AN EPIZOOTIC DISEASE OF FERRETS CAUSED BY A FILTERABLE VIRUS

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Plates 21 and 22

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In October, 1934, an epizootic disease affecting the respiratory organs appeared in a group of ferrets under use for experimental purposes at the College of Physicians and Surgeons of Columbia University, New York.[1] The number of ferrets at first affected was small but the disease spread with great rapidity and within 2 weeks all the animals in the colony had become infected. The first deaths occurred after the lapse of 14 days from the onset and at the expiration of 2 months all the animals in the colony had succumbed to the disease. During the early part of the outbreak the unaffected animals were separated from the sick but this maneuver did not prevent their contracting the disease. As far as could be determined the ferrets had not been exposed to canine distemper during their sojourn in the animal quarters. Administration of anti-canine distemper serum had no effect on the course of the disease in infected animals nor did it prevent apparently well animals in contact with the sick from contracting the disorder.

Several months later another group of ferrets was placed in the same quarters and a similar epizootic quickly broke out among them. The disease ran the same course, and exhibited the same high mortality rate with the exception that one animal in this group survived the infection. Concurrently with this outbreak a small breeding colony maintained in the country became infected, the disease resembling in

* A preliminary report including the complete histopathological study was presented at the 37th Annual Meeting of the Society of American Bacteriologists in 1935.

[1] Slanetz, C. A., Smetana, H., and Dochez, A. R., *J. Bact.*, 1936, **31**, 48.

every particular that observed in the experimental colonies. Young and old animals were equally affected and again the mortality rate was exceedingly high.

The spontaneous disease was characterized by elevation of temperature often to 106°F. A conjunctivitis developed which resulted in the closing of both eyes. Lethargy appeared and there was a gradual loss of appetite. A watery nasal discharge was frequently present which in some instances became mucopurulent as the disease progressed. Difficulty in breathing suggesting pulmonary involvement was observed in all animals surviving more than a few weeks. Sneezing was rarely observed and the weight loss was not great. The fatality rate for the three epizootics ranged from 70 to 100 per cent. Such animals as survived were usually older individuals and of the litters of young ferrets in the breeding colony none survived. Rapid removal of sick ferrets from the colonies did not check the spread of the disease amongst the uninfected animals.

Inquiries among ferret breeders reveal the occurrence in their stock from time to time of an apparently similar epizootic disease. Dr. C. H. Andrewes[2] has suggested the relationship of the disease described by us to a malady of ferrets designated by him as paradistemper. Kairies[3] has described a type of ferret influenza the cause of which he believes to be bacterial in nature. Brightman and Trask[4] in studying the effects of human influenza virus in ferrets observed that a number of animals died from secondary infection with *Streptococcus hemolyticus*. Normal carriers of hemolytic streptococcus were encountered and it seemed that inoculation of such carriers with influenza virus resulted in a fatal disease. So far we have not discovered in the literature any description of a disease precisely similar to the one encountered by us.

The Induced Disease

At the time the decision was made to study the disease described all animals infected during the recent outbreak had died and no fresh material was available for experimental investigation. An attempt was made to revive the infection by collecting dust from the room in

[2] Personal communication.

[3] Kairies, A., *Z. Hyg. u. Infectionskrankh.*, 1935, **117**, 12.

[4] Brightman, I. J., and Trask, J., *Am. J. Dis. Child.*, 1936, **52**, 78.

which the diseased animals had been harbored. This dust was added to the food of two normal ferrets. After the lapse of about a week both animals developed typical symptoms of the disease.

One of the above animals was chosen as the source of material for the experimental study of the disease. The infected tissues were prepared for inoculation by grinding lung and the nasal mucosa overlying the turbinate bones with sterile quartz sand and the final emulsion diluted to a 10 per cent suspension by the addition of physiological salt solution or of ordinary nutrient bouillon. After centrifugalization the supernatant fluid was passed through a Berkefeld N filter. The filtrate was proven free from bacteria cultivable on the common bacteriologic media and diluted 1 to 10 and 1 to 1000 with physiological salt solution. The diluted filtrate was administered in 1 to 2 cc. quantities intranasally, with the ferrets under light ether anesthesia. Occasionally the filtrate was injected subcutaneously and in a few instances intradermally. All the animals inoculated whether by the intranasal, subcutaneous or intradermal route became infected after an incubation period of 4 or 5 days, all showing the typical symptoms of the disease. Heart's blood diluted 1 in 1000 and 1 in 10,000 proved infective for ferrets when given intranasally. Ferrets were also inoculated intranasally with unfiltered lung suspensions. The disease so produced differed in no way from that caused by the inoculation of filtered material. Healthy animals could be infected by placing them in close cage contact with experimentally inoculated animals. In these examples of the disease the incubation period was somewhat longer, 7 to 10 days. In all 98 ferrets were inoculated with infective material, all developed the typical disease, and the fatality rate was 100 per cent.

