AN INVESTIGATION INTO THE NATURE

AND

ETIOLOGY OF ALEUTIAN DISEASE OF MINK

A Thesis

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by

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"One prime fault of man, over the whole spectrum of his activities, is that he so often fails to determine the nature of the problem before he sets about solving it."

Anon.

ABSTRACT

An Investigation into the Nature and Etiology of Aleutian Disease of Mink.

Evidence is presented which indicates that:

A. Aleutian Disease of mink is a genetically conditioned disease related to the progressive hyperplasia of multipotential cells. Such cells are situated mainly in tissues capable of active hematopoiesis. A feature of the disease is the relative increase in the number of plasma cells found in situ in the bone marrow of affected mink. An increase in quantity of one or more serum immunoglobulin types occurs with such in situ plasmacytosis, in a constant manner. Such dysproteinemia is often detected by an iodine precipitation test, prior to the onset of other manifest signs of the disease.

B. The development of the disease seems to depend upon prior genetic conditioning followed by a triggering stimulus which is non-specific in nature.

Evidence is presented that the triggering stimulus is unlikely to be a microbial contagious, self-replicating agent of the classically recognized type.

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I N T R O D U C T I O N

The subject of cancer, the uncontrolled proliferation of cells, is one of the most serious medical problems confronting humanity. Much study of this disease has been made in animals (1) and abundant evidence shows that certain forms, eg. mouse mammary tumors, fowl leucosis, (2) are associated with the presence of viruses. The role of these viruses has yet to be elucidated.

Aleutian Disease, (AD), of mink is characterized by a systemic uncontrolled proliferation of the plasma cell series (3) and, in the clinical form, is invariably fatal. (4) During recent years, several studies relating to the transmissibility of AD, (5, 6,7,8,9) its possible viral etiology and pathogenetic mechanisms, (10,11,12,13) have stimulated renewed medical and veterinary interest in this syndrome. One instance of the disease in a mink rancher has been reported, (14) although subsequent study of the immunoglobulins of mink-associated workers has revealed no abnormalities which are characteristic of the disease in mink. (15) Occurrence of the Chediak-Higashi Syndrome (CHS) in Aleutian mink (16,17) presents opportunities for the investigation of this disease in man. The possible implication of CHS in connection with AD might also prove worthy of further research.

With regard to AD, the precise nature of the etiology and pathogenetic mechanisms remain in doubt. Review of the literature indicates that, while a filter passing agent may be responsible, conclusive proof is lacking. No specific agent has been demonstrated in the tissues of affected mink, yet, when cell-free

extracts of such tissues are injected into (normal) mink, lesions, characteristic of the parent disease, are produced. In addition, the severity of the resulting disease may vary with the recipient genotype. Furthermore, it is impossible to say if a given mink harbours the disease or agent in a latent form. On these grounds, it is also impossible to deduce whether the disease results from a specific agent, which is present in the inoculums used for experimental transmission, latent in the test animal, both or neither.

It has been previously shown that certain non-infectious materials, when injected into susceptible animals, can induce the development of tumors. (18,19,20) The mink disease is, basically, one affecting the lympho-reticular system. An active and prolonged stimulator of this system is adjuvant, which for the same reason, is a common component of vaccines. The mode of action of adjuvants, eg. aluminum, and their ability to reinforce the immune response, is not fully understood but one explanation is that they cause aggregation of the antigens which are thus more readily phagocytosed by the reticulo-endothelial system (RES). (21)

The predisposition of certain genotypes, both for the spontaneous disease and the experimental form, poses the question that a possible defect exists in the RES of such mink. It may be fairly asked whether AD is a manifestation which results from the activity of an agent, latent or acquired, which possesses oncogenic properties in normal mink, or, the manifestation of an inherently defective RES, which responds blindly, excessively and non-specifically, to one or many repeated (antigenic) insults.

The present study was undertaken to determine if AD could be provoked or accelerated by a non-specific stimulus, (adjuvant), and to compare such manifestations of the disease with those resulting from the use of diseased organ suspensions.

REVIEW OF THE LITERATURE

GENERAL

Aleutian Disease of mink, as will later become apparent, meanders elusively among the major fields of medicine. To review, in an objective manner, the relevant work of medical and veterinary scientists which has appeared in the literature to date would be an extensive task and require a comprehensive knowledge. Because the disease is still conjectural in several respects, this review is intended largely to report the work of various investigators on different aspects of AD except with regard to the etiology which will be critically evaluated as it is of special significance in relation to this study.

The mink belongs to the family Mustelidae which also includes the weasels, otters, skunks and wolverines. The best known species are - Mustela vison of North America and M. lutreola of Europe. Another species, M. siberica of eastern Asia, links the true mink with the polecats. Sub-species of M. vison, notably M. vison vison, M. vison melanpeplus and M. vison ingens, have largely contributed to the development of the modern ranch mink.

The pelt or pelage consists of a dense soft matted underfur mixed with long stiff lustrous hairs. The color of the pelt in nature varies from light to dark brown. Selective breeding during the last 20 years has enabled mink ranchers to obtain a wide variety of pelt shades which depend on the shape, size and concentration of the pigment granules in the hair.

The coat color of the Standard Dark mink results from the interaction of at least 18 genes, of which five are dominant and the remainder either recessive or mutant. The inheritance of coat color appears to follow the pattern of simple Mendelian laws. Descriptions of recessive color phases among mink are given below:

NAME	GENETIC SYMBOLS	COLOR PHASE
Royal Pastel	bb (single)	Light brown
Platinum	bb (single)	Metallic grey
Aleutian	alal (single)	Clear dark grey
Sapphire	al al pp (double)	Clear blue-grey
Winter Blue	al pp bb (triple)	Clear pale blue-grey

The Sapphire was by far the most popular and profitable of the double recessives. In 1941 a spontaneous mutation occurred on a mink ranch in Oregon and the color phase was called Aleutian. The early Aleutians were difficult to raise and possessed various physiologic defects. (16,17) Out-crossing to the Standard Dark mink appeared to overcome some of these weaknesses and because of the attractive color of the Aleutian pelt, the strain was bred in large numbers and distributed to the major mink raising areas of America and across the world.

HISTORY, DISTRIBUTION AND INCIDENCE

In a review of the disease, (22) it is reported that the first observation of the malady was made by Hartsough in 1946. Carriers of the Aleutian gene were chiefly affected and displayed symptoms of oral bleeding and cachexia. Hepato-splenomegaly and renal lesions were consistent findings at autopsy. At that time, the disease was ascribed to the mutant character of the affected mink and little systematic investigation occurred until 1958 when losses were becoming increasingly severe in mink homozygous for the Aleutian gene. By the early 1960's, AD had been reported from all the major mink ranching countries of the world, not only in Aleutian mink, but other color phases. The economic losses caused by AD stimulated much interest in its pathology, causation and control. In 1961 it was discovered that affected mink developed serum protein abnormalities (22) and the following year a field test, (23) depending on the precipitation of abnormal serums by Lugols Iodine, (24) was used to detect subclinical cases and has become a popular control measure. [Iodine Agglutination Test, (IAT)].

Mass testing revealed a much greater number of mink with abnormal serums than had hitherto been suspected. (25) Reactors to the test have been found in twelve color phases, the highest percentage occurring in Aleutians and Standard Darks (greater than 20%) and lowest in Pearls and Sages (7%).

A survey of 1272 mink pelted in 10 Utah ranches showed incidences from 1 - 75% reactors, the mean incidence among all genotypes being 55%, as determined by gross and histological examination. (26)

The youngest mink which have been found with lesions were two months old. (4) Since mink are commercial animals and are not allowed to die of senility, the upper age limit is not known.

The incidence of clinical disease can be markedly reduced if breeding animals are mated once and pelted before the next breeding season. (27) This suggests that age may be an important factor in the expression of AD.

Attempts to explore the relationship of sex and the incidence of AD have not established a positive correlation. (28,10,29)

CONTROL

Methods to control AD have been based on the use of the IAT and on the supposition that an infectious contagious agent is responsible for the disease.

It has been shown that Lugol's iodine solution, such as is used in Gram's stain, causes the precipitation of proteins from certain abnormal serums. Precipitation is related to the albumin: globulin ratio and becomes apparent as this ratio approaches 1:1. (24) The degree of reaction increases as the ratio decreases from this point and is graded from fine, granular, slow precipitation, (1 plus), to rapid, complete, gelation of the mixture, (4 plus).

A field test, based on these reactions, has been used for the detection of dysproteinemia which occurs in established cases of AD in mink. (23)

Extensive use has shown that this test consistently indicates those animals in which serum gammaglobulin exceeds 30% of the total protein, but is unreliable in affected animals in which the serum gammaglobulin is below this level. (30) The normal range of mink gammaglobulin is 5% - 14%.

It will be apparent that, because of its failure to detect incipient disease, the IAT is of limited value. In the individual animal, the test may offer some guide regarding life expectancy because mink giving minor reactions may live a year or more. (31) Exceptional cases have been known where, in spite of consistent, moderate to severe IAT reactions, the mink survived and bred normally for several years. (27)

In general, the IAT results serve to indicate the presence and progress of AD in a mink herd and which animals might be suitable as breeding stock.

Whether eradication of AD has been accomplished in a given herd has not been reported. Electrophoretic analysis of serum proteins, which is far more sensitive than the IAT, still fails to find up to 10% of affected mink. Rigorous sanitation coupled with the latter method of detection, did not succeed in eradicating the herd disease after a two year program. (30)

It is evident from the above that, until further research reveals the true nature of the disease, control and/or eradication will be problematical.

CLINICAL AND PATHOLOGICAL FEATURES

The important clinical and pathological features of AD have been well described by Wilson in his review. (22) In the mink herd, infertility, poor litter averages and mortality in newly born kits, occur. Increased mortality and morbidity appear in older animals when stress conditions prevail.

In individual mink, loss of weight, enlargement of the cervical lymph nodes - followed by regression, hemorrhages from the mucous membranes, progressive debility, polydipsia and cachexia characterize the illness. Uremia and terminal infection are frequently observed before death. With the onset of signs, death invariably results, usually within a few weeks. The occurrence of an accompanying febrile reaction has not been definitely shown.

Autopsy findings comprise hepatosplenomegaly, lymphadenopathy and enlarged, pale, or shrunken, pitted kidneys. Evidence of secondary pneumonia is frequent.

Bone Marrow

The bone marrow appears greyish red and smears show plasma cells singly or in groups. (3) Frank plasma cell tumor formation and accompanying bone lesions as in human myelomatosis are absent. (13)

Lymphatic System

Carcass or visceral nodes may show atrophy of the lymphoid follicles and engorgement of the medullary cords by mostly mature plasma cells. (13) Histiocytic hyperplasia has also been recorded. (4)

Spleen

The organ is enlarged but maintains its architectural integrity. The red pulp becomes increasingly prominent due to the proliferation of endothelial and plasma cells of various ages. (4) Erythropoiesis may be evident and megakaryocytes are present. (3)

Liver

Lesions are found early in the disease. Variably sized collections of mature plasma cells and lymphocytes occur in the portal triads and around the sinusoids anywhere in the parenchyma. (4) Bile ducts of the triads appear to bud from the main duct. The epithelial cells of the buds, however, do not contain bile, but a proteinaceous material. Both basophilic and pink staining plasma cells with Russell bodies, have been observed in the halo of mononuclear cells surrounding the bile ducts. (32)

Kidneys

Although renal lesions tend to appear later in the course, they may coincide with, or precede, those in the liver. (4) Early lesions consist of scattered focal or diffuse groups

of mononuclear cells, chiefly mature plasma cells and lymphocytes, distributed interstitially throughout the cortex often in perivascular array. As the infiltration proceeds, mechanical and physiologic impairment of the nephrons ensues. (11) Fibrinoid arteritis is a regular finding in certain color phases. (33,32,22)

Endothelial cells of the glomerular tuft may be initially hyperplastic but eventually undergo degenerative changes. Ultrastructural observations as well as histochemical techniques, reveal subendothelial deposits of fibrinoid material accompanied by thickening and eventual disruption of basement membranes. (34, 32) The latter show changes in tinctorial characteristics indicative of the presence of glycoproteins. The basement membrane thickening may be secondary to the narrowing of capillaries resulting from the mesangial pattern of endothelial proliferation. The glomerular tuft ultimately degenerates into a hyalinized amorphous mass.

Bowman's capsule encloses a steadily increasing amount of glycoprotein material which may extend throughout the length of the nephron lumen. (11) Similar endothelial cell and basement membrane changes occur as in the capillary tuft.

The proximal and distal tubules are gradually separated - and later compressed - by infiltrations of mononuclear cells leading to distortion and atrophy of the nephron. Cytoplasmic glycoprotein inclusions are found in the tubular epithelium or free in the lumen. (35) Immunofluorescent studies suggest that

these are probably gammaglobulin. (46) Similar basement membrane changes, as in the glomerulus, take place. Hematuria and calcification have been reported in the medullary portions of the nephrons. (11)

Central Nervous System

In the central nervous system, diffuse and focal gliosis becoming nodular with lymphocytic tufts have been reported, as well as random, periarteriolar cuffing of vessels in the meninges, choroid plexus and pia mater. (4,3)

Lungs

Peribronchial and perivascular arterial cuffing with the typical cells (4) and alveolar cell proliferation and the presence of glycoprotein material within the alveolar spaces (33) have been reported.

Vascular System

Perivascular aggregates of mononuclear cells may be found in tissues or organs besides the prime locations in the liver and kidneys. Distribution is random, but the CNS, myocardium, bladder, kidney and genital tissues are commonly involved. Medium sized arteries and arterioles are exclusively affected. The lesions are constant in development but vary in the severity of vascular damage. The latter is greatest in mink of the Aleutian type of which about 20% develop fibrinoid arteritis during the

course of the illness. The sequence of changes in fibrinoid arteritis has been discussed by several authors. (11,32,33)

Henson (11) observed four stages in the development of fibrinoid arteritis, all of which may occur in the same animal and within the same organ. No thrombosis was present at any stage.

Initially, perivascular cuffing with histiocytes and plasma cells, in intimate association with the tunica adventitia, occurs. The normal agonal, sinuous contraction of the internal elastic membrane is absent. Scattered irregular areas of degeneration occur in the middle and peripheral zones of the tunica media. Within the areas, the circumfibrillar structure and normal tinctorial properties are replaced by amorphous granular material with increased affinity for Periodic Acid Schiff (PAS) Stain.

In the second stage, focal or diffuse myolysis with hemorrhage into the lysed areas is apparent. Structural integrity of the smooth muscle is lost and the lysed cells are replaced by pink or tan colored material which may be homogenous and granular or composed of fibrillar masses. Increased amounts of PAS positive material occur and are more prominent in the adventitial portions of the media. This stage is followed by frank fibrinoid necrosis of the periphery of the medial tunic, decreasing in degree towards the center of the vessel. The fibrinoid material stains homogeneously with Hematoxylin and Eosin (HE) and PAS and may contain

mononuclear as well as a few polymorphonuclear leucocytes. At this stage, proliferation of the endothelial cells of the intima, accompanied by mild mononuclear infiltration is apparent although the fibrinoid changes never extend past the internal elastic lamina. Finally, occlusion of the lumen by intimal cells and mononuclears occurs. The outer zones may show vestiges of fibrinoid among loose laminae of fibroblasts, and mononuclear cells. The description given by Karstad (33) is in agreement with the above, with the observation that the earliest changes described, appear to involve the cement substance between the smooth muscle cells, which becomes thickened and disorganized and stains prominently with PAS.

GENETIC STUDIES

Aleutian Disease was so named because it was first detected clinically in mink homozygous for the Aleutian gene. In spite of this, published research concerning the relationship of genetic factors to AD is scanty. Two reports, which appear to be somewhat conflicting, are available. The tendency of kits to develop hypergammaglobulinemia, (HGG), has been shown to be significantly greater if the dams are affected. (28) Another study (29) dealt with the incidence of dysproteinemia in progeny from the following matings - normal homozygous Aleutians, affected homozygous Aleutians, and normal Aleutian males crossed with

normal heterozygous females. No differences occurred in the number of affected kits, (as determined by serum electrophoresis), in litters derived from either of the homozygous matings. A significant decrease in the number of kits which developed HGG was observed in litters obtained from the homozygous/heterozygous cross. Failure to conceive, poor litter sizes, general morbidity and mortality up to the time of pelting, were also less serious in the latter group.

