

## A DISEASE RESEMBLING DISTEMPER EPIDEMIC AMONG FERRETS

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(With 1 Figure in the Text)

### INTRODUCTION

DURING the winter of 1934–5 an epidemic of acute respiratory disease occurred among the ferret stocks of dealers in South London who supplied the Wellcome Research Laboratories at Beckenham. In certain respects the disease resembled distemper, which is known to be a common disease among ferrets, but it differed in some points from canine distemper in the ferret as described by Dunkin & Laidlaw (1926). For this reason it was submitted to the following experimental investigation.

The plan adopted was to continue the disease under experimental conditions in Cambridge, well away from other ferrets and other distemper, so that a study could be made of its relation to distemper of the classical variety, and its course, symptoms and morbid anatomy recorded.

Recently, another spontaneous disease of ferrets has been observed in America by Slanetz *et al.* (1936). Their account suggests that the American disease was very similar to the London disease of 1935, but again there are points of difference. A comparison of the three diseases is made below, after the description of the London disease.

### SOURCE OF MATERIAL

Early in 1935, three sick ferrets were sent from London to Cambridge. Two of these were dead on arrival. The third, a medium-sized female, arrived in a very bad condition at midday on 10 January 1935. When first seen, this animal was drowsy and offered no resistance to handling. Respiration was noisy and obstructed. The eyes were clear and free from conjunctivitis though held partly closed. Yellow muco-pus was running over the corners of the mouth, and there was a brown crust around the nostrils. The rectal temperature was 105·2° F. In the course of the afternoon, the animal became very inert and seemed moribund, with a temperature falling to 104·0° F. It was therefore killed with coal gas and immediately dissected.

At dissection, the eyes appeared healthy, and no vesicles, pustules or other lesions were found in the mouth or on the chin or lips. The nostrils were blocked

with muco-pus. The anterior turbinates which were inflamed were removed with the precautions described below. One of them was placed in fixative, and the other used for the inoculation of other ferrets.

The trachea contained frothy muco-pus, smears of which showed large numbers of small Gram-negative bacilli, but no Gram-positive bacteria. On opening the thorax, the pleura appeared healthy. The lungs were pale pink in colour with about fifty small miliary lesions on their surface. On cutting into the lungs, frothy muco-pus escaped from the small air passages, but no large areas of consolidation were found. The spleen was large and a uniform pale chocolate in colour. The liver and kidneys were pale but not otherwise unusual. No abnormalities were seen in any other viscera.

Sections were stained, some with haematoxylin and eosin, others with Laidlaw's acid fuchsin inclusion-body stain. They showed the following microscopic appearances:

The mucous membrane of the turbinate was inflamed, and its epithelium contained many apparently typical "distemper inclusion bodies". The lungs showed a patchy scattered bronchopneumonia. The areas of consolidation frequently lay next to or contained small bronchioles which were full of pus. The bronchial epithelial cells contained numerous "distemper inclusions". The solid areas of lung had lost all visible lung structure and consisted of masses of cells—small round cells, polymorphonuclear leucocytes and larger mononuclear cells. At the edges of the solid areas the process appeared to be spreading into the neighbouring lung as an infiltration of the walls of the alveoli, the cavities of which contained varying numbers of cells. "Distemper inclusions" were seen only in bronchial epithelium. In the kidney, the parenchyma and connective tissue appeared healthy, but numerous "distemper inclusions" were seen in the transitional epithelium of the pelvis. The spleen showed no pathological change. The liver was heavily infiltrated with fat which was uniformly distributed in large droplets throughout the tissue.

Cultures were made from the trachea, lung, spleen, heart blood, etc., on horse blood agar plates, on Fildes' peptic blood digest agar, and in plain peptone broth. *Brucella bronchiseptica* was obtained in apparently pure culture on plates sown from the trachea and in a broth inoculated from the spleen. Plate cultures sown directly from the spleen remained sterile, as did cultures from the heart blood. The turbinates yielded a mixture of bacteria, including *Br. bronchiseptica*. No *Pasteurella* was obtained in direct cultures made from this ferret.

