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Monoclonal γ-Globulins in Ferrets with Lymphoproliferative Lesions.* (31529)

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(Introduced by Wesley W. Spink)
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In mustelids, Aleutian disease (AD) of mink has been the only disease described in which gammopathies resembling those observed in man had occurred(1-3). However, recently ferrets have been found to have a condition analogous to, if not identical with, AD in mink in which a systemic proliferation of lymphoid elements occurs with concomitant hypergammaglobulinemia and vasculitis(4).

Since previous attempts to transmit AD to ferrets resulted in infectivity as measured by transmission of AD back into mink, with no change in serum proteins(5), ferrets from different breeders were obtained for AD transmission to determine the influence of genotype and previous exposure to AD. During the course of these studies, ferrets from a particular ranch were observed to have hypergammaglobulinemia(4). As the result of this observation, the survey reported herein was made to establish the incidence of hypergammaglobulinemia and the occurrence of myeloma-like immunoglobulins in a representative ferret population.

Materials and methods. Ferrets of both light and dark color phases ranging in age from 1 to 4 years and about equally distributed in males(49) and females(43) were used in this survey. The sera harvested from 92 blood samples obtained from ferrets on 3 ranches, as indicated in Fig. 1, were analyzed electrophoretically to quantitate the level of *gamma* globulins and to determine if monoclonal hypergammaglobulinemia existed.

Quantitative estimates of the *gamma* globulins were made with paper electrophoresis at pH 8.6, 0.075 ionic strength veronal buffer in a Spinco model R electrophoretic cell with a potential difference of 75 volts, (2.5 mamps) for 16 hours. The electrophoretically resolved proteins were stained with bromphenol blue and the paper strips scanned with a Beckman RB integrating densitometer. Additional evidence of myeloma-like globulins was sought by zone electrophoresis on cellulose acetate and by immunoelectrophoresis. The Beckman model R101 Microzone cell was employed for electrophoresis with cellulose acetate using veronal buffer at pH 8.6, 0.075 ionic strength at 250 volts (4-6 mamps) for

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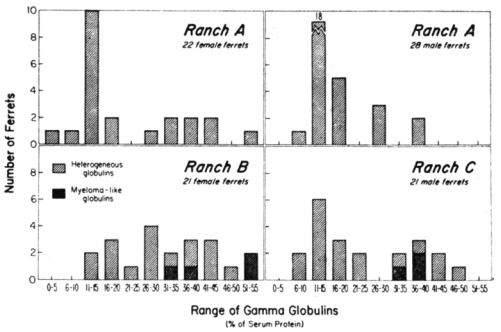

FIG. 1. The distribution of ferrets from each of the 3 ranches according to per cent *gamma* globulin of total serum protein.

20 minutes. The cellulose acetate strips were stained with ponceau S. Immunoelectrophoresis analyses were made by the method of Scheidegger(6) using antisera prepared in rabbits to whole mink serum and with rabbit anti-ferret serum, obtained from Hoechst Pharmaceuticals, Cincinnati, Ohio.

On the basis of the electrophoretic character of the serum, animals with extreme hypergammaglobulinemia or sharp monoclonal banding in the immunoglobulin zones were purchased and necropsied to establish if lymphoproliferative changes similar to those previously observed in ferrets were present (4). For comparison control normal ferrets were similarly processed. For this purpose tissues from kidney, liver, spleen, lymph nodes, and thymus were fixed in neutral buffered formalin, sectioned and stained with hematoxylin and eosin.

Sera from hypergammaglobulinemic and from normal ferrets were examined in the ultracentrifuge after the sera were diluted to 2.5% protein with phosphate buffer pH 7.6, 0.5 ionic strength. Analyses were conducted in a double sector cell at 59,780 r.p.m. In one experiment the proteins in the *gamma* region of a serum resolved on starch block electrophoresis were isolated and subjected to sedimentation analysis.

Results. Quantitation of *gamma* globulins in serum samples from the 3 ferret ranches revealed that a large number of ferrets (38 out of 92) had *gamma* globulin values above 20% which may be considered the upper limit of the normal level for most domestic animals(7). The data shown in Fig. 1 regarding sera from ferrets from Ranch A where both males and females were examined indicated that the incidence of hypergammaglobulinemia was greater in females than males, 8 of 22 as opposed to 4 of 23. A similar trend in sex distribution of animals with hypergammaglobulinemia may have existed in the females on Ranch B (16 of 21) and the males from Ranch C where 10 of 22 had hypergammaglobulinemia.