Inasmuch as coat color inheritance follows simple Mendelian laws, the tendency to develop HGG could not be explained on this basis. The presence of the disease in homozygous Aleutian parents did not change the incidence of the disease in their progeny, whereas it was reduced by the heterozygous condition. On this evidence, Hemmingsen concluded that the Aleutian gene exerts pleiotropic effects with regard to physiology and susceptibility to AD, and that the predisposition depends not on one gene, but a polygenic system.

CLINICAL PATHOLOGY

Serum Proteins

The fact that AD-affected mink had an accompanying HGG was first reported privately. (22)

Ranch mink, both Aleutian and non-Aleutian types have similar serum protein levels. The total protein, by biuret method, is 5.6 gms %, and contains 3.3 gms % albumin and 1.0 gms %

gammaglobulin, as determined by paper electrophoresis and densitometer tracings. Affected mink show an increased total protein (up to 8.1 gms %) and the albumin:globulin ratio is reversed. The albumin level is reduced to 1.7 gms % whereas the gammaglobulin is increased up to 4.5 gms %. (37) Similar results have been recorded elsewhere. (10,38,39,40) The degree of HGG seems to be regularly related to the degree of plasma cell proliferation present in a given mink. (37,38)

Ultracentrifugation of normal and AD serums shows that the increased globulin is a 7S* gamma component. (10,38) The presence of an increased 7S component and two additonal components of heterogeneous sedimentation behaviour, ranging from 9S to 17S and 22S to 25S and which form up to 6% of the total protein, has more recently been described. (39) Normal and AD euglobulins have also been shown to display differences in solubility related to pH, when added to potassium chloride solutions. (41)

When subjected to paper or starch-gel electrophoresis, AD gammaglobulins are heterogeneous in mobility. (10,41) This was also found in cellulose acetate electrophoresis. In the latter experiment, a small number of animals with the natural disease were serially studied and their serums showed transition from the heterogeneous diffuse HGG to a discrete homogeneous type of either beta or gamma mobility, similar to that of myelomatosis. Splitting

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*Svedberg units

of the gammaglobulin arc was also evident in some mink, when the serums were examined by immunoelectrophoresis (IEP). The split arc when present was constant for that particular animal and indicated some difference in the antigenic structure of the paraprotein. (13)

Attempts have been made to compare the metabolism of serum proteins in normal and affected mink. (39) Following the injection of radio-active (I 131) albumin or gammaglobulin, no differences were observed with regard to the biologic half-lives of the albumin in the two classes of mink but a significantly lower half-life of gammaglobulin was evident in the affected animals. No significant protein loss occurred in the urine of these mink during the experiment.

In summarizing the investigations of the serum protein changes occurring in affected mink, there is substantial evidence that an increase in total protein occurs and that the increase is related to an increase of gammaglobulin. Further, this gamma-globulin does not differ from that of normal mink on the bases of net surface charge, molecular density, sedimentation behaviour or electrophoretic mobility. Limited work suggests that there is also a similarity in the major antigenic components.

Blood Biochemistry

The blood chemical changes found in the serums of seventy-four Pastel and Aleutian mink of both sexes which were examined

during the pelting season have been reported. Mink from AD herds and from a ranch where the disease was absent, were used. Presence of AD was determined by histopathological findings and IAT reactions. Standard procedures compiled by the American Association of Clinical Chemists were adopted to measure serum protein distribution, prominent enzymes, electrolytes, and liver and pancreatic functional profiles. The results were evaluated statistically.

Mink with AD showed increases in blood urea nitrogen (BUN) and total protein, including beta and gammaglobulins. Increased levels of the serum enzymes SGOT*, SGPT** and amylase were also observed. Calcium was depressed. Mink of both groups had levels of lactic acid dehydrogenase (LD) comparable to extreme human homologous serum hepatitis. (40) Other workers found no detectable differences in SGOT and calcium levels (methods not given) but did observe increases in BUN, phosphorus, and alkaline phosphatase whereas sodium levels were depressed. (10)

Serology

To date, no serological test has been devised to identify a specific AD agent. Serums from AD mink when injected into normal mink, appear to induce proliferative lesions characteristic of the disease. (42) Thompson and Aliferis (10) found that latex

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*Serum glutamic oxalo-acetic acid transaminase
**Serum glutamic pyruvic acid transaminase

fixation tests for Rheumatoid Factor and LE* cell preparations, by direct and indirect methods, proved negative.

Recently Porter et al (39) confirmed these findings together with negative Syphilis VDRL and Mazzini tests. The antibody responses of AD and normal mink to bovine gammaglobulin (BGG), egg albumin and hemocyanin were compared. Neither group responded to egg albumin antigen. The response to hemocyanin was markedly reduced in affected mink and no response occurred with BGG which was also low in the controls. When AD was induced in the controls there was no corresponding increase in pre-existing antibody levels in spite of fivefold increases of gammaglobulin. Quantitative serum complement assays showed that similar levels occurred in both groups of mink.

Attempts to demonstrate auto-immune reactions by use of fluorescent diseased and normal serums on autologous tissues or cells of diseased and normal mink, met with failure. Double diffusion tests in agar-gel, consisting of rabbit anti-AD serum (absorbed with normal mink serum) and antigens of AD serum or AD mink tissue homogenates, were also negative. However, Saison and Karstad (12) have demonstrated that mink in which the disease was experimentally induced, developed positive direct Coombs tests in association with a positive IAT and HGG. The titre of the antiglobulin, which effected hemagglutination, increased as the disease

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*Lupus Erythematosus

progressed. To add to these somewhat confusing results, Williams et al (36) showed that anti-gammaglobulin factors of low titre occurred in the blood of normal and diseased mink and were apparently unrelated to the amount of infecting inoculum used.

Hematology

Obel (3) observed terminal anemia which she attributed to depressed hemopoeisis resulting from plasma cell infiltration of the bone marrow. Other workers (10) found that total white cell counts, hemoglobin and packed cell volumes (PCV) were significantly lower in affected mink which also showed a relative lymphocytosis. Erythrocyte sedimentation rates while variable, were similar in normal and affected mink.

Urinalysis

Obel (3) tested urine obtained at post mortem from five cadavers and found the heat coagulation test for Bence Jones (BJ) proteins positive in four. Thompson and Aliferis (10) also found consistent proteinuria accompanied by granular casts and in some cases hematuria. They did not detect the presence of BJ proteins however. Kenyon et al (43) examined urines obtained from 27 affected live mink, which were found to have plasma cell reactions in the usual organs, and also failed to demonstrate BJ proteinuria by the heat test and Jurgenson's propanol denaturation method. By zone electrophoretic analysis of urine, protein components with

mobilities of gamma and beta globulins were found. By UCF* analysis of urine from two mink, the main components were found to have sedimentation coefficients of 1.5 - 2.2S. Upon IEP and chromatography of AD urine, concentrated 20 times, 7S gamma-globulin and low molecular weight urinary gammaglobulins were found which were antigenically related to the gamma 7S of normal and AD serums. Daily urinary loss of protein in affected mink may range from 10 to 125 mg consisting chiefly of BJ proteins. The latter have the mobilities of gammaglobulins but differ in mobility from the homogeneous myeloma paraprotein observed in the corresponding serum of certain mink. (13)

PATHOGENESIS OF ALEUTIAN DISEASE

The pathogenesis of AD has been recently fully described by Henson et al. (11) Evaluation of the microscopic lesions in the kidneys suggests that the development of changes involves at least two general mechanisms. First, the agent initiates a proliferation of plasma cells in the kidneys and elsewhere. The collections of plasma cells increase in size and exert mechanical effects on the adjacent tubular structures. Second, glomerular lesions develop which lead to interference with vascular flow, increased permeability of the glomerular capillaries, hemorrhage and loss of plasma constituents. These in turn cause numerous casts which, by tubular blockage, further interfere with renal function.

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*Ultracentrifuge

The genesis of the glomerular lesions with progressive deposition of eosinophilic material could arise from several mechanisms and more than one may play a role. The correlation of the severity of glomerular lesions and HGG is significant although the two could possibly be independent of each other. The possibility of immunologic damage such as is seen in Serum Sickness, Nephrotic Serum Nephritis and LE, is discounted because of the absence of the membranous glomerular lesion and the mesangial pattern of deposited gammaglobulin, fibrin and albumin. The latter have been detected by fluorescent tagged serum protein antisera, and are considered to indicate nonspecific proteinuria.

Another possible mechanism is that of intravascular coagulation. In these circumstances, fibrinoid material which can be fibrin per se, polymerized fibrinogen and related products, is deposited subendothelially and in the mesangium. Gammaglobulin can be detected in the mesangium while fibrin and its products can be detected in the mesangium and beneath the endothelium. A capillary deposition pattern is lacking. Fibrin deposits in the mesangium, (39) fibrin thrombi in the glomerular capillaries of some affected animals (33) and preliminary investigations into the various clotting factors in normal and AD affected mink, (11) indicate that alterations consistent with intravascular coagulation, occur in the affected mink.

The third possible mechanism is the proteinuria itself. Homologous gammaglobulin infused into rats exerts a nephrotoxic effect with formation of intracapillary fibrin thrombi. Human cases of multiple myeloma develop renal lesions in relation to the severity of the hyperglobulinemia.

Henson concludes that AD may be a primary dysproteinemia initiated by a virus or filterable particle. The significance of the HGG and its relationship to the renal and arterial lesions remains to be clarified. The possible role of a direct effect of the agent on vascular and glomerular tissues as an initiating insult, must also be kept in mind.

Since the earlier descriptions of the pathology of AD (4,3) and the discovery of the attendant HGG, (22) detailed accounts of the syndrome in its established phase, have been provided by various authors. (34,11,33,32,10,44) Knowledge as to what constitutes the primary lesion and the early pathogenesis is incomplete. The significance of minor lesions related to the future outcome, i.e. regression or progression, remains an open question. Serial biopsies may be the only method to resolve this point.

For the moment, AD in its detectable form, presents a picture of a systemic multiplication of the plasma cell series. The effects of these space-occupying lesions, coupled with HGG and the resultant physiologic impairment of epithelial membranes, 'drenched' in useless gammaglobulin, would seem to be the most

reasonable explanations for the cause of death.

The Aleutian mink genotype possesses certain structural anomalies resembling the CHS of man. (16,17) It is significant that CHS human patients seldom survive beyond the first decade and that death commonly results from a malignancy of the lympho-reticular system. Hepatosplenomegaly and recurrent lymphadenopathy are detected clinically. Microscopic findings include extensive infiltrations by histiocytes and immature lymphoid cells, of the viscera, brain and parenchymatous organs. (45)

Such pathology is not unlike that of AD except with regard to the predominant cell type. Little is known of the serum protein changes in CHS. Later studies of CHS suggest that transformation to the malignant state may be related to abnormalities of the lysosomal membranes which render them or the host genome, more susceptible to viral attack.

Particles resembling viruses have been found in circulating cells - but not in cultured cells - of the peripheral blood of CHS patients, but the significance of these particles is not presently known. (46) It would seem that the lysosomal defects of Aleutian mink, the lympho-reticular malignancies associated with CHS in man, and the lympho-reticular proliferation found in AD, may be connected. The knowledge that CHS in mink is a genetic trait following the Mendelian laws of a simple recessive, should not exclude the possibility that, as in man, a lympho-reticular malignancy may result.

APPRAISAL OF THE LITERATURE REGARDING THE INFECTIOUS ETIOLOGY

The first recorded attempt to investigate the etiology of Aleutian Disease was made by Helmboldt. (4) Suspensions of affected organs inoculated into a diverse group of laboratory animals failed to produce evidence of disease after one month, neither was any consistent bacterial or mycotic organism demonstrated. Serological tests for leptospirosis and toxoplasmosis were also negative. When suspensions were inoculated into mink, changes resembling AD were observed in about one month. On repassage, "slight" lesions of AD were found in experimental mink. Other mink and ferrets failed to become ill.

A series of experiments were conducted (6) in which preparations made from diseased organs of affected mink were used as the inocula. Varying degrees of plasmacytic infiltration were induced in recipient mink. Natural death occurred only in Aleutian mink between 50 and 70 days after 10% w/v* crude tissue suspensions or 450 mu filtrates** were used as inoculums. When the same genotype received 100 mu filtrates in the same volume, clinical signs or significant amounts of plasma cell infiltration were not observed at sacrifice 88 days after injection. In Standard Dark mink similar responses were found except that no natural deaths occurred during the experiment. (It was noted

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*weight/volume
**Filtrates passing the predesignated filter size.

that a number of mink died following intra-cardiac puncture and the possibility of impaired coagulation mechanisms in affected mink was suggested.) High BUN levels were detected in mink prior to natural death.

In a subsequent experiment (47) designed to assess the immunogenic value of formalin-treated tissue suspensions, doses of 1 ml of 15% w/v suspension resulted in lesions of plasmacytosis observed on sacrifice between 65 and 113 days. Mink receiving suspensions exposed to 0.3% formalin for 48 hours at 36°C had not developed lesions at 113 days but on subsequent challenge with unformalinized suspensions the remaining mink developed typical lesions which were found at autopsy 60 days after challenge. Mink which received equivalent doses of suspensions from normal mink did not show lesions of plasmacytosis over the same period but did show lesions following challenge with suspensions of diseased tissues. The criteria of normality of the mink contributing such tissue inoculums were not indicated in this experiment.

An effort to demonstrate an agent by the use of mink tissue cultures was reported by Basrur et al. (7) Crude extracts obtained by centrifugation (2000 RPM - 10 mins.) of 10% suspensions of diseased and normal organs were inoculated in 0.25 ml amounts onto four day old cultures of mink testis or mink kidney cells. Cell cultures which received the crude extracts of diseased tissues developed nuclear and later cytoplasmic damage and overall mitoses in the cultures ceased. Other authors, while investigating the

relationship of Yersin tuberculosis and AD, found no evidence of nuclear damage in tissue cultures under similar experimental conditions. (48)

Basrur (7) found that when replicate titrations of the crude extract or the fluids obtained from inoculated cell cultures (incubation period not quoted) of the 2nd, 4th, 6th and 8th passages were evaluated, the $TCID_{50}$/0.1 ml remained constant at 10^{-6}. Primary tissue culture fluids and fluids of the 2nd, 4th, and 8th passaged materials were injected into Standard Dark and Pastel mink. Serum samples were drawn between 30 and 70 days post injection for IAT. Five of eight developed positive reactions (criteria not given), and six were found to have lesions of plasmacytosis but the severity was not described. One of two animals receiving 450 mu filtrate of a normal mink tissue also showed suspicious reactions of IAT and also by histopathological examination. On the bases of these results, Karstad and Basrur have presented a case for the transmissibility of AD and for the etiological factor to be viral in nature.

The main difficulty encountered in attempting to assess AD transmissibility experiments, lies in the present inability to categorically exclude the pre-existence of the agent, or early forms of the disease, in test mink which have normal serum proteins. It is reported that the ranch incidence may reach 25% before AD becomes evident to the owner. (25) It should also be kept in mind that AD homogenates are, ipso facto, enriched with the contents of

plasma cells, namely nucleic acids, enzymes and gammaglobulins, whereas normal tissue homogenates contain much lesser amounts of plasma cell constituents. The latter substances readily pass filters and therefore such materials ought not to be overlooked as being capable of physiological activity in susceptible animals. It is known that erythropoietin, a substance which occurs in increased amounts in the serum of rabbits suffering moderate to severe blood loss, stimulates erythropoesis when given to normal rabbits. (49)

The apparent decreasing severity of AD lesions in relation to the amount or degree of filtration of inoculums, might also be a quantitative reaction to a substance, similar to erythropoietin, which occurs in increased amounts in AD tissue.

The effects of formalinized AD inoculums, which did not provoke plasma cell proliferations within the period of trial, (16 weeks), and their apparent failure, based on clinical and pathological evidence, to evoke an immunity to subsequent challenge with unformalinized AD suspensions, are indicative of total destruction or denaturation of the excitatory substance.