The experimental disease produced in ferrets by intranasal inoculation had a relatively constant incubation period, 4 to 6 days, and a uniform clinical picture. From 4 to 6 days following inoculation an elevation of temperature to 105–106°F. took place and this fever usually persisted up to the time of death. From 4 to 8 days after infection the animals became lethargic, manifested loss of appetite, the eyes became closed with a sticky exudate and occasionally a thick purulent nasal discharge developed. Convulsions and other nervous manifestations were sometimes noticed a few hours before death. The major symptoms were referable to infection of the respiratory tract. The average period of survival following inoculation was 20 days and there was an individual variation of from 14 to 56 days. The infection was uniformly fatal to the inoculated animals.

A study of the infected ferrets' tissues was made for the presence of visible bacteria cultivable on ordinary bacteriologic media. If the

disease had been present for some time it was usually possible to cultivate hemolytic streptococcus from the tissues. In some animals a staphylococcus and a Gram-negative bipolar staining bacillus were also present in association with streptococcus. From the lungs of ferrets sacrificed within 16 days of inoculation it was frequently impossible to cultivate any form of visible bacterium.

That the primary agent of this disease is a filterable virus is shown from the following observations.

A lung emulsion was prepared from a single infected animal. This emulsion was divided into two parts, one of which was preserved as prepared and the other passed through a Berkefeld N filter. The filtered portion was proven free of bacteria by cultivation. Two series of ferrets were then inoculated, one with the unfiltered and the other with the filtered emulsion. Both sets of animals came down with the disease within the same incubation period and no appreciable difference in the clinical course of the disease was noted in either group. Histological examination of the tissues showed the lesions to be similar in both series of animals.

The virus-containing filtrate was completely inactivated by heating for ½ hour at 60°C. The virus is also inactivated by short exposure to a 0.5 per cent concentration of formalin. The virus survives a 3 month exposure to 50 per cent neutral glycerin but is no longer infective after a 5 month exposure to a like concentration of glycerin. When frozen and desiccated *in vacuo* the virus remains infective for at least 4 months.

The Pathological Changes

Autopsies were performed as routine on all ferrets dying of the disease and a number of these animals was selected for histological study. In addition to these diseased animals, three ferrets suffering from canine distemper and three normal animals were subjected to a careful study of their gross and microscopic pathology.

In contrast to the severity of the clinical picture the gross pathological changes were slight, even in animals dying at the height of the disease.

No significant changes, aside from evidence of loss of weight and shagginess of the fur, were noted externally except that all animals had marked conjunctivitis accompanied sometimes by considerable purulent exudate. The skin, subcu-

taneous fat tissue and skeletal musculature appeared essentially unchanged. As a rule ferrets dying spontaneously always showed areas of consolidation in the lungs although these were not necessarily present in animals sacrificed during the earlier course of the disease. From such consolidated lungs hemolytic streptococci and other organisms could be recovered and were thought to be secondary invaders. The spleen was consistently larger in infected animals. Its capsule was smooth and the consistency soft. On section the pulp appeared soft and congested. The liver likewise was larger than usual in infected animals which had died spontaneously. The heart, kidneys, suprarenals, genital organs and the gastrointestinal tract showed no characteristic gross changes. The brain and spinal cord appeared normal. The mucosa of the mouth, nose and pharynx of some of the infected animals was mildly injected or swollen.

The most characteristic and constant histological feature was the presence of inclusion bodies in the epithelial cells of many organs.

These bodies were round, oblong or sometimes sausage shaped, well defined, hyaline and eosinophilic, located usually within the cytoplasm but also intranuclearly. Intranuclear inclusions were always single, cytoplasmic ones frequently multiple; some were as small as nucleoli, others easily attained the size of nuclei of epithelial cells. They were surrounded by a clear halo, perhaps due to shrinkage. Special stains, including fat stains and glycogen preparations, failed to demonstrate any substance between the bodies and the cytoplasms or nuclear material. There was a suggestion of concentrical lamellae in some of the bodies, and larger inclusions frequently showed stippling and fine vacuolization which were brought out well by Giemsa stains.

Ordinarily the inclusions were easily seen in hematoxylin-eosin preparations. If only a few were noted Laidlaw's stain was employed, by means of which they became brilliant red. This was especially true if the tissue had been fixed in saturated mercury bichloride with 5 per cent glacial acetic acid added to destroy the mitochondria. Unfortunately erythrocytes and hyaline substances were similarly stained so that the Laidlaw method cannot be called specific. The Giemsa method is also an excellent way to demonstrate these bodies; they stain purplish red, while erythrocytes take a pale orange tint. Phosphotungstic acid and Gram stains can also be employed to demonstrate the inclusions which are, however, not affected by lipoid or glycogen stains.