The unfixed anterior turbinate and a small piece of lung were removed with precautions against contamination and ground with powdered glass and saline. The suspension, which was very thick, was allowed to settle at room temperature for 5 min., and the supernatant fluid was then used for the inoculation of two mice, one healthy ferret and one ferret which was immune to dog distemper. (This animal and the two distemper-immune ferrets used later were kindly provided by Mr Dalling of the Wellcome Research Laboratories.)

The distemper-immune ferret (No. 1) remained perfectly well and never showed the slightest sign of ill health.

The normal ferret (No. 4) developed an acute coryza and bronchopneumonia and was used to initiate a series of passages from which the following description is derived. (Ferrets are referred to by number on the pedigree chart.)

The two mice were inoculated by the intraperitoneal injection of 0·1 c.c. of turbinate-lung suspension. One died in the succeeding night and the other was killed next morning when apparently moribund. Both were found to be suffering from a septicaemia apparently due to a *Pasteurella* which was obtained in pure culture from the heart blood. This organism retained its pathogenicity for mice for at least 18 months; on the other hand, its pathogenicity for ferrets as tested by intranasal and intraperitoneal inoculation was negligible.

The properties of the strain of *Pasteurella* derived from these two mice were as follows:

*Morphology*: small, regular, bipolar, Gram-negative bacilli.

*48 hr. agar plate culture*: colonies 3–4 mm. in diameter, irregularly circular, with a slightly undulate edge, smooth surface and soft, easily emulsifiable consistency. No pigment. By transmitted light, colonies were iridescent, and translucent with darker centres.

*24 hr. broth cultures*: even turbidity, without pellicle or deposit. No motility seen.

*Biochemical tests*: litmus milk unchanged. Acid but no gas in dextrose, sucrose and mannitol, a little acid in maltose, but none in lactose or salicin. Indole positive. Voges-Proskauer negative.

With one possible exception, no other *Pasteurella* was obtained from any of the ferrets used in this investigation. There is therefore little evidence to connect this mouse *Pasteurella* with the ferret disease; it may even have been derived from the mice themselves. The exception was a small bipolar bacillus with cultural and biochemical reactions similar to those of the *Pasteurella* described, which was obtained from the eye of Ferret No. 12, one of the direct-line passage ferrets which suffered from an acute conjunctivitis. This strain died out in culture before a complete comparison had been made between it and the mouse strain.

## METHODS

The experimental disease was propagated through a series of healthy ferrets bought from dealers in the Cambridge neighbourhood and subjected to at least a fortnight's preliminary quarantine.

The healthy stock ferrets were kept in wood and metal cages in a separate room. No spontaneous disease occurred among them throughout the period covered by the investigation.

Ferrets which had been inoculated were kept in another room in galvanized iron cages closed above by thick wire netting. Different attendants looked after the healthy and infected animals, and all unavoidable handling was safeguarded by rubber gloves moistened with lysol. All cages vacated by sick ferrets were autoclaved.

The degree of isolation so obtained was sufficient to protect the healthy stock animals, but not to prevent spontaneous infection from cage to cage in the infected room. Two normal healthy uninoculated ferrets, kept in separate cages in this room in company with infected animals, both contracted the disease, one after 21 and the other after 30 days' exposure. It was therefore useless to attempt in this room any experiments on the relative infectivity of