It would seem feasible to expect that if a viral agent were involved in the transmission of AD, an optimal exposure to the effects of formalin exists which would result in inactivation, if the agent is a virus in the classical sense.

In the tissue culture studies, (7) the cytopathic effect described suggests evidence of toxicity. It is known that

gammaglobulin under certain conditions, is toxic to tissue cultures (50) and unless this substance could be excluded from tissue filtrates used to infect cell cultures, a generalized toxic effect rather than a specific CPE* may result. The finding of a uniform $TCID_{50}$ for all passage materials does not support the idea of propagation (replication) of the agent, since a progressive change in titre is the usual result of its adaptation to the medium. It is also of interest that an agent which in vivo appears to stimulate or otherwise cause proliferation of cells, should reverse its effects in vitro on a substrate which consists of cell cultures derived from the same host species. It is hoped that further studies will clarify the nature of these tissue culture reactions.

Russell et al (25) found that 9 out of 20 mink, both Aleutian and non-Aleutian types had lesions of plasmacytosis when killed four months after receiving 1 ml of positive 15% tissue suspensions. The latter had been previously exposed to the action of 0.5% formalin for 24 hours at 37°C. When similar suspensions were exposed to 1% formalin the same manner, only 2 out of 20 animals were found to have lesions.

These results differ from those of Karstad (47) and may be related to the concentration of formalin used or the length of the exposure time. Unfortunately Russell did not describe his methods in detail.

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*cytopathogenic effect

The latter author also reported that mink which received tissue emulsions, previously passed through a Berkefeld N filter, or a similar filtrate subsequently exposed to 0.2% beta-propriolactone at 37°C for 24 hours, showed lesions of plasma-cytosis. The incidence was 14 and 2 out of a total of 20 mink respectively; i.e. 70% and 10%. Mink which received untreated suspensions developed lesions which were found in 24 of the 26 animals inoculated (92%). All these test groups were sacrificed four months after inoculation. The incidence of natural death or the criteria by which lesions of AD were assessed were not described in this experiment. The question arises as to why filtration of the emulsion should result in a fairly substantial reduction in the proportion of animals which showed lesions if, as is suggested, a virus agent is responsible for these lesions.

In this experiment, 19 out of 19 mink which received 2 mg prednisolone daily in the feed, in addition to an initial injection of positive tissue suspension, succumbed within six weeks. Lesions of plasmacytosis however were found in 17 of these 19 animals (90%). Russell suggested that the increased mortality was due to increased susceptibility to the infectious agent which resulted from the use of prednisolone.

In this case it is noteworthy that the overall incidence of lesions found after the use of positive suspensions (92%) and positive suspensions with predisolone (90%) is very similar. The difference is in the resulting mortality. It is difficult to relate

this mortality with the pathogenesis of AD in which progressive renal impairment over an extended period of time is usual. It is also of interest that two animals showed no lesions but nevertheless succumbed. It would be perhaps more feasible to explain the effects of the drug as a depression of the inflammatory and immune response, and mortality resulted from increased susceptibility to infections in general.

The rapid-passage studies reported by Russell involved the use of five Fawn male mink which were IAT negative. The first animal received 5 ml of pooled 40% w/v AD challenge suspension intraperitoneally. After two weeks, the mink was destroyed and its spleen, liver and kidneys used to prepare a further 40% suspension, of which 2 ml were given to the next animal. The latter procedure was repeated at weekly intervals. Whereas the first four animals were sacrificed the final recipient was found dead 15 days after the injection. The lesions observed in all mink consisted of marked hyperplastic changes in the lymphoreticular organs. The kidneys were enlarged and showed whitish foci and petechiation. None of the animals became IAT positive. Russell thought the last recipient might have died of 'acute' AD. No histopathology was described in this experiment.

In the next experiment, 50 Pearl male mink, negative to the IAT, received log dilutions of a 10% tissue suspension derived from an experimentally affected mink. The experiment was evaluated

on the bases of IAT reactions and the gross lesions at the end of a four week period. Discrepancies occurred between IAT reactions and the gross pathology. End points were therefore determined by the latter. Mink receiving the final dilution (10^{-5} gm of tissue per ml) had gross lesions in 5 out of 10 instances. All others were positive by gross examination. The end point, assessed by gross examination, was estimated at $10^{-5}ID_{50}$ although this was clearly arbitrary.

A similar experiment was conducted using diseased organs resulting from the third passage of the AD agent. Pastels, in groups of three, received log dilutions of a 10% tissue suspension through 10^{-7} in 1 ml amounts intraperitoneally. The IAT was performed at four weeks post injection and again at six weeks at the time of autopsy. The results showed that IAT positivity occurred later with increasing log dilution. The severity of macroscopic and microscopic lesions tended to decline after an initial increase in severity. No end point was reached at log 10^{-7} dilution. All three mink in this group showed evidence of AD by microscopic examination which was scored on the basis of minor round cell and plasma cell infiltration in the kidneys.

In general, Russell's experiments support the findings of Karstad, in the sense that the disease onset and severity appears to be partly related to the dose of pathological tissue or filtrate used. The same uncertainty exists with regard to disease <u>a priori</u>.

Animal titration experiments echo the results observed in tissue culture by Basrur in that extinction of the agent did not take place after dilution to 10^{-7} or 10^{-8} (i.e., 1 in 10 million).

Coincident with the reports of Karstad and Basrur, transmissibility was described by Trautwein and Helmboldt. (44) A group of eight 9 week old Aleutian mink received a total of 6.5 ml, of 10% positive tissue supernate, intraperitoneally over a 10 day period. All showed extensive lesions when examined eight weeks later. A similar group, which received negative equivalent supernate, showed non-specific RE hyperplasia. In the third group of uninoculated controls, three deaths occurred. The survivors showed splenic lymphoid hyperplasia and hematopoeitic foci. In the mink which died, gastric hemorrhage, interstitial pneumonia and lymphoid hyperplasia were observed at post mortem. It is noteworthy that all these lesions though not specific, have been seen in association with AD.

In three additional experiments, Trautwein observed slight proliferation of plasma cells in 4 out of 6 Pastels which were six months old, four months after receiving 2 ml positive supernate. Somewhat more severe lesions were found in six Pastels of similar age, which received 1 ml of positive supernate of first passage material. In a final experiment, two groups of 12 month old mink received 4 ml intraperitoneally, in divided doses, of Seitz filtrates of either naturally or experimentally affected mink organs. Similar results were observed in each group when killed

77 days post injection. Two animals died after one and two months. Autopsy revealed gastric hemorrhage and pathognomic lesions. The remaining mink showed slight or severe plasma cell infiltrations in the liver and kidneys plus lesions elsewhere in no particular relation to either group. Trautwein pointed out the relative mildness of the experimental disease as evidenced by the absence of vascular lesions and low fatality rate. He suggested other as yet undetermined factors were responsible for the fatal outcome under natural conditions.

Henson et al (8) used 147 mink which were two months of age, obtained from a ranch where spontaneous AD had not been observed in the previous 10 years, for their transmission experiments. Inoculums were obtained from the organs of cases of spontaneous AD in Aleutian type mink. The organs were ground and diluted to 10% tissue suspensions* which were then exposed to 0.3% formalin (in final dilution) at 5°C. Reactions to various inoculums were determined by serum protein analysis as shown by paper electrophoresis. No abnormal patterns were observed over a four month period in animals (three genotypes) which received 0.3 gm of either bovine serum albumin, 0.6 gm "feline tissue," or 0.6 gm of normal mink tissue. Of the formalinized suspensions, diseased mink tissue (0.6 gm) exposed for 40 weeks or normal mink tissue (0.6 gm) exposed for two weeks failed to elicit a response. Mink which

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*Weight/volume presumed.

received 0.6 gm of diseased tissue or 0.6 gm of diseased tissue exposed to formalin for two weeks, developed HGG in a similar degree but with a frequency of 15 out of 23 (64%) and 8 out of 13 (62%) respectively. The frequency was 100% in homozygous recessive Aleutian mink and approximately 50% in non-homozygous Aleutian mink.

In a later experiment, Henson et al (9) initiated HGG in three mink genotypes by the injection of cell-free filtrates of affected organs. The filtrate was obtained by the use of a Krueger E pad and was thereafter centrifuged at 95,000 g for one hour at which time a visible pellet was obtained. The supernatant fluid and the resuspended pellet were used as inoculums. The results showed that the incidence of affected animals was markedly less with the pellet inoculum or the original filtrate compared with that of crude tissue suspensions. The fact that a visible pellet was obtained in the above manner was represented as evidence of the particulate nature of the agent of AD and as further substantiating a viral etiology.

The results of this experiment may again be interpreted as a quantitative type of reaction to the inoculums. The cell-free filtrate, though lacking intact cells, could well contain the various cell constituents which are capable of passing such a filter. It is further possible that after such centrifugation, these constituents could form a visible pellet. The ability of the supernatant to induce HGG in one of the five test animals also calls for some explanation.

In the course of an investigation into the natural history of AD, Gorham et al (5) found a high incidence of AD lesions in mink which had received preparations of urine, blood, saliva or serum obtained from affected mink. The preparations were administered orally. A lower incidence resulted from the similar use of fecal suspensions and no lesions were found after the oral administration of colostral milk. Bone marrow given intravenously and spleen emulsions administered by an aerosol method also demonstrated a high level of activity in the production of lesions. The authors commented that, "In spite of the presence of an active agent in so many body fluids of affected animals natural transmission among mink does not occur to extent expected." One may also add that the failure of colostrum to induce lesions, in contrast to serum, saliva, etc. is itself an unexpected finding since newly born mink receive gammaglobulin via the colostrum during the first week of life. (51)

Genetic studies similarly have produced conflicting results. Henson (28) reported the familial occurrence of HGG in mink. The kits of 31 families showed an incidence of HGG which was significantly higher if the mother was affected. In contrast, Hemmingsen and Heje (29) compared the incidence of HGG in mink litters derived from homozygous Aleutian parents, which were either both affected or not affected, and found no difference in the number of kits which developed the HGG. However when negative heterozygous females were mated with homozygous Aleutian

males, the incidence of HGG in the progeny was significantly reduced. These workers suggested that the disease was genetically conditioned.

In evaluating the results of experiments dealing with the etiology of AD, most of which have involved transmission of the disease with various tissues or tissue filtrates derived from affected mink, it must be emphasized that no test exists for the presence of the agent or early disease - in which serum protein changes have not yet reached detectable levels - in the experimental mink.

In the light of the foregoing description of AD and the results of experimental investigation, it is evident that the disease, if such it is, is not a simple entity. The impression is gained that after an initial enquiry, the difficulties encountered in the control of experimentation and the evaluation of the results, have discouraged several workers from further efforts. A conservative view of AD as it now stands would be as follows:

1. AD became a clinical entity with the appearance and subsequent multiplication of certain mutation mink.
2. The disease appears to be confined to mink, as other experimental animals have not yet proved susceptible to the typical disease.
3. There is marked individual and genetic predisposition.
4. Natural contagion among mink, if it occurs, is highly erratic and less than expected in view of the presence of the agent in excreta, saliva, etc.

5. Pathologically, AD in its established clinical form, is characterized by:
    1. A sustained activity of the lympho-reticular system.
    2. Hypergammaglobulinemia and its related physiologic effects.
    3. Terminal, physical and biochemical interference with renal function.
    4. The absence of the chief features of the inflammatory response, e.g. necrosis, neutrophilic reaction and fibrosis.
6. Tissue emulsions and filtrates derived from them appear to provoke proliferative changes whether given orally or by parenteral injection to susceptible mink.
7. Certain genotypes react more rapidly and severely to such materials.
8. Chemically-inactivated materials do not provoke an immune response as determined by subsequent challenge with non-inactivated tissue suspensions.
9. In artificial transmission experiments, the lesions produced are mild and usually non-fatal.
10. The responses observed appear to bear a relationship to the absolute amount of inoculum employed.
11. Animal and tissue culture titration experiments indicate that if an agent is present, it does not replicate in the usual manner of viruses.
12. A specific agent has not been isolated in pure culture.

M A T E R I A L S A N D M E T H O D S

Previous investigations have shown that AD tissue suspensions, or filtrates of them, stimulate the formation of lesions of the parent disease, when injected into other mink and these effects are apparently serially transmissible. It is believed that such suspensions contain a specific agent which is most probably a virus. Suspensions exposed to formalin do not produce these effects nor do they prevent the effects of subsequent challenge with unformalinized AD suspensions.

No evidence regarding the heat susceptibility of the agent has so far been published neither have experiments been performed in mink to compare the effects of injections of non-infectious materials which can stimulate the RES or evoke tumor formation in laboratory animals. The purpose of this experiment was to compare the tissue reactions occurring in mink after the inoculation of one of the following: Adjuvant, AD tissue and Adjuvant, Heat-treated AD tissue and Adjuvant.

In order to control the experiment it was considered necessary to include six test groups of sibling mink as follows:

Group	Inoculum	Object
A	Saline	Control spontaneous AD
B	Adjuvant	Non-specific RES stimulator
C	Adjuvant plus Heated Negative Tissue	Heated positive tissue control
D	Adjuvant plus Heated Positive Tissue	Effect of heat treatment
E	Adjuvant plus Negative Tissue	Positive tissue control
F	Adjuvant plus Positive Tissue	Reproduction of AD in test mink.

Since the substance of major interest was adjuvant, it was included in all groups except the saline control. To account for the effects of tissue bulk injections, normal tissue was also included. Because normal and AD affected tissue may vary considerably in cellular content and fluid, both forms were first lyophilized before suitable amounts were weighed for the preparation of the suspensions. In order to test the heat stability of the AD agent, raw diseased tissue was autoclaved at 15 lbs. for one hour, prior to injection into mink. The possible effect of autoclaved tissues alone (denatured substances) was controlled by similar heat treatment of normal mink tissues.

An attempt to control the differences in genetic susceptibility within mink was made by using litters of six with one kitten in each test group.

It was realized that the design of the experiment was not likely to show whether a specific virus-like agent was present in the test mink. However it was felt that an inherent tendency to manifest signs of AD would be revealed. The experiment might also reveal whether previous success in reproduction of the syndrome depended upon a specific entity in the inoculums employed, or if the latter contained substances which acted as unspecific stimuli on substrates within the recipient mink.

MANAGEMENT OF TEST ANIMALS

The animals used in this experiment were provided by and maintained at the Manitoba Fur and Game Station, University of Manitoba. The test animals consisted of 10 litters of six kittens from parents which were clinically free of AD. The kittens were bled and tested prior to experimental procedures and subsequently housed in a separate barn. A member of each litter was placed in each test group. Each group therefore contained 10 kittens of different litters (Table I). The mink in the test groups were placed in separate pens which were serially numbered (Fig. 1).

After experimental inoculums were given, the test animals were maintained in equal conditions regarding environment, feed and water. Separate gloves were used for handling each test group throughout the experiment from June 1963 until February 1964.

SOURCE OF INOCULUMS

Two Aleutian male siblings 12 months of age, were used as tissue donors. One animal (AJ 19) was positive (3+) to the IAT and exhibited gross lesions of AD at autopsy. Histological examination of the liver, spleen and kidneys confirmed the presence of the disease.

The second animal (AJ 11) was negative to the IAT and showed no gross or microscopic pathology. These mink

were subsequently destroyed by euthanasia and certain tissues removed. The sequence and pertinent details are illustrated in the flow diagram (Fig. 2).

Adjuvant used consisted of a mixture of:

Arlacel A - 1 part (Atlas Powder Company)

Bayol F - 9 parts (Imperial Oil)

ROUTE OF INOCULATION

After reconstitution with sterile distilled water, the 10% suspensions were cultured routinely for bacterial growth and were negative.

Tissue inoculums were mixed with equal volumes of adjuvant immediately prior to injection and administered by 18 gauge disposable needles into the peritoneal cavity of each test mink (Table II).

DISPOSAL OF TEST ANIMALS

After administration of inoculums, the test groups were reduced by the sacrifice of one complete litter at intervals of approximately one month. The mink in the lowest numbered control pen determined which litter was removed for investigation. When death occurred in any group, the corresponding littermates were also sacrificed. Table III indicates the ages at sacrifice and the origin and color phases of the 10 mink litters.

