"	1, 11, 25	87	K186	"
SD105	Std. Dark	1, 11, 25	87	K205	"
SD106	" "	1, 11, 25	87	K205	"
A133	Aleutian	none	87	K205	"
A134	"	"	87	K205	"
A135	"	"	87	K205	"
A136	"	"	87	K184	"
A137	"	"	87	K205	"
A138	"	"	87	K179	"
SD139	St.d Dark	"	87	K205	"
A140	Aleutian	none	87	K205	"
SD141	Std. Dark	"	87	K205	"
A142	Aleutian	"	87	K205	"
SD143	Std. Dark	"	"	K205	Normal
SD144	" "	"	"	K205	"
SD145	" "	"	"	K205	"
SD146	" "	"	"	K205	"
A147	Aleutian	"	"	K205	"
A148	"	"	"	K205	"

*Numbers in columns 3, 4 and 5 represent days of the experiment on which mink received doses of vaccine (column 3); were given virulent inoculum (column 4); or were found dead (D), or were killed (K) for examination.

been stored for two weeks at 5°C but not after 40 weeks storage at this temperature.

In our experiments, the demonstrated high virulence of fractions of the diseased tissue suspension which were not treated with formalin and conversely, the demonstrated innocuousness of suspensions of tissues from normal mink, may be taken as further evidence of the presence in the diseased tissues of a transmissable agent, an agent which can be inactivated by formalin.

SUMMARY

A formalin-treated suspension of tissues from experimental cases of plasmacytosis (Aleutian disease) in mink failed to cause development of the disease when inoculated into susceptible mink. Mink vaccinated with one, two or three doses of this formalin-treated diseased tissue suspension did not possess demonstrable immunity when later challenged with virulent inoculum.

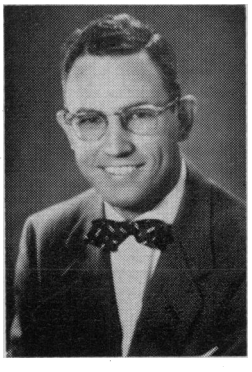
REFERENCES

1. **KARSTAD, LARS,** and **PRIDHAM, T. J.** Aleutian Disease of Mink. I. Evidence of its Viral Etiology. Can. Jour. Comp. Med. & Vet. Sc. **26** (5) May, 1962. pg. 97-102.
2. **HENSON, JAMES B., GORHAM JOHN R., LEADER, ROBERT W.,** and **WAGNER, B. M.** Experimental Hypergammaglobulinemia in Mink. Jour. Exp. Med. **116** (3) Sept., 1962. pg. 357-364.
3. **TRAUTWEIN, G. W.,** and **HELMBOLDT, C. F.** Aleutian Disease of Mink. 1. Experimental Transmission of the Disease. Am. Jour. Vet. Res. **23** (97) Nov., 1962. pg. 1280-1287.
4. **HENSON J. B., LEADER, R. W.,** and **GORHAM, J. R.** Hypergammaglobulinemia in Mink. Proc. Soc. Expr. Biol. Med. **107**. 1961. pg. 919-920.
5. **HELMBOLDT, C. F.,** and **JUNGHERR, E. L.** The Pathology of Aleutian Disease in Mink. Am. Jour. Vet. Res. **19**. 1958. pg. 212.
6. **OBEL, ANNA-LISA.** Studies on a Disease in Mink with Systemic Proliferation of the Plasma Cells. Am. Jour. Vet. Res. **20** (75) 1959. pg. 384-393.

News and Views

Setting for AVMA Centennial

Symbolizing the growth of the AVMA during the past 100 years is the striking contrast between the 50-story Americana Hotel, site of the Centennial convention, and the 5-story Astor House (inset), site of the Association's first meeting, June 9-10, 1863. The Astor House, which was located in the heart of downtown New York, no longer exists, but AVMA records reveal that the first meeting occupied one room and was attended by 40 veterinarians, physicians, and other scientists from 7 states. AVMA's Centennial convention will use 6 ballrooms, 29 meeting rooms, 30,600 sq. ft. of space for commercial exhibits, and 5 restaurants in the Americana Hotel alone, and more than 2,000 guest rooms



Dr. Jack O. Knowles, AVMA president-elect, who will begin his term as AVMA president at the conclusion of the Centennial Convention.