*Distemper-like Disease in Ferrets*

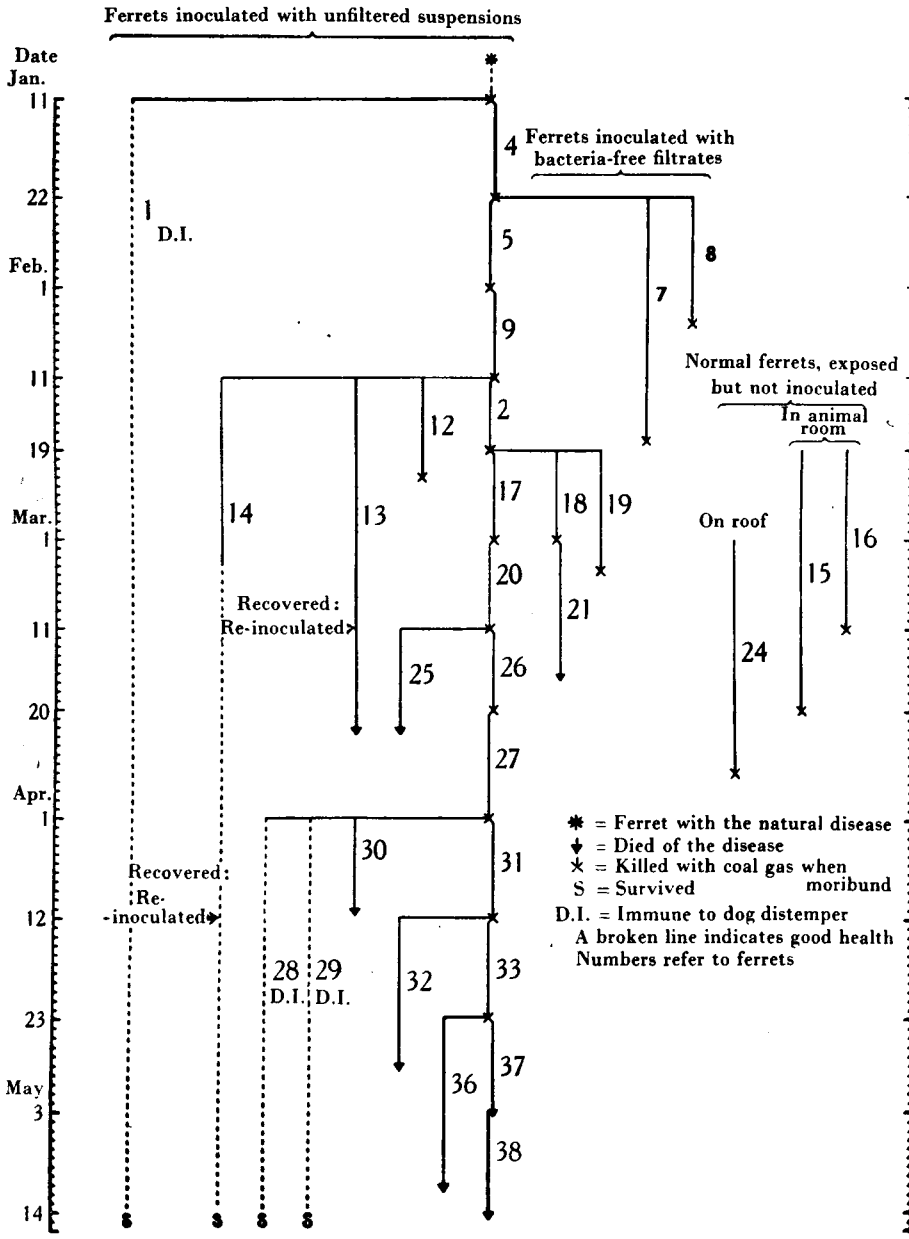


Fig. 1. Table of succession of ferrets experimentally infected with a disease resembling distemper.

different tissues or the relative value of different routes of inoculation. The development, morbid anatomy and histology, and bacteriology of the disease could be observed, and so could the resistance of immune ferrets; but more complicated questions needed for their solution a different and more isolated environment.

Only one such question was attempted: this concerned the infectivity of material filtered through bacteria-proof filters. Ferrets inoculated with filtrates were kept apart from all the others on the roof of the Department, in a wooden cupboard which protected them and their iron cages from the weather. The isolation obtained in this way was probably adequate because the cupboard on the roof was farther away from the infected room than was the healthy stock room.

Since the disease seemed to be primarily one of the upper respiratory tract, and since there was a possibility that several agents, bacteria or viruses, might be at work, material for transfer was always taken from the anterior turbinates. These were removed with precautions against contamination, and ground with broth or saline and a little glass in a closed hand-mill. The suspension so obtained was allowed to settle for a few minutes at room temperature, and the supernatant fluid was then used as an inoculum, either filtered or unfiltered.

At first, ferrets were inoculated by two routes simultaneously, intranasally by the instillation of one or two drops, and subcutaneously by the injection of 0.5–1.0 c.c. Later, intranasal instillation alone was found to be effective and subcutaneous injection was abandoned.