PROCEDURES AT SACRIFICE

Commencing with the control animal, individual members of the same litter were successively removed, anesthetized, and exsanguinated by intra-cardiac puncture, using an 18 gauge disposable needle and 30 ml or 50 ml syringes.

A set of sterile instruments was used for each animal. After the skin incision from the pelvic brim to the throat, a second pair of scissors and forceps were used to open the abdomen and a third set to remove organs for laboratory examination.

Portions of liver, spleen and intestine were removed for aerobic and anaerobic culture. Similar specimens were held at -20°C pending virological examination.

LABORATORY STUDIES

1. Histopathological examination of tissue sections and organ imprints.
2. Hematological examination of blood smears and bone marrow; hematocrit values and sedimentation rates.
3. Serum electrophoresis and IAT.
4. Virological investigation.
5. Bacteriological examination.

Histopathological Examination

At autopsy, representative organs were fixed in 10% formalin. Sections of liver, spleen, and kidney were made routinely and stained HE and PAS.

Some doubt exists regarding the presence of minor lesions and a positive diagnosis of AD. In this evaluation, the occurrence of one characteristic lesion in either the liver, or kidney was considered diagnostic. A characteristic lesion consisted of an aggregate of mononuclear cells including histiocytes, plasma cells and lymphocytes. The frequency of lesions in a given section was arbitrarily classed as single or multiple.

Clinically healthy animals, negative to IAT and exhibiting no lesion in either the liver or kidney sections were regarded as negative with respect to AD.

Hematology

Smears of peripheral blood and smears of bone marrow were air dried, fixed in absolute methyl alcohol and treated with Wrights and/or dilute Giemsa stain.

Hematocrit values were determined directly by the International Micro-capillary Centrifuge and Reader. Blood was collected in heparinized capillary tubes 0.8 mm x 90 mm. Samples were centrifuged on collection at 15,000 RPM for two minutes.

Erythrocyte Sedimentation Rates (ESR) were ascertained by the method of Landau-Adams. (52) Only samples taken at autopsy were used. The blood sample of 4 - 5 ml was added to 0.25 ml of 5% ethylene diamino tetra-acetic acid (EDTA) for

transport to the laboratory. For this method it was required to further dilute the samples with a fixed volume of 4% sodium citrate. Readings of the ESR in millimeters were taken at 30 and 60 minutes.

Mink Serum Antigens

Whole blood, removed aseptically under ether anesthesia, was allowed to clot at room temperature, then stored overnight at 40°F. The serum was withdrawn and, unless in use, stored at -20°C. Before use, serum was diluted 1:10 in sterile saline (0.85%) (Table IV).

Preparation of Rabbit Anti-Mink Serum

Twelve New Zealand White rabbits approximately four months old, were used for the production of anti-mink serum.

Mink serum antigens were obtained from four animals - two Aleutian mink (AJ 19, AJ 11) which also contributed tissue inoculums, and two adult Standard Dark mink (141 J, 49 J). The pertinent details are shown in Table IV.

Two rabbits were "immunized" with each mink serum. One of each pair also received 1 ml adjuvant intramuscularly. Four rabbits were "immunized" with pooled sera of the four donor mink. (Those rabbits not receiving adjuvant failed to produce satisfactory precipitating sera.)

All animals were pre-bled for control serums. The ear was clipped and a few drops of Xylol applied to the central

outer surface. The ear was then massaged to attract blood to the area. Using a scalpel blade, the inferior peripheral vein was nicked and sufficient blood collected. Xylol was removed by swabbing the ear with 70% methyl alcohol. The immunization schedule is shown in Appendix A.

Examination of Mink Serum

Procedures used -

1. Double Diffusion in agar gel.
2. Immuno-electrophoresis in agar gel.
3. Iodine Agglutination Test.

Double Diffusion and Immuno-Electrophoresis

The serums of mink were tested by the micro-methods described by Parker et al. (53) Mink serum antigens were initially tested against rabbit anti-mink serums by double diffusion in agar gel. For IEP it was necessary to concentrate rabbit precipitating serums by an adaptation of Kohns method*. (54)

Examination of mink sera by IEP was performed simultaneously on each family of six siblings by the use of glass slides 3 1/4" x 4" coated with 12 ml of 2% agar gel. Two serums, one of which was negative to the IAT and the other strongly positive, were included in each set for comparative purposes.

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*See Appendix B.

The sera of several mink which had been blood-tested at monthly intervals and which showed changes in reactions to the IAT were stored and subsequently tested together by IEP.

Iodine Agglutination Test

Fresh serum samples, obtained at autopsy and at monthly bleedings, were tested by the established method. (23)

VIROLOGY

Attempts to Isolate a Filterable Agent

Efforts were made to determine whether the tissues of mink affected with AD contained an agent capable of producing a CPE on tissue cultures derived from animals with no (detectable) evidence of the disease.

Mink tissue cell cultures were prepared in the following manner:

Four mink, aged four months, including a Pastel, Standard Dark, Aleutian, and Sapphire, which were negative to the IAT and which subsequently showed no histological evidence of AD in the liver, spleen, or kidneys, were exsanguinated under ether anesthesia, and the kidneys and testes removed aseptically. Duplicate specimens were fixed in formalin for histological examination.

Mink Kidney Cell Cultures (MKCC)

After removal from the donor animals, the kidneys were stripped of fat and the capsules removed. The organs were minced

with scissors into pieces 1 - 3 mm and pooled. The minced tissues were washed four times with Hanks Balanced Salt Solution (HBSS) by which time the supernatant fluid was clear. The latter was decanted and replaced with 0.25% trypsin in HBSS. The minced tissue cells were incubated at 36°C for one hour, after which the supernatant was replaced with fresh 0.25% trypsin solution.

After two hours incubation at 36°C, the mixture was filtered through three layers of sterile gauze and the filtrate centrifuged for 15 minutes at 1000 RPM. The supernatant was discarded, the cells resuspended in 300 ml propagating medium* and aliquoted in 2.0 ml volumes in 40 coverslip preparations and 60 roller tubes.

After four days incubation at 37°C the cells were observed growing out but there were many dead cells floating in the supernatant. The media was removed and replaced with propagating medium containing 10% calf serum. After a further six days incubation, a confluent sheet of healthy epithelial cells was visible at which time the medium was replaced with maintenance medium (5% calf serum).

Mink Testes Cell Cultures (MTCC)

These cultures were prepared in a similar manner to the kidney cell cultures. After digestion with trypsin the cells were resuspended in 25 ml propagating medium (20% calf serum)

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*See Appendix C.

and dispersed into 12 coverslip preparations in 2.0 ml volumes. A good growth of fibroblasts occurred after three days incubation and the medium was replaced with H 597* + 2% calf serum.

Tissue Culture Inoculums

Four mink contributed tissues for the inoculums. AJ 19 (A1) and 141 J (SD) were animals having well established lesions of AD and were positive to the IAT (3+). The other two animals, AJ 11 (A1) and 171 (SD) were negative with regard to histopathological evidence of AD. Since the time of sacrifice two weeks earlier, organs of these animals had been stored at -20°C.

The type and number of tissue cultures made with mink kidney cells and the source and quantities of the various inoculums used, are shown in Table V.

*Connaught Laboratories

R E S U L T S

SERUM PROTEIN EXAMINATIONS

1. Iodine Agglutination Tests

The female parents of the test animals showed a variety of reactions to the IAT at the time the litters were weaned in May. The occurrence of dysproteinemia in the related litters is also shown on Table VI and appears to vary independently of the status of the dam.

Of the young mink, a higher incidence of positive IAT reactions occurred among the males, throughout the experimental period (Table VII). Initially five positive animals, all males, were found in the experimental group prior to the injection of inoculums. The IAT reactions were either 1+ or 2+. One positive animal appeared in each treatment group except group C (adjuvant plus autoclaved negative tissue) and comprised minks numbered 3, 17, 38, 42 and 53 and which were confined to the Sapphire litter and one litter of Standard Darks.

During the test period one Sapphire (#3) in the control group was positive at the beginning of the experiment and another animal, a Standard Dark, became positive after six weeks. Otherwise no positive IAT reactions occurred in the control group. The incidence of IAT reactors at various times, in all groups, is given in Table VIII.

One month after tissue inoculums were administered, 30 of 50 mink had positive IAT reactions. Two animals (#24 and #34)

became positive at the subsequent test, and another mink (#25) became positive 12 weeks later. Three minks (#40, #48 and #46) which became positive, were negative three months post-inoculation. All three animals had lesions at death. The incidence of dysproteinemia and histopathological lesions of AD occurring among the various test groups is given in Table IX. A lower incidence of IAT reactors is found consistently.

2. Double Diffusion in Agar Gel

Both positive and negative serums showed fine distinct lines of precipitation. The line nearest the antigen well varied in density and breadth being more diffuse in positive serums. The leading edges of this precipitation line corresponded with that at the negative serum and was taken to be identical antigenically. All serums particularly Sapphire and Aleutian which were strongly positive to the IAT, showed precipitation at the perimeter of the well in addition to the usual lines. Such precipitation is consistent with the presence of an increased amount of lipoprotein.

Comparisons between tissue juices obtained from the spleen and other organs and serums occasionally showed one line corresponding with the albumin component of the serum sample. The presence of this line did not appear to be related to the AD status of the animal.

3. Immuno-Electrophoresis in Agar Gel

An international nomenclature for the human immunoglobulins was agreed in 1964. (55) Similar terminology for the immunoglobulins of different species was also recommended and will be used here.

To date, no reports have been found in the literature regarding the electrophoretic patterns of mink serums or natural factors which affect them, e.g., age, sex, or genotype. This study was made to compare the immunoglobulins of normal and diseased mink in relation to the IAT and the presence of AD histologically. The former depends on a decreasing albumin: globulin ratio and precipitation increases as the ratio narrows. Immuno-electrophoresis is best suited to compare mixtures of blood proteins but may, to a limited extent, reflect quantitative changes. Some of the important features noted in the electrophoretograms are indicated on Fig. 3.

Examination of IEP patterns showed regular relationships with the IAT. The ratio of the product of the lengths of the respective IgG and albumin arcs and the width of their respective electrophoretic axes was related fairly consistently to IAT positivity and the presence of AD histologically. For example, electrophoretic patterns shown in the serums of litter 284 (Fig. 4) indicate readily by the abnormal length of the IgG arcs and the distance at which precipitation occurs from the wells, distinct

differences between positive and negative serums. Also the IgG arcs of two minks (#23 and #34) are diffuse and a definite splitting of the arc is apparent in the latter. The other positive animal (#44) shows a shorter arc which begins to split at the cathodic end. In the same figure, #45 also shows a comparatively long IgG arc, but the corresponding increase in the albumin arc indicates that a greater sample of serum was placed in the well. The three positive serums show a small precipitation line around the well. This component is a slowly diffusible substance having the mobility of a lipoprotein or macroglobulin and is not apparent in the negative serum.

In the serial electrophoretograms, (Fig. 5) the reduced albumin:globulin ratio of the positive serum is apparent and double arcs of IgG mobility are also clearly visible. The January sample shows an extra precipitation line emanating from the well into albumin arc. The other mink serial samples exhibit a consistent lip on the IgG arc towards the cathodic end which is faintly suggestive of a myeloma-like protein. The first sample (September) shows a definite reduction in the number of precipitation lines near the well except for a diffuse zone of precipitation leading to the IgG component. A similar reduction of lines occurs in the same month in the positive sample (#47).

Turning to Fig. 6 which shows serial electrophoretograms of a positive and a negative mink, it is observed that the IgG

arc again shows a fairly homogeneous component near the cathodic end which appears to lengthen in the following sample. The next sample illustrates an artifact caused by an eccentric well, i.e., the left arc is quite discrete, being further from the antibody trough, whereas its counterpart appears longer, more diffuse and closer to the antibody trough. Otherwise, no remarkable change is apparent in the IgG arc in that series. The neighbouring series of a mink (#19) which was positive and eventually died, shows a consistent double IgG arc which is increased in length. The sample taken at death (January) shows a disturbed pattern where the faster moving components appear prominent within the distorted albumin arc. The IgG appears to have gained overall mobility and seems to extend almost into the alpha-1 zone. A lipoprotein is obvious and also there is a prominent line in the region of the IgA which itself is preceded by a diffuse band merging with the IgG around the well. The IgM macroglobulin which is present in the four preceding samples is not evident in the sample recovered at death.

In Fig. 7 which shows immuno-electrophoretograms of a litter of male Sapphire mink all of which were positive, a splitting of the IgG arc occurs in four mink and in addition, an unusual pattern is present. In mink #11, a prominent arc of alpha-2 mobility extends from the well, but the components of slower mobility are faint or absent. This animal had severe renal lesions including necrotizing arteritis.

The features depicted in the various electrophoretograms are many and varied and require intensive study before valid interpretations can be made. A great number of specimens showed splitting of the IgG arc which in serial samples was constant for a given animal. A split arc was seldom seen in a negative animal. The serum of one animal (#19) which died, exhibited a discrete myeloma-like precipitin arc with an IgA or fast IgG mobility (Fig. 3). Generally, arcs of IgA proteins were not prominent in serums of these mink. On the other hand many positive serums possessed slowly diffusible components of slow mobility in the region of the beta-lipoprotein and often a spontaneous dense precipitation of this substance occurred around the well.

In several instances a discrete component migrating as part of the IgG appeared in animals negative to IAT but which were positive histologically.

A disturbance of serum protein metabolism seemed to occur in a number of mink between September and October and was manifested by a reduced number of arcs of medium mobility proteins near the well. Instead a diffuse, broad zone continuing from the fast IgG was evident.

CLINICAL FINDINGS, MORBIDITY AND MORTALITY

Initially all mink appeared healthy. Following the administration of inoculums a number of animals vomited; otherwise no reactions to the inoculums were observed.

During the remainder of the experimental period, five mink died naturally of which four showed gross lesions of AD at autopsy. The fifth carcase (#21 Sapphire) was inadvertently discarded and was not autopsied. The prior IAT had given a strong positive reaction and it was assumed that this mink died of AD. The pertinent data of these mink are given in Table X. Three of the deaths occurred in treatment group C. (See Table IX). No other symptom of clinical disease was observed.

HISTOPATHOLOGICAL EXAMINATIONS

Sections prepared from the livers and kidneys of the test mink were examined for evidence of AD. The critical lesion was an aggregate of mononuclear cells (Fig. 8). The incidence of such lesions in these organs and whether single or multiple is shown in Table XI. The number of mink in which one or more lesions occurred relative to each test group is also indicated. Among the five treatment groups the incidence of mink with AD lesions ranged from 70% - 100% being lowest in the adjuvant group and highest in the raw positive tissue:adjuvant group. By Chi-square analysis, the numbers of affected mink in the five treatment groups did not differ significantly. ($P = 0.01$)

Affected animals varied widely with regard to the extent and severity of the lesions. Sapphires and Aleutians were most seriously affected among which three mink were found to have

fibrinoid changes in the renal arterioles. Likewise, the proportion of typical plasma cells varied among mink. No particular pattern was noted except in young animals in which typical plasma cells were not plentiful.

The overall histopathological findings and IAT results with reference to each test group, age and genotype are depicted in Table XII.

BACTERIOLOGY

The isolates found on routine examination of tissues removed at autopsy are shown on Table XIII. No pathogenic organism was consistently recovered from the clinically healthy animals or the four cadavers which were available for cultural examination. A Sapphire mink (#5) which also was affected with AD, yielded Mycobacterium avium. In general, the cultural findings were scanty and irregular and were regarded as incidental. The isolation of M. avium is not unusual since Sapphire mink are unduly susceptible to this infection.

VIROLOGY

Results of the Applications of Various Tissue Inoculums on Mink Kidney Cell Cultures

Details of these results are given in Tables XIV and XV.

The constant finding in all cell cultures was a cytotoxicity first noted in the cultures which had received the crude

suspension. Cytotoxity was evidenced by a uniform increase in cell granularity, observed seven days after exposure. Within three days, large numbers of cells contained pyknotic nuclei and dark shrunken cytoplasm. Breaking up of the cell sheet was apparent and after a further seven day interval only a few cells remained attached to the glass. These changes occurred later and were less pronounced in the cultures which had received inoculums of the supernatant and filtrate. The inoculated control cultures showed similar changes to those receiving filtrate, commencing with increased granularity of the cell cytoplasm and breaking up of the cell sheet after 10 days of culture.