A total of 7 ferrets were found which showed sharp monoclonal banding of the *gamma* globulins, 4 on Ranch B and 3 on Ranch C. The typical myeloma-like banding observed on cellulose acetate electrophoresis with ferret serum may be seen in Fig. 2 (arrows). Immunoelectrophoresis of these sera revealed a narrow dense bow of precipitated *gamma* globulin when diffused against either antisera prepared to whole mink serum or ferret serum.

The sera examined in the ultracentrifuge showed increases in the 7S components in both the myeloma-like and heterogeneous types of hypergammaglobulinemia (Fig. 3). In an experiment where the sharply defined monoclonal globulin was isolated from starch block and sedimented, it was established as being a 7S component.

A total of 16 ferrets selected on the basis of having either monoclonal γ-globulin components or very high *gamma* globulin values were examined for histologic lesions similar to those previously described in ferrets(4). In all cases where the *gamma* globulin level was elevated, plasmacytic and lymphocytic infiltrates were observed in the kidney and liver sections. In 5 ferrets the thymus was markedly hypertrophied filling the entire anterior mediastinum. In these animals the mesenteric lymph nodes were also enlarged. Vasculitis was apparent in some of the organs. No pronounced difference in lesions was detectable between those ferrets with "monoclonal" and those with "polyclonal" hypergammaglobulinemia.

Discussion. A plasmacellular disease with lesions similar to those described in AD of mink was found to be as widespread among ferrets as AD is in mink. Although most of the ferrets with hypergammaglobulinemia

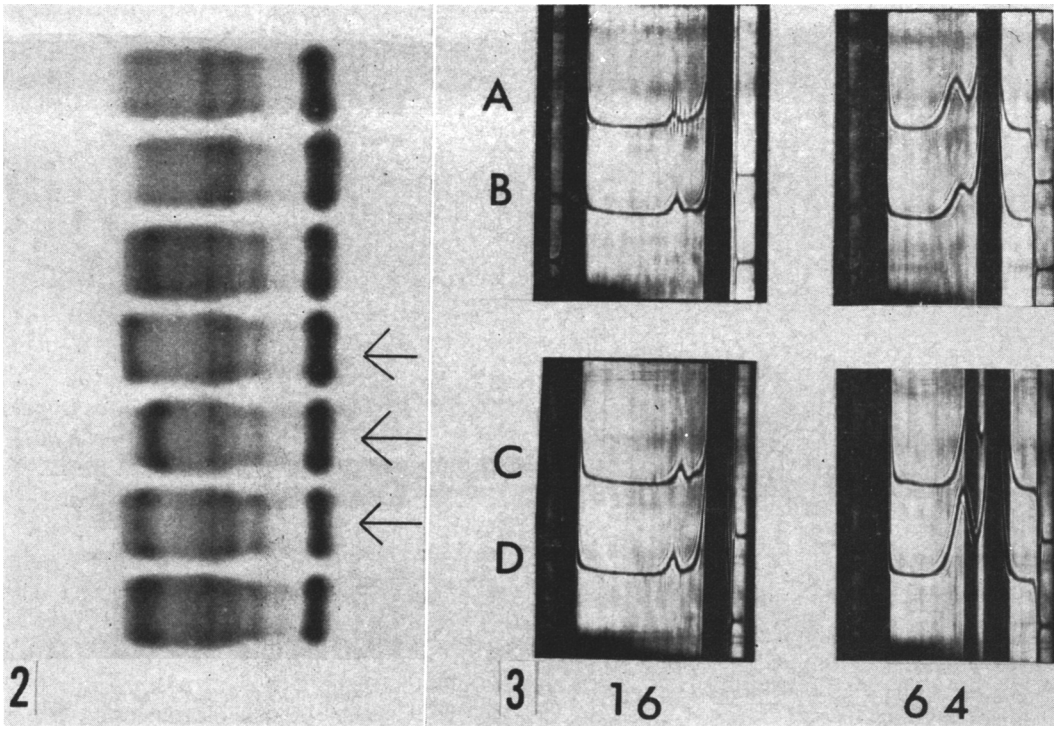

FIG. 2. Electrophoretic pattern of 2 normal ferret sera above and 5 with hypergammaglobulinemia. Arrows indicate those with very sharply defined monoclonal banding in γ-globulin region.
FIG. 3. Ultracentrifuge pattern of ferret sera showing increased 7S components (A, C. D) and a pattern from a normal ferret (B). Direction of sedimentation is from right to left. Frames were taken at 16 and 64 minutes, after the machine had reacted 59,780 r.p.m.