Animals which died or were killed with coal gas were examined as soon after death as possible. Cultures were made from the trachea and various other organs on blood agar and in peptone broth. Pieces of tissue for histological examination were fixed either in formol saline or in a mixture of saturated corrosive sublimate three parts, strong formalin one part. Turbinates were fixed in 5% glacial acetic acid in saturated corrosive sublimate. Formalin-fixed tissues were stained with haematoxylin and eosin, and mercury fixed specimens by Laidlaw's acid fuchsin orange G inclusion body stain. By these methods, the disease was passed in a direct line through twelve ferrets. The chart shows the number of ferrets used and the order of their treatment.

Five other ferrets were inoculated in the same way with similar material derived from various members of the passage series, and five were inoculated with suspensions of other tissues or by other routes. Of these, all of those inoculated intranasally developed the disease.

Three distemper-immune ferrets, kindly provided by Dr O'Brien and Prof. Dalling, were inoculated intranasally and subcutaneously with strongly infective turbinate suspension, but none of them showed any signs of disease at all.

Five ferrets were inoculated with bacteria-free Berkefeld filtrates; these all developed a disease similar in most respects to that produced by inoculation with suspension.

So far, then, the uniform and high infectivity of the disease, its transmissibility by bacteria-free filtrates and the resistance shown by three ferrets immune to dog distemper pointed to a true strain of distemper virus as whole or part cause of the disease. This idea is supported by a consideration of the course and pathology of the experimental disease.

#### COURSE AND PATHOLOGY OF THE EXPERIMENTAL DISEASE

After an incubation period of about 5 days, during which the animals seemed well, an acute coryza developed. The first sign of this was usually a noisy, wheezing respiration rather than the conjunctivitis which Dunkin & Laidlaw (1926) described as the first sign of canine distemper in the ferret. Frequent sneezing attacks were followed by definite nasal obstruction, violent efforts to breathe out through the nose, vigorous nose licking and uncomfortable movements of the head and neck. Although the coryza was the first sign, eleven of the ferrets developed conjunctivitis during the first few days of the disease. The eyelids were sometimes glued together so that the conjunctival sac became converted into a bag of pus. Corneal ulcers were not seen, but the palpebral conjunctiva contained microscopic lesions which will be described later.

Typical distemper pustules did not appear except in two of the last ferrets in the series, Nos. 31 and 33. However, the lips and snout in many cases became red and covered with a brown crust, removal of which exposed small bleeding points; microscopic sections of the lips showed small lesions similar in all essentials to those described by Dunkin & Laidlaw, but these very seldom attained macroscopic dimensions.

For 4 or 5 days the animals became weaker, did not eat, and suffered from increasing respiratory embarrassment. Those that died did so on the tenth to the fifteenth day after inoculation, but most ferrets were killed with coal gas when apparently moribund on the tenth or eleventh day. Of six ferrets inoculated with turbinate suspension and not killed with coal gas, four died (Nos. 25 and 38 on the eleventh day, Nos. 30 and 37 on the tenth day after inoculation), one, No. 14, recovered and proved immune on reinoculation; and one, No. 13, recovering after a comparatively mild attack, was reinoculated 3 weeks later, developed the disease and died of it 11 days after the second inoculation.

It is therefore evident that the mortality of the disease was high, though no reliable figure can be given on so small an experience.

The literature concerning the inclusion bodies of distemper has recently been reviewed by De Monbreun (1937). These structures were described in detail by Sanfelice (1915), to whose paper Laidlaw (1930) refers. Sir P. Laidlaw kindly supplied full details about them, together with instructions for their demonstration, and histological material from dogs and ferrets which had died with canine distemper, for comparison.

Distemper inclusions are round or oval structures, varying in size from small

spheres about  $1\mu$  in diameter to large elongated bodies  $14$  or  $15\mu$  by  $9$  or  $10$ . They lie in the cytoplasm of various epithelial cells and occasionally of large mononuclear cells in lymph glands. They are usually surrounded by a clear zone or vacuole in the cytoplasm. Sometimes they distort the nucleus of the cell in which they lie. Stained by the acid fuchsin orange G method, they are coloured a bright red, similar to that taken by the red blood corpuscles, though in a suitably differentiated preparation they may possess a slightly paler and more purple tint. Seen under the  $\frac{1}{12}$  in. objective, and often even under the  $\frac{1}{8}$  in., they appear to possess a foamy or vacuolated structure, the vacuoles showing as pale circular areas inside the inclusion body, each measuring rather less than  $1\mu$  in diameter. When present they are generally numerous.