For comparison 20 mink kidney monolayer cultures were infected with the following viruses: Poliomyelitis 1, Echo 6, Adenovirus 3, Coxsackie A9.

No CPE was observed except with Adenovirus 3, which caused characteristic changes, similar to those seen in monkey kidney cell cultures, seven days after inoculation.

Results of 10% w/v Crude Tissue Suspensions on Four Day Old Coverslip Preparations of Mink Testes Cultures (Fibroblasts)

In this experiment 0.2 ml amounts of crude suspension obtained from the four donor mink were inoculated into four pairs of coverslip preparations with three left as controls.

During the six days following exposure no CPE was observed in the inoculated preparations. Toxicity effects manifested by

uniform granularity, degeneration and cellular debris were found consistently. Some degenerative changes were observed in the uninoculated controls. The results are summarized in Table XVI.

HEMATOLOGY

Hematocrit Readings

Mink in the experimental group were periodically bled by toe-nail clipping. The microhematocrit readings obtained at these times and at death were grouped by age, sex and the presence or absence of AD lesions at subsequent autopsy and are presented in Table XVII.

An increase in values for all groups occurred between three to four months of age. No difference was found in the mean values among the sexes; neither was a difference apparent in the mean values of animals which were grouped by the presence or absence of lesions. (See Table XVIII).

Bone Marrow

Samples of bone marrow were removed from the medullary cavities of the femurs which were broken open with difficulty. The first smears prepared were densely cellular and later preparations were made by either gently teasing out the marrow into elongated strips or direct imprinting.

Wrights stain was found to be disappointing and did not appear to penetrate the cells. Smears stained overnight in 1:40

Giemsa gave good results regarding the morphological details and tinctorial characteristics.

The mink marrow preparations generally indicated great activity judged by the proportion of early cell lines. Plasma cells usually single or loosely arranged in groups of two or three were observed in marrow smears of animals with advanced disease. A number of differential marrow counts were performed and the results of three typical specimens are shown in Table XIX. This illustrates the findings in three Aleutian minks of the same litter. One female was IAT negative and showed no histopathological lesions in the liver or kidney or abnormal patterns by immuno-electrophoresis. Impression smears of the spleen of this mink showed active erythropoiesis. The two AD affected mink, which also showed erythropoiesis in spleen imprints, revealed plasma cells in the lymph nodes and kidney and to some extent in the liver. A forty-fold increase in the numbers of plasma cells occurred in the bone marrow.

Erythrocyte Sedimentation Rate

Forty-three blood samples taken at autopsies were tested by the micro-sedimentation method. The majority of tests were run about six hours after death of the mink.

The mean readings at 30 and 60 minutes were 0.54 mm and 0.93 mm for 19 animals which were negative to IAT. The corresponding figures for 24 animals positive to the IAT were 0.42 mm and 1.1 mm. (See Table XX).

A number of Sapphire and Aleutian mink, in the test group, which were quite severely affected, yielded blood samples at autopsy which were watery and which coagulated spontaneously on collection. These samples were unfit for test.

Other than a slightly higher range in the IAT positive samples, no differences were noted.

Differential Blood Counts

Animals with serum protein changes showed reduced neutrophils and a relative lymphocytosis (Table XXI). These changes, however, were not apparent in animals which were negative to the IAT but which showed histological lesions (Table XXII).

Organ Imprints

Imprints taken from the cut surfaces of various organs revealed cell types not readily apparent in the corresponding paraffin sections. Granulopoiesis, as evidenced by cell types with a morphology and staining reactions of eosinophil myelocytes, were observed in the spleen, and similar granular cells with neutrophilic staining properties were observed in the liver imprints.

Many imprints from the spleens of negative and positive animals showed a definite erythropoiesis in the form of megakaryocytes or cells of the erythroid series. The latter were very prominent in animals with advanced disease. The overall incidence of the various cell types in the liver, spleen and kidney imprints is summarized in Table XXIII.

ELECTRON MICROSCOPY EXAMINATIONS

Specimens of lung, spleen and bone marrow obtained from two positive and one negative mink at sacrifice were fixed and embedded by the method of Kellenberger (56) and scrutinized at magnifications from 35 - 135,000 diameters.

No differences in structural detail, of the corresponding cell types found in the various tissues were noted except with regard to the plasma cells. In the negative animal these cells showed the normal extensive development of the endoplasmic reticulum with associated ribonucleoprotein particles. Plasma cells in the affected mink showed increased dilatation of the cysternae of the endoplasmic reticulum, indicative of increased physiologic activity.

No structures suggestive of virus particles were observed in the tissues studied.

EFFECTS OF MINK SERUMS IN RABBITS

Of the 12 rabbits used for the production of anti-mink serum, four died of known causes. The remaining eight rabbits grew well and behaved normally throughout the period of immunization. Each of these animals received the equivalent of 2 ml of mink serum - primary immunization and maintenance doses - over a period of 10 months at which time they were destroyed by euthanasia.

Autopsies revealed no gross pathology. Results of histopathological examinations of the parenchymatous organs are summarized below.

Livers Periportal fibrosis with varying degrees of chronic inflammatory cellular reaction.

Lungs Hyperplasia of the RES and perivascular cuffing with occasional endarteritis.

Spleens Hyperplasia of the RES, scattered foci of degeneration and necrosis, nuclear debris, hemosiderin deposits and phagocytosis.

Kidneys Patchy cortical congestion, occlusion of cortical arterioles by swollen endothelial cells. Occasional perivascular cuffing with mononuclear cells and neutrophils, as seen in the lungs.

These changes were regarded as results of hyper-immunization, since the perivascular lesions resulted from the use of either hyperglobulinemic or normal serum antigens.

TABLES AND FIGURES

TABLE I

COLOR PHASES AND DISTRIBUTION OF
TEST MINK BY PEN NUMBERS

Litter Color	Test Groups					
	A	B	C	D	E	F
Pastel	1	18	28	39	50	60
Sapphire	3	11	21	32	42	53
Pastel	5	16	26	37	48	59
Aleutian	7	13	22	33	43	55
Pastel	2	15	24	35	46	56
Standard	4	17	27	38	49	58
Standard	6	12	23	34	45	54
Pastel	8	14	25	36	47	57
Pastel	10	20	30	40	44	51
Pastel	9	19	29	31	41	52

TABLE II

COMPOSITION AND QUANTITIES OF INOCULUMS GIVEN TO TREATMENT GROUPS

Group	Saline	Adjuvant	Autoclaved Neg AD 10% w/v	Autoclaved Pos AD 10% w/v	Raw Neg AD 10% w/v	Raw Pos AD 10% w/v	Total Volume of Inoculum
A	2 ml						2.0 ml
B	1 ml	1 ml					2.0 ml
C		1 ml	1 ml				2.0 ml
D		1 ml		1 ml			2.0 ml
E		1 ml			1 ml		2.0 ml
F		1 ml				1 ml	2.0 ml

TABLE III

ORIGIN, COLOR PHASE OF MINK LITTERS AND AGES AT SACRIFICE

Dam	IAT of Dam	Color Phase of Litter	Age (Months) at Sacrifice
132 J	4+	Pastel	3.5
242	Negative	Sapphire	4.5
114 J	1+	Pastel	5.5
AJ 10	Negative	Aleutian	6.0
120 C	2+	Pastel	7.0
82 J	1+	Standard	7.5
284 J	3+	Standard	7.5
48	Negative	Pastel	8.0
280 J	Negative	Pastel	8.0
12 J	Negative	Pastel	9.0

TABLE IV

MINK SERUM ANTIGEN - DESCRIPTION OF DONOR MINK

No.	Color Phase	Age	Sex	IAT*	Histology
AJ 19	Aleutian	1 year	M	4+	+ve AD
AJ 11	Aleutian	1 year	M	-ve	-ve AD
49 J	Standard	1 year	M	2+	+ve AD
141 J	Standard	1 year	M	4+	+ve AD

*Iodine Agglutination Test

TABLE V

TYPE AND NUMBER OF TISSUE CULTURES MADE WITH MINK KIDNEY CELLS AND THE INOCULUMS EMPLOYED

	Crude Suspension*		Supernatant**		Filtrate***	
Donor Mink	Roller Tube	Coverslip	Roller Tube	Coverslip	Roller Tube	Coverslip
171	2	2	2	2	2	2
AJ 11	4	4	4	4	4	4
141 J	2	2	2	2	2	2
AJ 19	2	2	2	2	2	2

Uninoculated Controls - 10 Roller Tubes, 10 Coverslips

*Crude Suspension - 0.1 ml 10% suspension of liver, spleen and kidney of minks 171, AJ 11, 141 J, AJ 19.

**Supernatant - 0.1 ml of supernatant after centrifugation at 1000 RPM/15 minutes of the crude suspension.

***Filtrate - 0.1 ml of filtrate obtained after centrifugation of supernatant 6000 RPM/15 minutes and passage through a 450 mu millipore membrane.

TABLE VI

THE INITIAL IODINE AGGLUTINATION TEST (IAT) RESULTS OF EACH DAM AND THE INCIDENCE OF POSITIVE IAT REACTIONS IN THE CORRESPONDING LITTER OF MINK

Dam	IAT	IAT+ Kits	Color	Post-Inoculation Period (Days)
1	4+	0:6	Pastel	49
2	Negative	6:6	Sapphire	78
3	1+	3:6	Pastel	106
4	Negative	4:6	Aleutian	119
5	2+	4:6	Pastel	147
6	1+	5:6	Standard	169
7	3+	3:6	Standard	181
8	Negative	3:6	Pastel	193
9	Negative	2:6	Pastel	224
10	Negative	2:6	Pastel	270

TABLE VII

COMPARATIVE INCIDENCE OF MALE AND FEMALE MINK WITH DYSPROTEINEMIA AS SHOWN BY POSITIVE IODINE AGGLUTINATION TEST AT THREE STAGES OF THE EXPERIMENT

Time of Test	Males	Females
14 days pre-inoculation	5:36 (14%)	0:24 (0%)
20 days post-inoculation	21:36 (58%)	12:24 (50%)
Death	19:36 (53%)	11:24 (46%)

TABLE VIII

THE NUMBER OF MINK IN EACH TREATMENT GROUP WHICH WERE POSITIVE TO THE IODINE AGGLUTINATION TEST AT THREE SUCCESSIVE STAGES OF THE EXPERIMENT

Group	Inoculum	14 Days Pre-inoculation	20 Days Post-inoculation	Death
A	Saline Control	1:10	2:10	2:10
B	Saline/Adj	1:10	5:10	5:10
C	Adj/Auto Neg Tissue	0:10	5:10	7:10
D	Adj/Auto Pos Tissue	1:10	5:10	5:10
E	Adj/Raw Neg Tissue	1:10	6:10	4:10
F	Adj/Raw Pos Tissue	1:10	9:10	9:10

Auto = Autoclaved
Adj = Adjuvant

TABLE IX

COMPARISON OF THE NUMBER OF MINK IN EACH GROUP WHICH WERE POSITIVE TO THE IODINE AGGLUTINATION TEST (IAT) AND THE NUMBER SHOWING LESIONS OF ALEUTIAN DISEASE AT DEATH

Treatment Group	Number Positive IAT	Number with* Observed Lesions	Total Mink in Group
A	2	4	10
B	5	7	10
C	7	9	10
D	5	8	10
E	4	9	10
F	9	10	10

*All animals positive to the IAT showed lesions.

A - Saline Control
B - Saline Adjuvant
C - Adjuvant and Autoclaved Negative Tissue
D - Adjuvant and Autoclaved Positive Tissue
E - Adjuvant and Raw Negative Tissue
F - Adjuvant and Raw Positive Tissue

TABLE X

PERTINENT DATA CONCERNING MINK WHICH DIED NATURALLY DURING THE PERIOD OF TEST

Pen No.	Age	Color	IAT*	Histological AD Lesions	Test
21**	4.5 mos.	Sapphire	4+	No carcase	C
22	6.0 mos.	Aleutian	3+	Multiple	C
34	7.5 mos.	Standard	3+	Multiple	D
25	8.0 mos.	Pastel	2+	Multiple	C
19	9.0 mos.	Pastel	4+	Multiple	B

*Iodine Agglutination Test
**21 carcase not available for autopsy.

TABLE XI

THE NUMBER OF MINK IN THE VARIOUS TEST GROUPS WHICH SHOWED FOCAL LESIONS OF ALEUTIAN DISEASE IN THE LIVERS OR KIDNEYS AT DEATH

Group	Inoculum	Organ	Absent	Single	Multiple	Total Mink Pos AD
A	Saline Control	Liver	6	2	2	
		Kidney	6	2	2	4
B	Saline/Adj	Liver	3	2	5	
		Kidney	6	1	3	7
C	Adj/Auto	Liver	1	2	7*	
	Neg Tissue	Kidney	3	2	5	9
D	Adj/Auto	Liver	2	2	6	
	Pos Tissue	Kidney	5	0	5	8
E	Adj/Raw	Liver	1	5	4	
	Neg Tissue	Kidney	5	1	4	9
F	Adj/Raw	Liver	0	4	6	
	Pos Tissue	Kidney	3	2	5	10

*#21 of Group C, 4+ Iodine Agglutination Test. Carcase not available for autopsy. Assumed multiple liver and kidney lesions were present.

Auto = Autoclaved
Adj = Adjuvant

TABLE XII

THE RESULTS OF HISTOPATHOLOGICAL EXAMINATIONS OF THE LIVERS AND KIDNEYS OF ALL MINK IN THE EXPERIMENT AND THE RESULTS OF IODINE AGGLUTINATION TESTS (IAT) ON THE SERUMS COLLECTED AT DEATH

	Treatment Groups											
	A		B		C		D		E		F	
Litter Color	#		#		#		#		#		#	
Pastel	1	L K 0	18	L θ 0	28	L K 0	39	L θ 0	50	L K L	60	L K 0
Sapphire	3	L K 4	11	L K 4*	21	**4	32	L K 4*	42	L K 4	53	L K 4
Pastel	5	θ θ 0	16	L K 2	26	L K 3	37	L θ 0	48	L θ 0	59	L θ 2
Aleutian	7	θ θ 0	13	θ θ 0	22	L K 4*	33	L K 4	43	L K 4	55	L K 4
Pastel	2	θ θ 0	15	L θ 1	24	L θ 1	35	L K 3	46	L θ 0	56	L θ 2
Standard	4	L K 3	17	L K 3	27	θ θ 0	38	L K 4	49	L K 4	58	L K 2
Standard	6	θ θ 0	12	θ θ 0	23	L K 3	34	L K 4	45	θ θ 0	54	L K 4
Pastel	8	θ θ 0	14	L θ 0	25	L K 3	36	θ θ 0	47	L K 4	57	L K 4
Pastel	10	L θ 0	20	θ θ 0	30	L K 3	40	L θ 0	44	L θ 0	51	L K 3
Pastel	9	θ θ 0	19	L K 3	29	L θ 0	31	θ θ 0	41	L θ 0	52	L 0 2

L = Liver lesion K = Kidney lesion Numerals 0 - 4 = IAT Reaction *Renal arteritis
**No autopsy θ = No lesion Underlined = fatality
A - Saline Control B - Saline Adjuvant C - Adjuvant and Autoclaved Negative Tissue
D - Adjuvant and Autoclaved Positive Tissue E - Adjuvant and Raw Negative Tissue
F - Adjuvant and Raw Positive Tissue

TABLE XIII

THE BACTERIAL FLORA RECOVERED AT AUTOPSY BY ROUTINE CULTURE OF THE SMALL INTESTINE, LIVER, SPLEEN AND KIDNEY

Isolates	Small Intestine	Liver	Spleen	Kidney
Clostridium perfringens	7	2	1	2
Escherichia coli	7	2	3	1
Micrococcus	3	1	1	
Proteus ssp.	4			
Streptococcus fecalis	5			
Pasteurella ssp.	1			
Coagulase + Staphylococcus		1		
Mycobacterium avium			1	

TABLE XIV

CHANGES OCCURRING IN MINK KIDNEY MONOLAYER CULTURES FOLLOWING INOCULATION WITH 10% w/v CRUDE TISSUE SUSPENSION, SUPERNATANT FLUIDS AND 0.45u FILTRATES OBTAINED FROM MINK POSITIVE OR NEGATIVE FOR ALEUTIAN DISEASE

Donor Minks	Tube	7 days	10 days	17 days
AJ 11 Crude Suspension	4	2 2 1 1	3 3 3 3	4 4 4 4
AJ 19 Crude Suspension	2	0 0	2 2	4 4
171 Crude Suspension	2	2 1	2 1	4 4
141 J Crude Suspension	2	2 2	2 4	4 4
AJ 11 Supernatant	4	1 0 0 0	1 1 1 0	1 1 1 1
AJ 19 Supernatant	2	0 0	1 3*	1 1
171 Supernatant	2	0 0	3 3	3 3
141 J Supernatant	2	0 0	0 3	0 3
AJ 11 Filtrate	4	4 0 0 0**	4 2 2 2	4 1 1 1
AJ 19 Filtrate	2	0 0	2 2	1 1
171 Filtrate	2	0 0	2 2	1 1
141 J Filtrate	2	0 0	2 2	1 1
Uninoculated Controls	10	0 - 0 (10)	1 - 1 (10)	1 - 1 (10)

*pH change observed.
**No cells visible.