Inclusion bodies were most frequently and constantly seen in the respiratory tract and were present in the lining cells of the mucosa of the nose, sinuses, trachea and bronchi, where they were especially numerous and easily demonstrated (Fig. 1). Most of them were cytoplasmic but intranuclear ones were also present. The cytoplasmic bodies were located between the nucleus and the ciliated border of the cells. Stippling and vacuolization had occurred in large inclusions. The mucous glands of the tracheal and bronchial mucosa likewise contained many inclusions but they were never found in the alveolar ducts nor in the alveoli.

The inclusion bodies did not occur as regularly in the gastrointestinal tract as in the respiratory tract although here too they could commonly be seen. They could be found in the lining cells of the tonsils, pharynx, esophagus and stomach (surface epithelium, principal cells and acid cells). They were not seen in the small intestine but were sometimes present in the colon (Fig. 2). No uniformity concerning their polarity was observed. The submaxillary and parotid glands sometimes contained a few inclusions some of which were intranuclear, but usually these glands proved to be negative (Fig. 3). Inclusion bodies were never found in liver cells but they were rather frequently encountered in the lining cells of the bile ducts (Fig. 4). There was no uniformity regarding their polarity. Sometimes bipolar bodies were present in the same cell. The gall bladder epithelium never contained inclusions. In the pancreas inclusions could be seen in the lining cells of ducts but not in cells of the acini or islands of Langerhans.

The finding of inclusion bodies in the lining cells of the kidney pelvis and of the urinary bladder was as constant as in the respiratory tract. In the cells of the kidney pelvis they frequently were multiple and varied in size but there was no constancy as to their polarity. In the bladder epithelium their orientation was from the nucleus toward the surface and they were especially numerous and large. Here the stippling and vacuolization was especially common and intranuclear bodies in addition to the usual cytoplasmic inclusions were more frequently encountered than elsewhere (Fig. 5). Sometimes there was proliferation of epithelial cells especially of those lining the kidney pelvis, often with formation of multinucleated giant cells (Fig. 6). Inclusion bodies were never found in cells of the fallopian tubes, uterus, ovaries, epididymis or testes.

The cortical cells of the suprarenals sometimes contained small cytoplasmic and occasional intranuclear inclusions (Fig. 7).

A few cytoplasmic bodies were present within the epithelial cells of the cornea (Fig. 8), the lining cells of the conjunctiva bulbi and palpebrarum. Occasionally they were also seen in sweat glands and in the mammary glands but not in the squamous epithelium of the skin, hair follicles or sebaceous glands. Inclusion bodies were never found in any of the cells of the brain, spinal cord or meningi.

Occasionally, in addition to the inclusion bodies, mild hyperemia and signs of irritation were evident in the respiratory tract. The lungs of some animals presented bronchitis and patches of lobular pneumonia. The splenic follicles contained cellular debris and appeared depleted of lymphocytes; the pulp was congested and hemorrhagic and contained immature white and red blood cells together with many megakaryocytes. Irregular fatty changes and occasional focal necroses were seen in the liver. All other organs including the brain and spinal cord presented no characteristic histological findings.

The pathology of animals dying of the epizootic disease was essentially the same as that of those dying of the experimental disease.

Comparative Studies on Canine Distemper and Ferret Virus Disease

In order to compare the clinical picture in ferrets induced by inoculation of ferret virus and canine distemper virus, four animals were selected from one source and were inoculated with the respective viruses on the same day. The ferrets inoculated with ferret virus showed during the course of the disease little change externally except conjunctivitis and slight emaciation. On the other hand the ferrets inoculated with canine distemper virus showed conjunctivitis, reddening and swelling about the mouth, the abdominal region, the scrotum and adjoining areas. In many places there were petechial hemorrhages. The incubation of the ferret virus disease was 4 days whereas the incubation period of the canine distemper infection was 10 days. Both series of animals showed continuous elevation of temperature, that of the animals infected with ferret virus being higher and more sustained than that of the ferrets suffering from canine distemper. The temperature curves are shown in Chart 1. From these observations it is evident that the clinical pictures of the two infections are easily distinguishable one from the other.

Histologically cytoplasmic and intranuclear inclusion bodies could be found in epithelial cells of the respiratory tract, mucous glands, salivary glands, in the lining cells of the kidney pelvis and urinary bladder, in the epithelial cells of bile ducts and pancreatic ducts, esophagus, stomach, intestines, cornea, conjunctiva, mammary glands and sweat glands. These inclusion bodies were essentially of the same character and were found in the same locations as those described above; their staining reactions were also identical.

The susceptibility of a number of animal species to infection with the ferret virus was next investigated.