In the ferrets of this series, typical inclusion bodies were constantly found in the inflamed mucous membrane covering the anterior turbinates. The blood vessels of this area were widely dilated, sometimes the epithelium showed signs of desquamation, and the air passages contained plugs of pus.

The trachea usually contained frothy muco-pus. Its epithelial lining showed a variable but slight degree of inflammation, with frequent typical cytoplasmic inclusions.

The lungs showed various lesions, the most constant of which was a diffuse and patchy bronchopneumonia. This was either recognizable to the naked eye as a series of scattered miliary lesions, or it involved large portions of the lung in continuous consolidation. In some cases the bronchopneumonic areas were very small and early, and only discovered with the microscope. Four ferrets showed a pneumonia sufficiently massive to be described as lobar.

Microscopically, the pneumonia was seen to be distributed with relation to the smaller air passages, which were filled with pus and usually contained inclusion bodies in their epithelium. The frequency with which bronchopneumonia occurred among the ferrets of this series is a point which differentiates this disease from canine distemper in the ferret as described by Dunkin & Laidlaw (1926), who only observed pneumonia in a small proportion of their ferret autopsies. They describe, however, another lesion of the lung in the following words: "Small flat raised yellow areas are to be found in about one-third of the cases on the surface of the lungs. These are much commoner on the dorsal aspect of the lungs and may be numerous, but the number is very variable. They have a superficial resemblance to abscesses but they present no surrounding injected zone and they are solid on section. Nodules of a similar appearance have been found in three normal ferrets." They describe the microscopic appearance of these lesions in some detail, and conclude: "The frequency of the lung lesions in the distemper animals and their numbers in many cases indicate that the virus is one cause of the lesions; but their peculiar character must be attributed to a characteristic response of the ferret's tissues to injury and not to a special response induced by the distemper virus."

In the ferrets of this series lesions corresponding to this description have been found in seven of the diseased animals, and in one normal apparently



healthy ferret which had never had distemper. Nothing can be added from these results to the description given by Dunkin & Laidlaw. It seems that lesions of this kind probably have little significance in the pathology either of canine distemper or of the disease under investigation.

Examination of the other internal organs of diseased ferrets revealed little of interest. The spleens were usually large and soft and rather chocolate coloured, but they showed no change either macroscopic or microscopic that was in any way characteristic.

There was usually a rather extensive fatty change in the liver. Some of this must be discounted because normal healthy ferrets on a diet of bread, milk and meat store a certain amount of fat in droplet form in their liver cells. Nevertheless, the fatty change seen in a few of the livers of diseased ferrets was very severe, and in two of them typical inclusion bodies were seen in the epithelium lining the bile ducts.

A search was made for cytoplasmic inclusions in the liver cells proper, since these were described by Pappenheimer & Hawthorne (1936) in liver section derived from ferrets which had died with the disease described by Slanetz *et al.* (1936), but none were found.

Inclusion bodies of the ordinary distemper type were seen in the epithelium of the pelvis of the kidney in some cases. They occur in this situation also in canine distemper (Laidlaw, personal communication) and in the American disease referred to above.

Typical "distemper inclusions" were seen in the tissues of all ferrets of the direct passage series, including the original sick ferret sent from London. They were found in all others that died of the typical disease, in ferrets inoculated with bacteria-free filtrates, and in two which contracted the disease spontaneously as a result of being housed in the infected room. They were not found in normal controls.

#### BACTERIOLOGY

*Brucella bronchiseptica* was isolated from the respiratory tract of the twelve ferrets of the passage series; no cultures were made from the twelfth.

This organism was also found in various other ferrets which developed the disease after inoculation with various other tissue suspensions, and in one which contracted the disease spontaneously during exposure in the infected animal room.

It was not, however, recovered from ferrets which were inoculated with bacteria-free filtrates and kept on the roof of the building, nor did it appear in cultures made from two of the three ferrets which developed the disease spontaneously after exposure.

It was obtained from the spleen and respiratory tract of the original ferret, but only from the respiratory tract of the experimental animals, where it was usually present in great numbers and almost pure culture.