0 = Non-granular cells - Sheet intact.
1 = Uniform slight granularity visible in cells.
2 = Uniform pyknosis.
3 = Uniform pyknosis - Partial disruption of cell sheet.
4 = Total disruption of cell sheet.

TABLE XV

CHANGES OCCURRING IN COVERSLIP PREPARATIONS OF MINK KIDNEY CELL CULTURES FOLLOWING INOCULATION WITH 10% w/v TISSUE SUSPENSION, SUPERNATANT AND FILTRATE

Donor Minks	Coverslip	10 days	17 days
AJ 11 Crude Suspension	4	4 4 4 4	4 4 * *
AJ 19 Crude Suspension	2	3 0	0 *
171 Crude Suspension	2	3 3	4 *
141 J Crude Suspension	2	3 3	4 *
AJ 11 Supernatant	4	2 2 2 2	1 1 * *
AJ 19 Supernatant	2	2 2	1 *
171 Supernatant	2	2 2	1 *
141 J Supernatant	2	2 2	1 *
AJ 11 Filtrate	4	2 2 2 2	1 1 * *
AJ 19 Filtrate	2	2 2	1 *
171 Filtrate	2	2 2	1 *
141 J Filtrate	2	2 2	1 *
Uninoculated Controls	10	0 - 0 (10)	1 - 1 (10)

*Removed for staining.

0 = Non-granular cells - Sheet intact.
1 = Uniform slight granularity.
2 = Uniform pyknosis.
3 = Uniform pyknosis - Partial disruption of cell sheet.
4 = Total disruption of cell sheet.

TABLE XVI

CHANGES OCCURRING IN FOUR DAY OLD COVERSLIP PREPARATIONS OF MINK FIBROBLASTS FOLLOWING INOCULATION WITH 0.2 ml VOLUMES OF 10% w/v CRUDE TISSUE SUSPENSION OBTAINED FROM ORGANS OF MINK LITTERS POSITIVE OR NEGATIVE FOR ALEUTIAN DISEASE

	Coverslips	1 day	2 days	5 days	6 days	16 days
AJ 19	6	6 x 1	5 x 1*	5 x 1	5 x 2**	4 x 4
Controls	6	6 x 0	5 x 0*	5 x 0	5 x 2**	4 x 4

*Removed for staining.
**Removed at 10 days' incubation.

0 = Non-granular cells - Sheet intact.
1 = Uniform slight granularity.
2 = Uniform pyknosis.
3 = Uniform pyknosis - Partial disruption of cell sheet.
4 = Total disruption of cell sheet.

TABLE XVII

MEAN VALUES OF MONTHLY MICROHEMATOCRIT READINGS OF THE TEST MINK GROUPED BY SEX, AGE AND THE PRESENCE OR ABSENCE OF ALEUTIAN DISEASE LESIONS AT AUTOPSY

	Positive AD at Death				Negative AD at Death			
Age in Months	No. of Males	Ht	No. of Females	Ht	No. of Males	Ht	No. of Females	Ht
3.5	29	39.2	19	43.3	4	40.0	5	44.0
4.5	26	51.4	20	52.2	4	55.5	5	54.0
5.5	28	47.8	17	52.2	8	56.2	6	54.5
6.0	14	52.5	15	53.3	6	55.1	5	56.5
7.0	14	51.3	14	50.0	5	56.0	4	53.0
8.0	6	53.6	5	51.2	5	55.0	1	55.0

Ht = Microhematocrit

TABLE XVIII

MEAN VALUES OF MICROHEMATOCRIT READINGS OF TEST MINK GROUPED BY AGE AT THE TIME OF SAMPLING AND RELATED TO THE PRESENCE OR ABSENCE OF ALEUTIAN DISEASE LESIONS, AT THE SUBSEQUENT AUTOPSY OF EACH

	AD Lesions Present at Death		No Lesions Observed at Death	
Age of Group in Months	Mean Hematocrit	No. of Samples	Mean Hematocrit	No. of Samples
3.5	41.0	(35)	41.0	(13)
4.5	50.3	(31)	55.4	(13)
5.5	51.2	(30)	54.5	(13)
6.0 or older	52.0	(35)	55.5	(23)

TABLE XIX

DIFFERENTIAL (500 CELL) MARROW-COUNTS OF THREE ALEUTIAN MINK LITTERMATES AT SIX MONTHS OF AGE

	Mink -	A*	B**	C***
	Sex -	Female	Female	Male
	Aleutian Disease -	Negative	Positive	Positive
Neutrophils				
Mature		16.8	15.8	2.4
Bands		22.2	12.4	6.0
Metamyelocytes		11.4	11.1	23.0
Myelocytes		8.0	10.5	14.4
Promyelocytes		0.4	4.5	5.2
Eosinophils				
Mature		3.8	11.5	2.2
Immature		5.4	3.1	1.4
Lymphocytes		5.6	8.7	1.0
Plasmacytes		0.2	8.0	7.2
Normoblasts				
Basophilic		6.6	2.1	6.4
Polychromatic		0.8	0.0	1.4
Eosinophilic		19.0	11.5	33.2

*Saline control
**Autoclaved positive AD tissue and adjuvant
***Raw, negative tissue and adjuvant

TABLE XX

ERYTHROCYTE SEDIMENTATION RATES OF THE 43 MINK BLOOD SAMPLES TAKEN AT AUTOPSY

IAT* Negative Samples		IAT Positive Samples	
30 Minutes	60 Minutes	30 Minutes	60 Minutes
0.30	0.30	0.30	0.30
0.30	0.50	0.30	0.30
0.30	0.50	0.30	0.50
0.30	0.50	0.30	0.50
0.30	0.50	0.30	0.50
0.30	0.50	0.30	0.50
0.30	0.50	0.30	0.50
0.30	1.00	0.30	0.50
0.50	0.75	0.30	0.50
0.50	1.00	0.30	0.75
0.50	1.00	0.50	0.75
0.50	1.00	0.50	1.00
0.50	1.00	0.50	1.00
0.50	1.00	0.50	1.00
0.50	1.00	0.50	1.00
0.75	1.20	0.50	1.00
0.75	1.50	1.00	2.00
1.50	2.00	1.00	2.00
1.50	2.00	1.00	2.00
		1.00	2.00
		1.00	2.00
		1.50	2.00
		1.50	3.00
$\bar{X}$ = 0.54	$\bar{X}$ = 0.93 (19)	$\bar{X}$ = 0.42	$\bar{X}$ = 1.10 (24)

*Iodine Agglutination Test

TABLE XXI

MEAN VALUES OF DIFFERENTIAL BLOOD COUNTS OF 52 NEGATIVE AND 47 POSITIVE MINK (IAT* 1+ OR GREATER)

	Negative IAT	Positive IAT
Neutrophils	43.00	32.50
Bands Neutrophils	2.70	4.20
Eosinophils	3.10	2.20
Basophils	0.19	0.40
Monocytes	3.27	3.26
Lymphocytes	41.60	52.00
Squash Forms	6.05	6.30
Normoblasts	0.05	0.21

Scores were grouped according to the IAT status of the animal at the time of sampling.

*Iodine Agglutination Test

TABLE XXII

MEAN DIFFERENTIAL BLOOD COUNTS AT DEATH OF MINK
RELATED TO THREE CLASSES* OF MINK

AD Lesions	Absent	Present	Present
IAT**	Negative	Negative	Positive
Neutrophils	52.0	56.7	34.0
Eosinophils	2.6	2.0	4.0
Basophils	0.1	0.3	1.4
Monocytes	4.0	6.0	4.0
Lymphocytes	41.0	35.0	56.6
Total Mink	10	7	24

*Grouped by either positive or negative IAT and a positive histopathology.

**Iodine Agglutination Test

TABLE XXIII

THE INCIDENCE OF VARIOUS FORMS OF HEMATOPOIESIS OBSERVED IN IMPRINTS MADE FROM THE CUT SURFACES OF VARIOUS ORGANS OF TEST MINK AFFECTED WITH ALEUTIAN DISEASE

Organ	Granulopoiesis*	Erythropoiesis**	Lymphopoiesis***	Plasma Cells
Spleen	19/27	27/34	27/34	6/34
Liver	9/13	2/13	0/13	10/13
Kidney	0/15	6/13	0/15	5/15

*Eosinophil metamyelocytes
**Normoblasts and/or Megakaryocytes
***Lymphoblasts

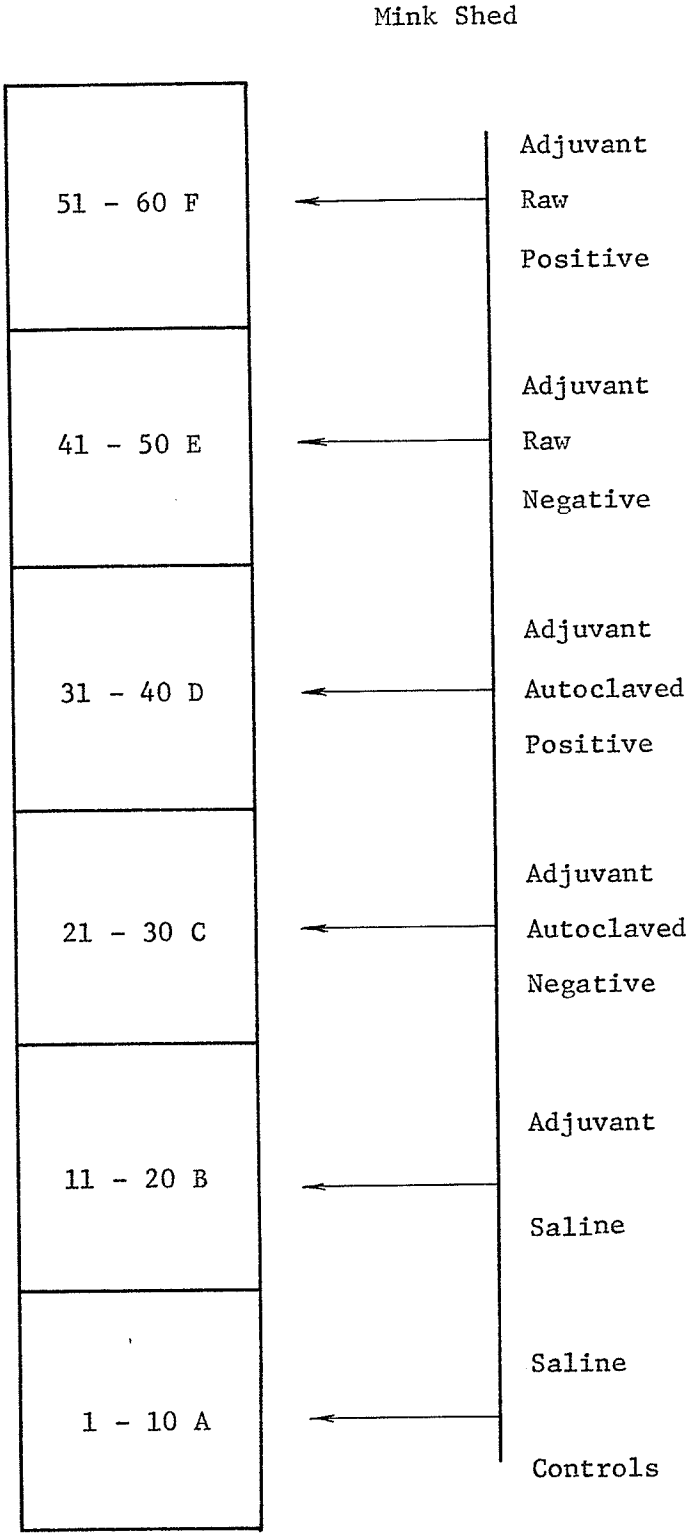


Fig. 1 - Sequence of test groups in isolation shed.

Adult Male Aleutian Siblings

(AJ 19, Positive AD; AJ 11, Negative AD)

May 14 - Etherized and exsanguinated

Organs aseptically removed and stored at -20°C

June 20 - Kidney, liver, spleen of each blended in sterile distilled water at room temperature

Suspensions lyophilized, aliquoted and stored at room temperature

July 6 - Lyophilates reconstituted to 10% w/v in sterile distilled water. Aliquots autoclaved one hour at 15 lbs.

July 7 - Four tissue inoculums used consisted of:

1) Raw 10% w/v Negative Suspension

2) Autoclaved 10% w/v Negative Suspension

3) Autoclaved 10% w/v Positive Suspension

4) Raw 10% w/v Positive Suspension

Fig. 2 - Source and preparation of tissue inoculums from donor minks AJ 19 and AJ 11.