In view of the possibility that the ferret disease might be a modified form of canine distemper the first animals selected were young dogs. Seven puppies 3 months of age, the period of greatest susceptibility to canine distemper, were each injected subcutaneously with 10 cc. of a filtrate of a 10 per cent lung suspension of freshly prepared infected ferret lung. All the animals remained healthy, exhibiting no symptoms of disease at any time. Daily rectal temperatures over a period of 4 weeks revealed no significant rise in temperature. Subsequent to this time all the animals were inoculated with desiccated canine distemper virus. All developed fever and ran a course characteristic of canine distemper.

Two strains of mice, Rockefeller Swiss and Bagg, were inoculated intranasally with bacteria-free filtrate obtained from the lung of a ferret infected with the ferret virus. No symptoms of infection were manifested by the Swiss mice.

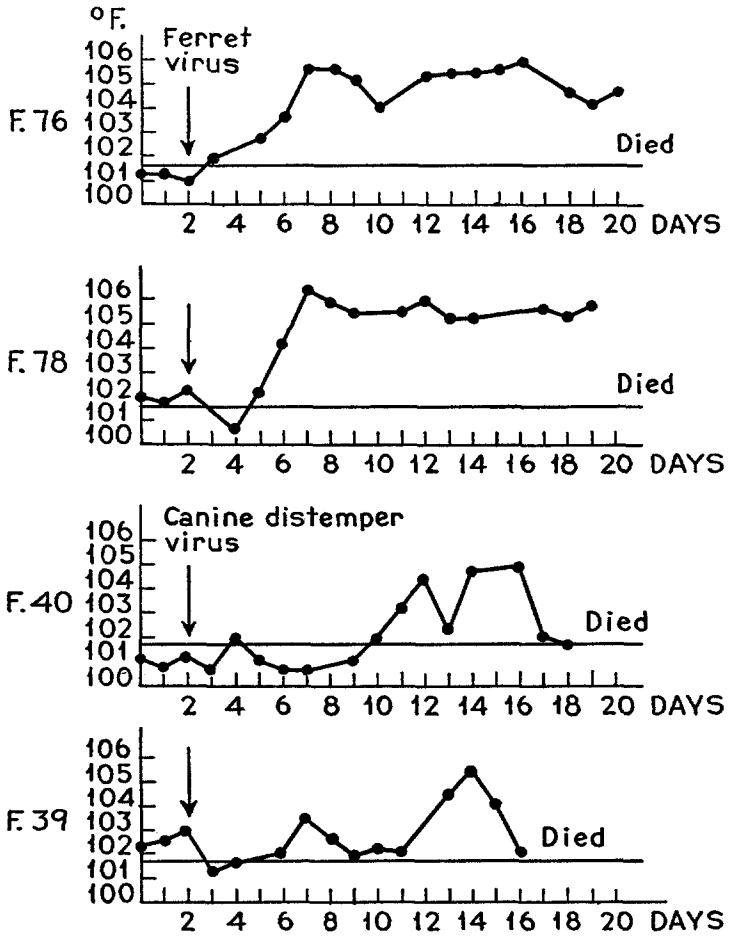


CHART 1. Comparative temperature records on ferrets inoculated with canine distemper virus and ferret disease virus.

When sacrificed 8 to 10 days after injection no objective signs of disease were discovered at autopsy. The mice of the Bagg strain died within 10 days following inoculation. The lungs of these animals at autopsy showed areas of hemorrhage and consolidation. Emulsions of the lungs of the Bagg mice were inoculated

intranasally in ferrets but no disease resulted in the ferrets from this inoculation. Subcutaneous inoculation into Swiss mice of filtered ferret virus did not result in infection. Rats, guinea pigs and rabbits were also proven insusceptible to infection with the ferret virus.

Immunological Studies

The capacity of a vaccine prepared from ferret virus was tested for its power to induce active immunity in ferrets.

The vaccine was prepared by grinding infected lung tissue in a sterile mortar with quartz sand, adding physiological saline and straining through several layers of sterile gauze. Formalin was then added to a concentration of 0.3 per cent and the mixture stored at a temperature of 4°C. for several days. After this time the mixture was no longer infective. Before injection the vaccine was neutralized with normal sodium hydrate. The vaccine thus prepared seemed to retain its immunizing power for at least 4 months.

Normal ferrets were immunized by giving two or three subcutaneous injections of the inactivated virus at intervals of 10 to 14 days in quantities of 2 cc. By this method ferrets were readily immunized actively against many times the fatal dose of living virus. Immunity has been tested after the lapse of 1 year and still found to be effective against living virus. During the last 2 years ferrets used for experimental work have regularly been immunized with formalinized ferret virus suspensions. Since the beginning of this procedure no spontaneous epidemic ferret disease has appeared amongst the immunized animals.

The formalinized vaccine also appears to have curative value.