Failure to grow *Br. bronchiseptica* from the three filtrate ferrets and from



two of the ferrets spontaneously infected does not prove its absence from these animals; on the other hand, failure of this kind did not occur with ferrets inoculated with unfiltered suspensions. It seems probable that bacteria-free filtrates or air-borne infection may produce a disease very similar to the passage disease, but one which is independent of *Br. bronchiseptica*.

A consideration of the incidence of pneumonia among the ferrets studied shows that bronchopneumonia was almost constantly present among those animals from which *Br. bronchiseptica* was isolated, but absent or very slight in those ferrets from which the bacterium was not obtained. Only one ferret—No. 19, a filtrate-inoculated ferret kept in isolation on the roof—showed a slight bronchopneumonia but failed to yield *Br. bronchiseptica*.

It seems probable therefore that *Br. bronchiseptica* may have increased the liability of sick ferrets to contract pneumonia, but that it did not play any more essential role in the causation of the disease. This conclusion is supported by the failure of this strain of *Br. bronchiseptica* to produce more than a transient pyrexia in ferrets inoculated intranasally and subcutaneously with young broth cultures of the organism.

One ferret so inoculated showed no signs of illness at all; two others developed slight signs of respiratory embarrassment and temperatures above 104° F., but quickly recovered.

The strains of *Br. bronchiseptica* obtained from these ferrets were all alike and all typical. They gave a strongly positive nitrate-reduction test, and were agglutinated by antisera prepared against a strain obtained from the National Collection of Type Cultures. These sera also agglutinated two strains of *Br. bronchiseptica* derived from ferrets at Hampstead, and kindly sent to Cambridge by Dr Wilson Smith. These two strains (which were numbered 520 and 521) failed to give a positive nitrate-reduction test.

Various other bacteria were obtained from different sites, but no other organism was found so constantly associated with the disease or so plentifully distributed through the respiratory tract. No haemolytic streptococci were encountered—a point of some interest, since Slanetz *et al.* (1936) describe haemolytic streptococci as constantly associated with the American ferret disease which they observed.

#### DISCUSSION

The conclusion that the disease of ferrets studied was due to a distemper virus not unlike the classical strains described by Dunkin & Laidlaw (1926) is made probable from the infectivity of bacteria-free filtrates, the resistance of distemper-immune animals alone of those inoculated, and the constant presence of typical inclusion bodies.

The points at which this disease differed from classical canine distemper in the ferret are: the short incubation period, the absence from most of the animals of macroscopic pustules around the mouth, and the high incidence of bronchopneumonia.

With regard to the first of these three, the incubation period might have been related to the size of inoculum. No attempt to measure this was made; it must have been very large, consisting as it did of up to ten drops of a strong suspension of turbinate. The second point of difference was not as impressive as it seemed at first sight, for the microscope demonstrated that pathological changes similar to pustule formation, but on a smaller scale, were usually present in the skin of the lips. The failure to form large pustules must be regarded as a peculiarity of this particular strain of the distemper virus; the point may be of some importance because of the diagnostic value of typical pustules.

The high incidence of bronchopneumonia is thought to have been associated with the presence of a strain of *Brucella bronchiseptica*. The resemblance of this disease to that described in America by Slanetz *et al.* is also striking. In both, the incubation period is short, bronchopneumonia is common, and inclusion bodies are found in profusion and in similar situations; but animals sick of the American disease died as a rule later, and cultures from their respiratory tracts yielded haemolytic streptococci.

The American workers tried and failed to infect puppies with infective material taken from their ferrets. Unfortunately, this test was not applied to the 1935 English disease.

#### SUMMARY

1. Material taken from a ferret sick of a disease epidemic among breeders' stocks in 1935 was shown to produce a similar disease when inoculated into normal ferrets.
2. The behaviour of the experimental disease and its pathology indicated that it was a form of distemper, antigenically related to dog distemper, but differing in minor points from the description of canine distemper in the ferret given by Dunkin & Laidlaw.
3. Associated with the disease was a strain of *Brucella bronchiseptica* which may possibly have been responsible for the high incidence of bronchopneumonia observed.

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*Note.* Case histories of the ferrets in this series are not published but have been preserved.

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