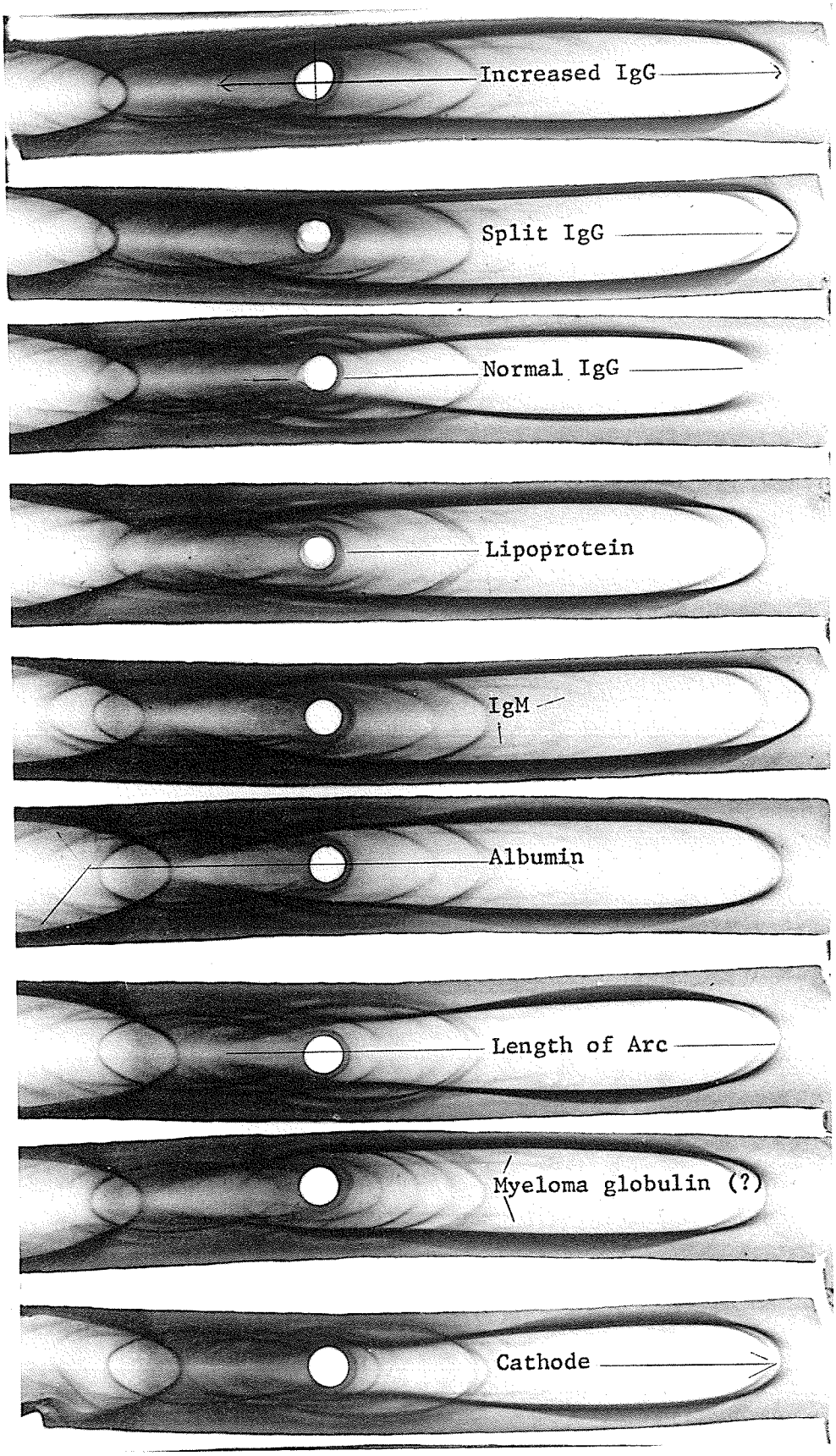
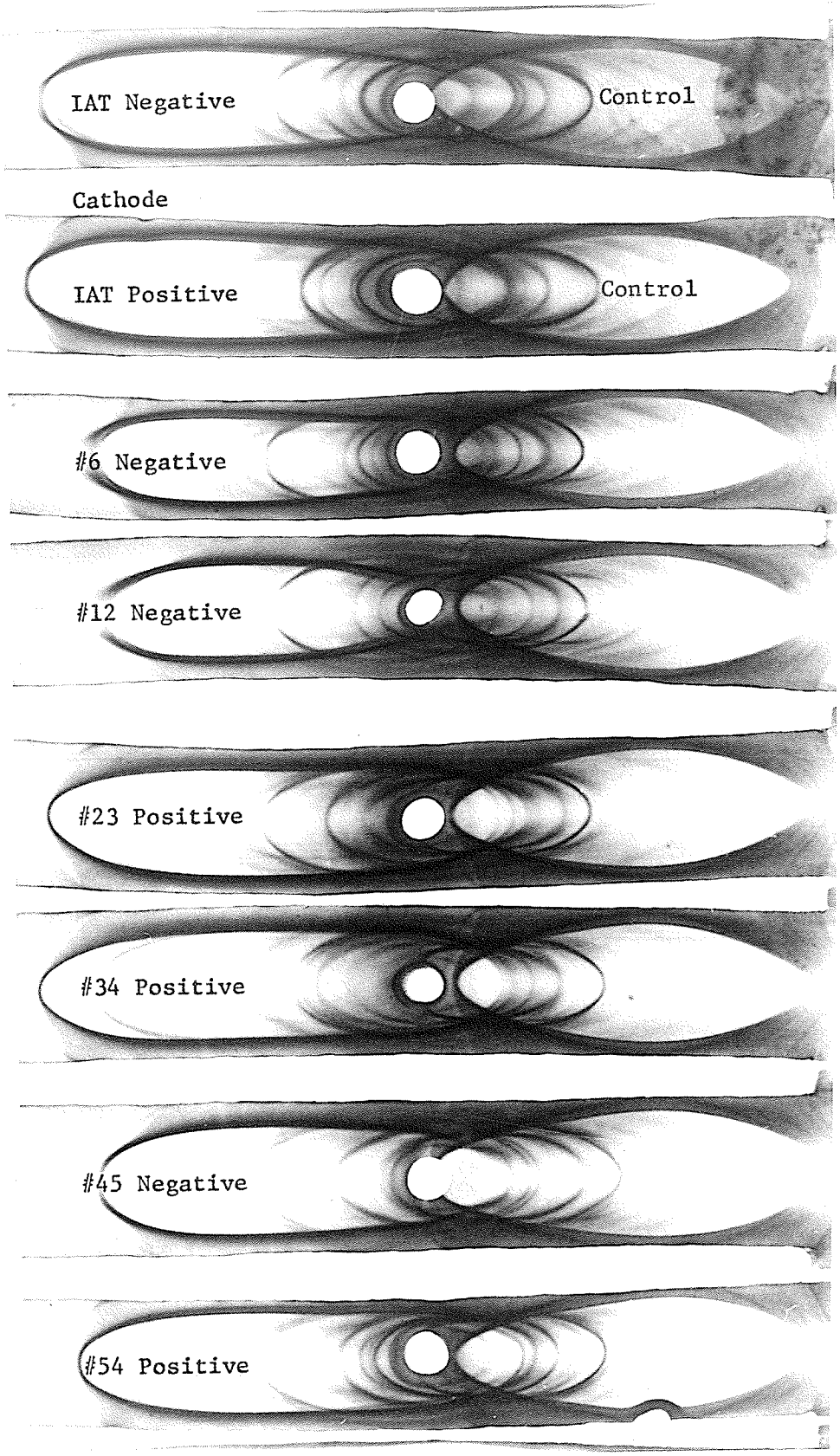


Fig. 3 The important precipitation arcs observed in immuno-electrophoretograms of mink serums.

Agar gel. Rabbit anti-whole-mink serum in all troughs.



Agar gel. Rabbit anti-whole-mink serum in all troughs.

Fig. 4 Immuno-electrophoretograms of litter 284 (Standard Dark mink) 7.5 months. Note the dimensions of the IgG arcs in relation to Aleutian Disease status.

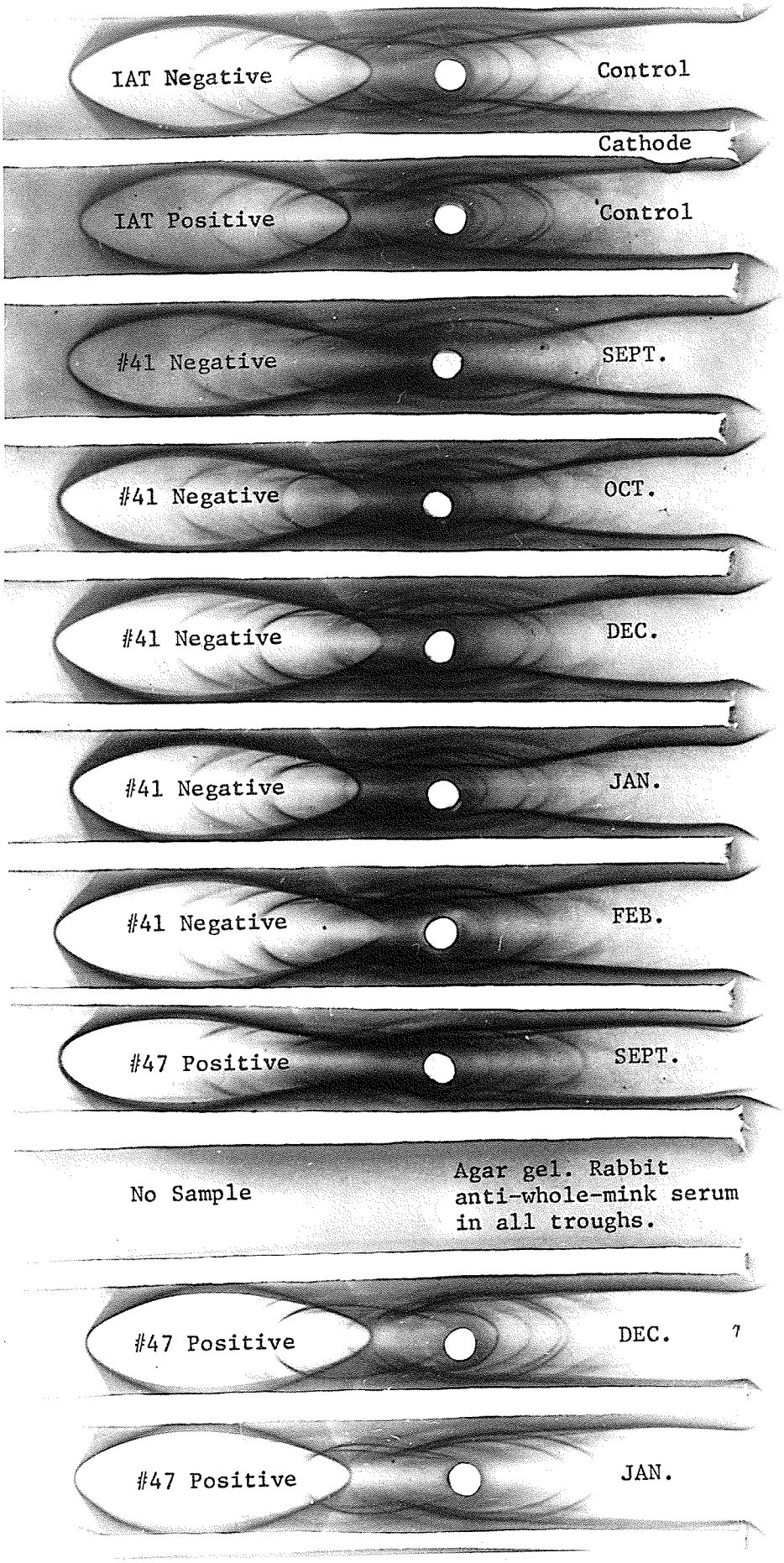
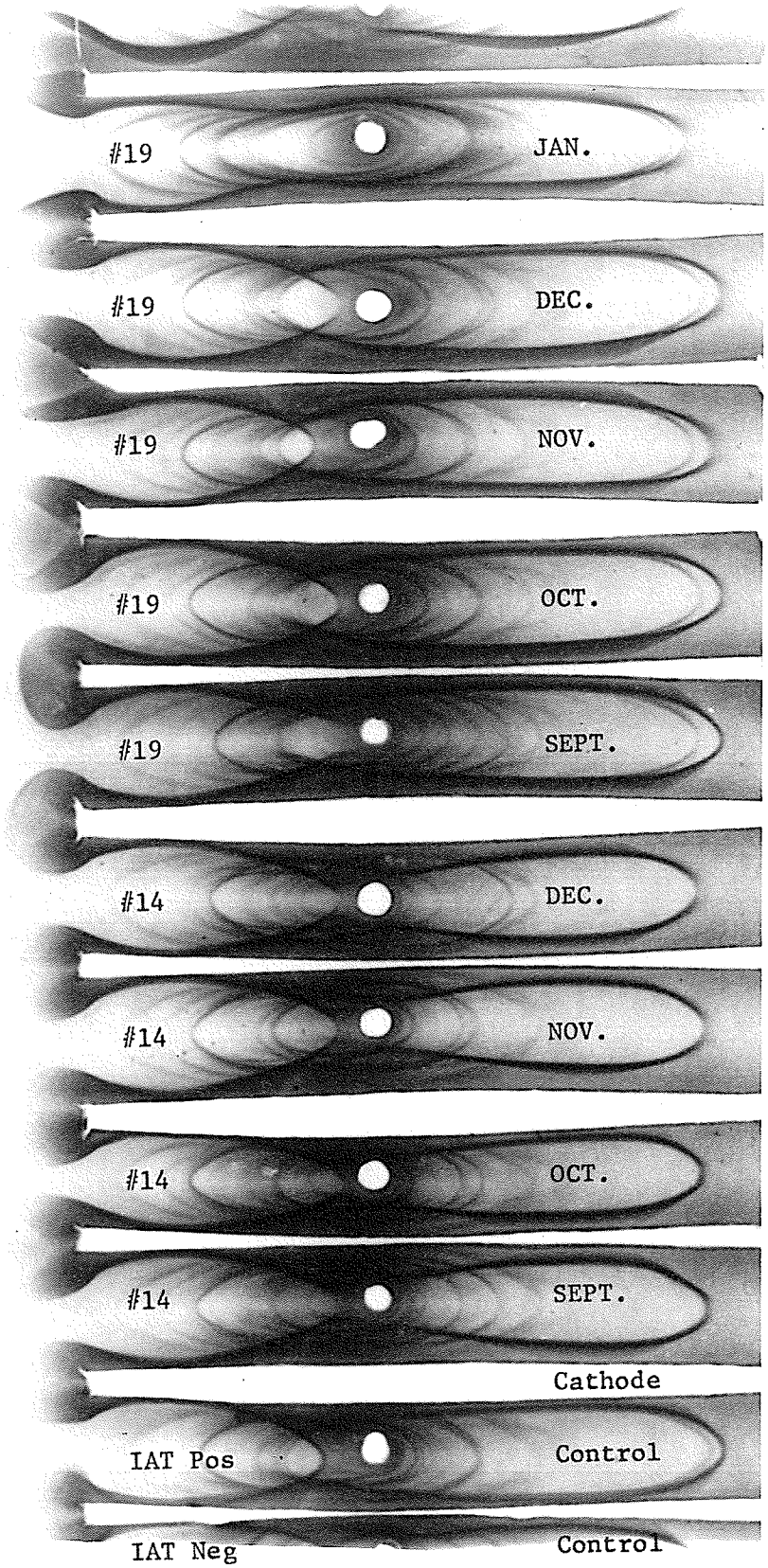


Fig. 5 Serial immuno-electrophoretograms of a normal and Aleutian Disease affected mink. The reduction of medium mobility proteins in the September patters is probably associated with molting.



(Death)
Note severe pattern changes.

Rabbit anti-whole-mink serum in all troughs. Agar gel.

Fig. 6 Illustrates serial immuno-electrophoretograms of a normal and Aleutian Disease affected mink. The positive animal maintains a split IgG arc.