A ferret which showed early symptoms of the disease was injected subcutaneously with the vaccine. Within 3 weeks the animal had entirely recovered. It was then sacrificed, autopsied and the tissues subjected to histological examination. No gross or histological lesions were discoverable. Subsequent to this eight ferrets in the early stages of the disease were given two subcutaneous injections of vaccine at 10 day intervals. Approximately 3 weeks following the last injections all eight animals had entirely recovered. They were subsequently proven immune to the disease by the inoculation of living virus.

The capacity of certain sera to protect animals against experimental inoculation of living ferret virus was also examined.

A mixture of equal parts of normal human serum and a 1–1000 dilution of ferret virus was allowed to stand at room temperature for 30 minutes. The mixture

was then inoculated intranasally into a normal ferret. The animal died of typical ferret disease 14 days later. A similar mixture of anti-canine distemper serum and ferret virus was inoculated intranasally into another ferret. 4 days later this

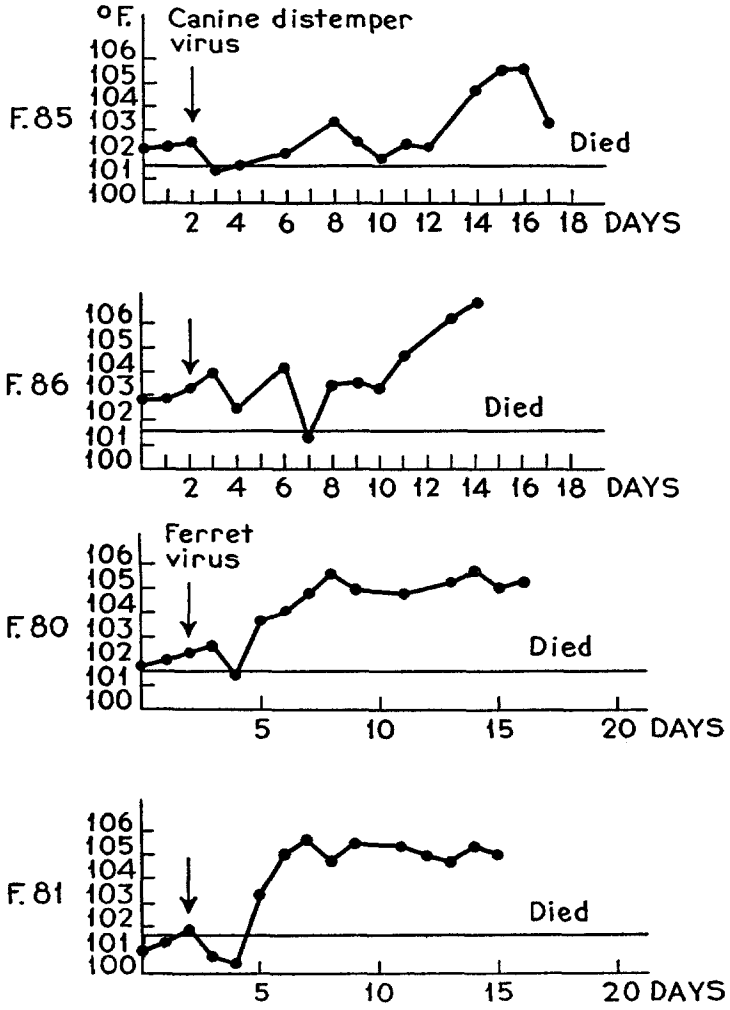


CHART 2. Temperature records on ferrets F. 85 and F. 86 immune to ferret virus and on ferrets F. 80 and F. 81 immune to canine distemper virus.

animal developed a rectal temperature of 105°F., showed a watery nasal discharge and difficulty in breathing. 38 days later the animal was in bad condition exhibiting marked dyspnea, high fever and occasional convulsion. It was sacrificed at