were not overtly ill, on necropsy they all were found to have pronounced plasma cell proliferation with hypertrophy of lymph nodes and frequently the thymus.

Monoclonal γ-globulin components have been observed in a few mink which have survived AD for periods longer than 1 year. In a survey of 859 mink with histologic lesions of AD, 16 were found to have myeloma-like hypergammaglobulinemia(3). In our study with ferrets, 7 animals had myeloma-like globulins out of a total of 38 having *gamma* globulin levels above 20%. The protein response in mink infected with AD is initially a broad heterogenous response probably representing polyclonal type of synthesis of immunoglobulins. In some mink surviving AD, γ-globulin components become more homogeneous resembling that seen in monoclonal neoplasia. Since the mortality does not appear to be as great in ferrets with this condition as in mink with AD, the possibility of more survivors having monoclonal γ-globulins would be increased. Thus, in ferrets with a prolonged illness sufficient time may have elapsed for the transition to or the ascension of a single clone of synthetic plasma cells.

Since all varieties of mink may be infected with the AD agent, it is not surprising that other members of the *Mustelidae* (mink, ferrets, skunks, weasels, wolverines) also may be infected with the agent or a variant of it. However, certain histologic features observed in ferrets seem to set this disease apart from that described as AD in mink. Massive involvement of the thymus in mink with AD has not been reported, whereas it was commonly observed in ferrets with hypergammaglobulinemia. In general, the disease in ferrets resembles AD in mink more closely than it does the lymphoproliferative changes associated with other diseases such as that seen in inbred strains of mice with an antoimmune disease(8,9) and in mice with transmissible

lymphomas(10).

Summary. Electrophoretic analyses of sera from 92 ferrets revealed 38 to have *gamma* globulin values above 20%, 27 above 30%, and 12 above 40%. Sixteen of the ferrets with hypergammaglobulinemia were necropsied and found to have systemic plasmacellular infiltrates with lymph node and frequent thymic hypertrophy. Seven of the ferrets had a monoclonal type of hypergammaglobulinemia.

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The Virus Watch Program. IV. Recovery and Comparison of Two Serological Varieties of Adenovirus Type 5.* (31530)

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From January 1961 through March 1965, during the Virus Watch program(1,2,3), a continuing surveillance of viral infections in metropolitan New York families, adenovirus type 5 was found in 66 respiratory and fecal specimens. Isolates from 32 specimens resembled previously described type 5 viruses (4,5,6) in that they exhibited at most a minor hemagglutination-inhibition (HI) cross-reactivity with rabbit antiserum against adenovirus type 1. The 34 remaining specimens, however, yielded type 5 isolates which showed considerably increased HI cross-reactivity with the same type 1 antiserum. The observation of two such readily distinguishable varieties of adenovirus type 5 prompted this comparative study.

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Materials and methods. Specimen collection and processing. Routine surveillance of each Virus Watch family consisted of a regularly scheduled bi-weekly collection of a family illness history and simultaneous collection of respiratory and fecal specimens from one or more family members designated as index persons. In addition, "special" respiratory and fecal specimens were obtained from as many family members as possible when suspected viral illness occurred in any family members. Respiratory samples generally were inoculated into tube cultures of primary rhesus monkey kidney (MK) and HEP-2 cells within a few hours of collection, while fecal specimens were extracted overnight as previously described(2) and usually were inoculated into both cell cultures the day after collection. For serologic studies, serum samples usually were obtained from all family members on admission to the program and roughly twice a year thereafter.

Typing antiserum. Stock antiserum to adenovirus 1 was produced in four 8½ to 10 lb female rabbits of non-pure basically New Zealand white breed which were bled and