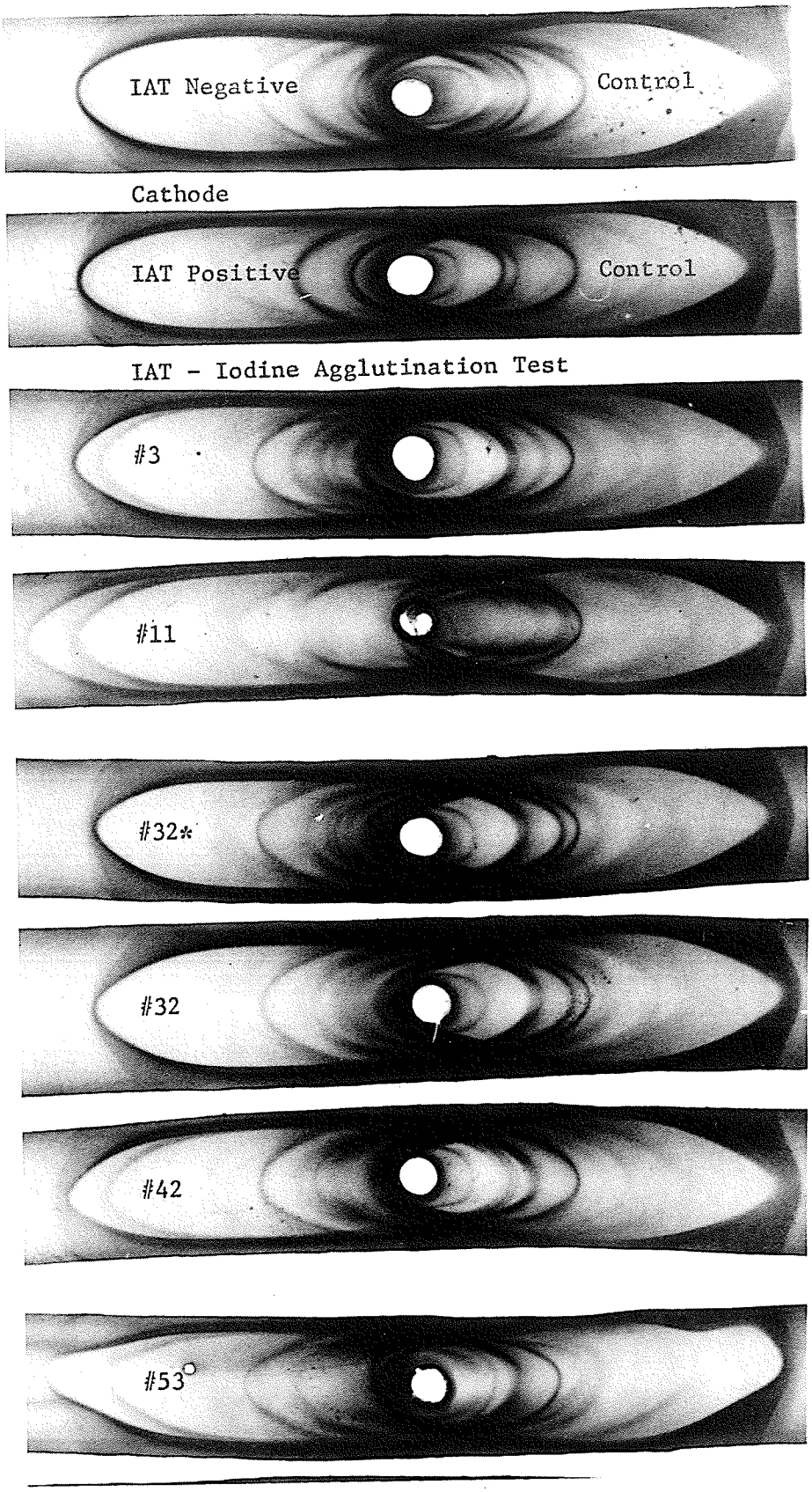
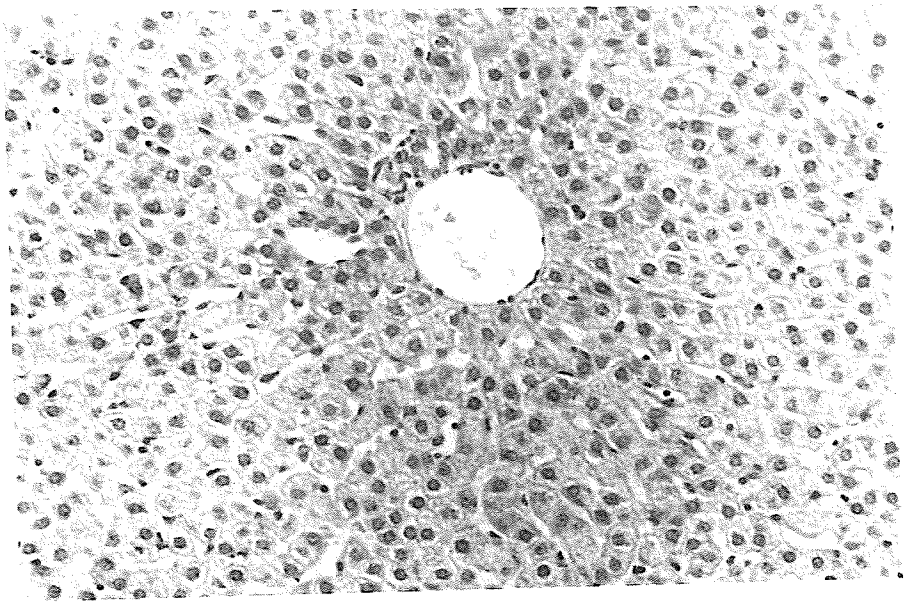
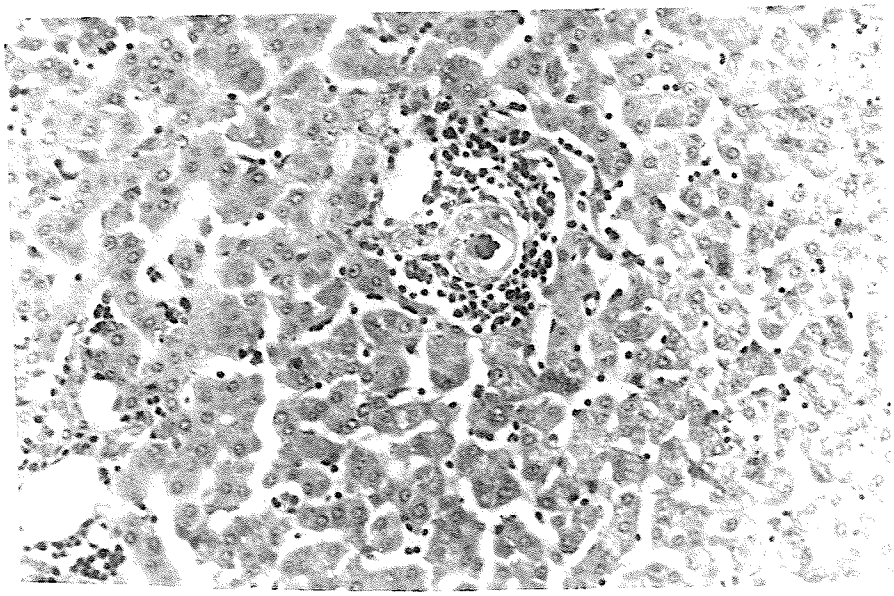


Fig. 7 Immuno-electrophoretograms of mink litter 242 (Sapphires) 4.5 months. All are hyperglobulinemic. #11 mink shows a prominent arc of alpha-2 mobility. Agar gel. Rabbit anti-whole-mink serum. *Replaces #21.



x 160



Note focal collection and the interstitial distribution of mononuclear cells.

x 80

Fig. 8 Sections of normal and Aleutian Disease affected mink livers. The critical lesion is shown in the lower photomicrograph.

D I S C U S S I O N

In reviewing the scientific literature concerning the etiology of AD, it becomes apparent that control of transmission experiments has been consistently undermined by several difficulties inherent in the syndrome itself.

At present no method exists to determine whether incipient disease, either in terms of a specific agent or early tissue changes, is present in a given mink. Further, it has been difficult to say with certainty when minor lesions, typical of AD, are found in an inoculated mink, if such an animal is truly "diseased." If the criterion of animal "infection" or transmission were taken as the ability to consistently produce clinical illness or death, it would be more convincing. The present failure to isolate a specific agent by the inoculation of other laboratory animals or tissue cultures, combined with the inconstant results obtained by mink inoculation, tends to oppose the concept of a biological agent in the inoculum.

Another general aspect concerns the lack of knowledge regarding the physiological parameters of wild or ranch mink. Does AD, in any degree, occur in wild mink? The answer would seem very pertinent to a study of the problem in the domesticated strains.

The results of experimental transmission are usually non-fatal which suggests that the genetic substrate for the inoculum might be more important than the presence of the agent in achieving a definite response. If the viral etiology is put

aside, the reactions observed following the use of certain inoculums, might be interpreted simply as physiologic responses which differ according to the genotype (predisposition). It is conceivable that AD suspensions contain organic substances which are stimulators of the RES. This could account for the self limiting type of "disease" seen in non-susceptible mink.

In the present study, it was hoped to elucidate some of these points by the use of raw and autoclaved AD suspensions combined with adjuvant in test groups of sibling mink.

It was next important to define by what means the diagnosis of an established case of AD could be made consistently. Previous workers have taken the presence of plasmacytosis - consisting of aggregates of mononuclear cells including plasma cells - and dysproteinemia as detected by a positive IAT, as being the most reliable methods of diagnosis. The results of this study support the validity of these criteria as follows: plasmacytosis was evident in affected animals in all cases where a positive IAT occurred (Table XII). However, the converse of this was not true. This is consistent with previous experience in that the IAT is not a reliable test in the control of the disease. The results indicated that whereas 31 mink were IAT positive, the number of mink which showed lesions of AD was 47. This suggests that the size, extent and degree of tissue involvement is important as to whether the IAT is positive or not.

Plasmacytosis is best supported by the myelograms of the three Aleutian mink littermates, two of which were positive, outlined on Table XIX. It will be noted that in spite of the variability seen in the proportions of other cell types, the constant difference between the positive and negative animals is a much higher percentage of plasma cells.

In the four animals examined of the five that died prematurely, all showed hepatosplenomegaly and enlarged kidneys. Microscopic examination of these organs revealed numerous focal areas of mononuclear cells many of which showed the morphology and staining characteristics of plasma cells. Some doubt has been expressed as to whether these abnormal aggregates are the result of infiltration or proliferation in situ. The types of cells observed in organ imprints of positive animals suggest by their relative size and mitotic activity that replication was occurring in situ. This phenomenon was not as readily observed in HE paraffin sections of the same organ. That in situ replication was occurring is further supported by the fact that such cells were never found circulating in the peripheral blood. Albeit the mitotic cells were not necessarily plasma cell precursors, the fact that no typical plasma cells were observed in smears of peripheral blood, tends to suggest that the plasma cells seen in association with the aggregates, were formed and remained at the site. Although similar aggregates of cells were found occasionally in small numbers in the tissues of young mink, plasma

cells were rarely if at all seen. This would suggest a more than accidental association between the presence of plasma cells in these aggregates of cells and the presence of disease in that particular animal.

Thus the three criteria, dysproteinemia, (IAT positivity) aggregations of mononuclear cells in tissues, and the presence of readily perceived numbers of plasma cells in such aggregates, would all appear to be good criteria to support the diagnosis of AD.

It would seem then that AD is a distinct entity and that as such, is potentially, a progressive disease which terminates fatally. Furthermore it is likely that varying degrees of severity of the disease may exist. This is supported by the fact that the disease may progress at different rates in litter mates, evidenced by five fatalities seen in this experiment when other identically treated animals were clinically quite healthy at this time; in fact, all other animals had to be sacrificed.

Other factors must therefore modify the severity and rate of progress of the disease. The most prominent predisposing factor would appear to be a genetic one. It is known, and has been supported in this study with rabbits, that AD cannot be reproduced in any animal species other than ranch mink.

The results of the study support the presence of a genetic predisposition for this disease. This is shown in Table XII where every member of a complete litter of Sapphire mink which are double recessive for the Aleutian gene, was affected. This, when

compared to a litter of Pastel mink in which only 1/3 of the litter was affected reflects a difference in susceptibility which appears to be associated with a genetic factor. One of the more convincing experiments reported (29) showed that AD, as evidenced by dysproteinemia, was genetically conditioned. The results of IEP of serums of diseased and non-diseased mink substantiate the onset and progressive development of hyperglobulinemia - chiefly the immunoglobulins - in affected mink which was related to presence of plasma cells in the aggregates and a corresponding positive reaction to the IAT (Fig. 4).

Further, the degree of tissue involvement and systemic effects of hyperglobulinemia whether related to renal impairment or general metabolism were reflected in unusual electrophoretic patterns whether compared in serial time studies of an individual mink or compared to the patterns of other negative or positive animals (Fig. 4).

Since the parameters of the genetic age or sex influences reflected in the IEP patterns of mink* are not presently known, the small number of mink utilized in this study (which was dictated by economic considerations) did not permit the parameters of genetic and sex influences to be significantly evaluated. However, the results show that major changes in the constituents of the serum proteins do occur in mink at the age of molting or correctly

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*An initial IEP study of normal mink serum proteins has recently been published. (57)

"furring out," which normally takes place in the late fall of the year (Fig. 5).

The implications of genetically linked susceptibility would suggest that AD is an expression of an inherent defect of the animal the consequences of which are provoked by one or many different stimuli. This is borne out by the use of five different mixtures in this experiment all of which were closely similar in effect and which produced a significantly higher incidence of mink with lesions of AD than was found in the uninoculated controls.

There are many parallel situations described in the literature where previously undetected genetic defect was revealed only after contact with the appropriate triggering mechanism (e.g.) six glucose phosphatase deficiency of the erythrocytes of humans who develop hemolytic anemia following ingestion of the Favus bean. The common factor of the five different mixtures which induced similar responses, was Freunds Incomplete Type Adjuvant. Substances of this type are known to act as non-specific stimuli which cause hyperplasia of the lympho-reticular system and increase the rate of metabolism in these cells, particularly in immature animals.

Evidence that a specific agent exists in AD tissues and which causes a particular cytopathic effect in mink tissue culture as described by Basrur, was not confirmed. The changes observed in the tissue cultures were suggestive of a non-specific toxicity.

These toxic effects were seen in cultures which were inoculated with either AD affected or normal tissue suspensions.

In its approach to the question of the cause of AD, the present study differs in some respects from that of previous workers. The use of adjuvant, heat-treated suspensions, immuno-electrophoretic analysis and the examination of organ imprints, have not so far been described in relation to this disease. It is therefore difficult to make comparisons in these respects, but it is hoped that the findings of this study will prove of interest and value to other workers, especially the possible effects of adjuvant.

C O N C L U S I O N S

Aleutian Disease is a species specific malady of mink which appears to be the result of a defective hyperplasia of one or more stem cell types of the lympho-reticular system and which is genetically conditioned.

The disease is manifested by:

Varying degrees of hyperglobulinemia.

Varying degrees of plasmacytosis.

Aggregates of mononuclear cells present in the bone marrow, spleen, liver, and in advanced cases, kidneys, and which may well represent erythroid and myeloid precursors.

Although the basic defect is genetically dependent, the overt disease requires some form of prolonged stimulus which is more probably non-specific than specific in nature.

There is little evidence that the stimulating factor is a contagious replicating agent of the classical type because autoclaved diseased tissues produced the disease together with unheated non-diseased tissues as well as Freunds Incomplete Type Adjuvant plus saline alone.

Animals given untreated diseased tissue showed no higher incidence of the disease than any other group which suggests that contagion due to a replicating infectious agent is unlikely.

Tissue culture experiments using diseased and non-diseased tissues as inoculums either as crude tissue suspensions or supernatants or filtrates derived from them, showed no virus like

effects in those cultures exposed to any of the three types of inoculums prepared from diseased tissues.

In those cultures exposed to the effects of similar inoculums prepared from non-diseased tissues a similar effect was produced. The effect was consistent with toxic changes often seen in any cell culture system following exposure to aggregated gammaglobulin preparations whether present in serum or tissue homogenates. This finding was further enhanced by the fact that both non-diseased and diseased crude tissue suspensions showed a greater toxicity than either the separated homologous supernatant or filtrate.

Routine bacteriological and fungal cultures from autopsy tissues removed with aseptic techniques, failed to reveal any pathogen common to diseased mink and absent from non-diseased tissues.

Controlled IEP of litters and similarly treated groups of mink, when performed simultaneously under identical conditions, showed marked variations in pattern attributed to the following:

a) differences in age

b) physiological state

c) genetic differences

d) artifact

e) presence or not of AD

f) severity of AD with respect to the degree of renal involvement or impending death.

Aleutian Disease affected mink showed irregular differences in length and distance of diffusion from the electrophoretic axis, of at least one immunoglobulin arc. This increase was regularly associated with IAT positivity and indeed was often detectable beforehand.

Differences in patterns due to genotype alone were not obvious.

Serial time studies of serum samples showed significant changes at the time of molting between September and October which consisted of obvious loss in quantity of most medium mobility proteins which normally could be separated from one another antigenically, and general merging of the IgG and albumin arcs.

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APPENDIX A

PRIMARY IMMUNIZATION SCHEDULE FOR RABBIT ANTI-MINK SERUM

Day	1:10 Serum	Route	Adjuvant	Route
1	0.5 ml	S/c	0.5 ml	S/c
14	0.5 ml	S/c		
36B*	1.0 ml	I/m	1.0 ml	I/m
44	1.0 ml	I/m		
52B*	1.0 ml	I/v		
58	1.0 ml	I/v		
68B*				

*Days on which blood was harvested.

Adjuvant - Arlacel A 1 part
Bayol F 9 parts

APPENDIX B

Method for Concentration of Antiserum

Bulk antiserum was placed in Visking dialyzing tubing and the ends secured. The latter was covered with flakes of polyethylene glycol 20M* and left at room temperature for 6 - 8 hours. The resinous material was rinsed off the outer surface of the tubing and the concentrated antiserum transferred to bijou bottles. The antiserum was reduced to approximately one-fifth of the original volume.

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*Carbowax - Union Carbide

APPENDIX C

PROPAGATING MEDIUM USED IN TISSUE CULTURE

Calf Serum	-	20 parts
Hanks Balanced Salt Solution	-	60 parts
Tryptose Phosphate Broth	-	20 parts
Penicillin	-	100 I.U./ml
Streptomycin	-	50 ugm/ml