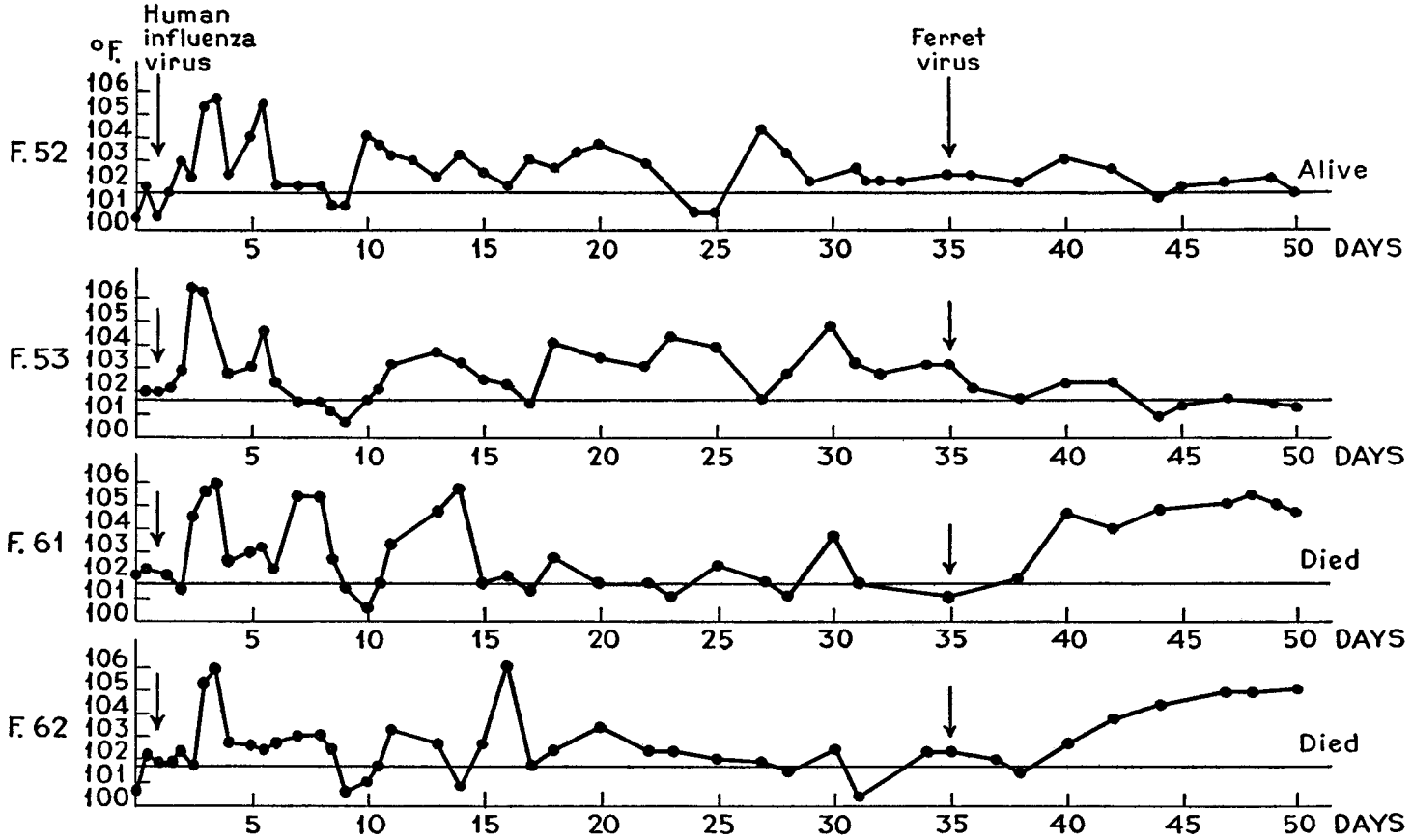


CHART 3. Temperature records on ferrets F. 52 and F. 53 immune to ferret virus and on ferrets F. 61 and F. 62 normal controls.

this time. A series of six ferrets in the incubation period of the disease were treated by giving 10 cc. of anti-canine distemper serum subcutaneously. All the treated animals died within the same period of time as six control ferrets which had received no serum.

A second test was made of the immunological relationship of the ferret virus to the virus of canine distemper.

Two ferrets, F. 80 and F. 81, were actively immunized against canine distemper by subcutaneous injection of canine distemper virus. After immunization the animals were inoculated with the ferret virus. Both developed the typical ferret disease and died within 21 days. In addition two ferrets, F. 85 and F. 86, which had been vaccinated against and were immune to the ferret virus were inoculated with the virus of canine distemper. Both animals died after manifesting the typical symptoms of canine distemper in ferrets. No cross immunity between the two viruses was observed. Chart 2 shows the temperatures obtained following inoculation with the virus indicated.

The immunological relationship of the ferret virus to human influenza virus was also investigated.

Five normal ferrets and five ferrets vaccinated against the ferret virus were inoculated intranasally with human influenza virus. All ten animals manifested the usual syndrome that follows the inoculation of ferrets with human influenza virus. Four of the normal and three of the vaccinated ferrets survived the inoculation of influenza virus. When they had completely recovered, all the animals were inoculated subcutaneously with ferret virus. The four normal ferrets that had survived the influenza inoculation developed the ferret disease and died within 4 weeks. The three vaccinated ferrets, however, showed no evidence of the ferret disease and remained alive. The temperature reactions of some of these animals are shown in Chart 3.

There seems to be no cross immunological relationship between the virus of the ferret disease and the virus of human influenza.

SUMMARY

1. An epizootic disease of ferrets with a very high case fatality rate is described.

2. By the use of suitable material the natural disease can be transmitted experimentally.

3. The primary causative agent of the disease is a filterable virus.

4. Secondary invasion by bacteria of the respiratory tract of infected

animals frequently occurs. The most important secondary invader is hemolytic streptococcus.

5. There seems to be no immunological relationship between the virus of the ferret disease and the viruses of canine distemper and of human influenza.

6. Histologically the disease is characterized by cytoplasmic and intranuclear inclusion bodies in epithelial cells of many organs.

7. These inclusion bodies are indistinguishable from those occurring in canine distemper.

The writers are indebted to Dr. A. R. Dochez, at whose suggestion the work was started, for his assistance and advice throughout the course of this investigation.

EXPLANATION OF PLATES

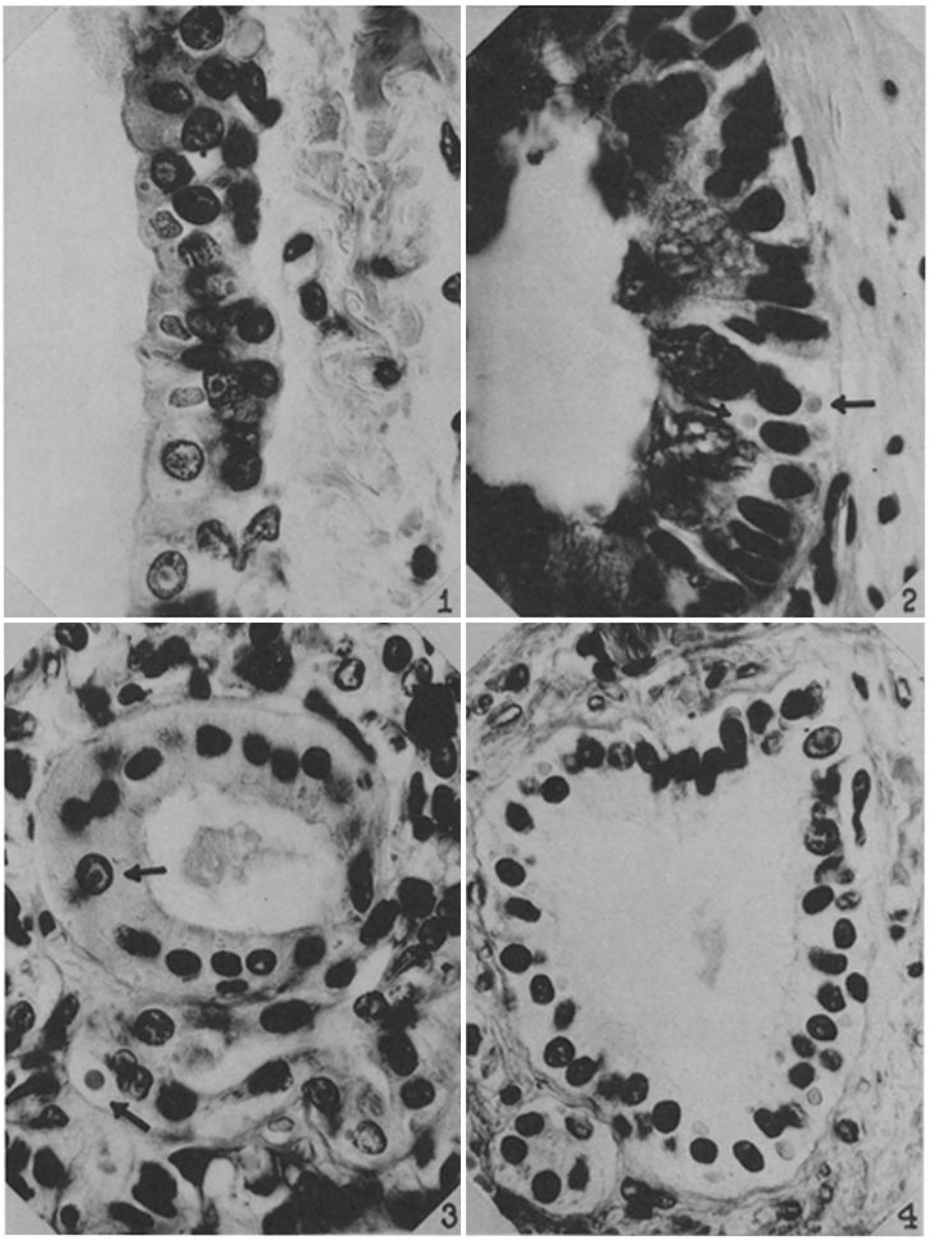
PLATE 21

FIG. 1. Bronchial mucosa, oil immersion. Cytoplasmic and intranuclear inclusions. Note the different sizes of the cytoplasmic inclusions and their stippled and vacuolated appearance. Hematoxylin and eosin stain. × 853.

FIG. 2. Mucosa of colon, oil immersion. Two cytoplasmic inclusions (arrows). Hematoxylin and eosin stain. × 853.

FIG. 3. Submaxillary glands, oil immersion. Cytoplasmic and intranuclear inclusions (arrows). Hematoxylin and eosin stain. × 853.

FIG. 4. Intrahepatic bile duct, oil immersion. Cytoplasmic inclusions. Hematoxylin and eosin stain. × 853.



(Slanetz and Smetana: Epizootic disease of ferrets caused by virus)

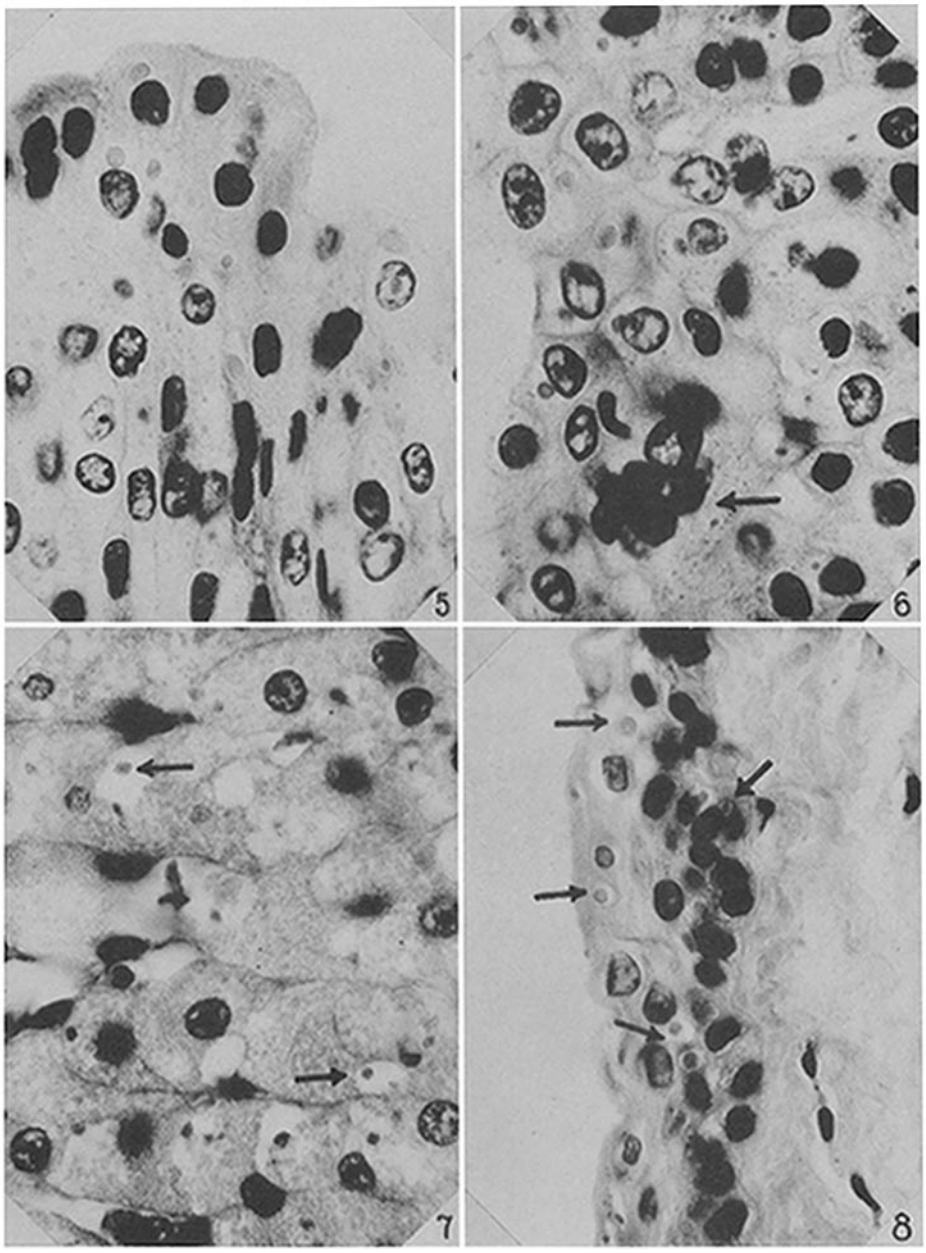
PLATE 22

FIG. 5. Mucosa of urinary bladder, oil immersion. Cytoplasmic and intranuclear inclusions. Note the different sizes of inclusion bodies. Hematoxylin and eosin stain. × 853.

FIG. 6. Mucosa of kidney pelvis, oil immersion. Cytoplasmic inclusions. Proliferation of epithelial cells with formation of giant cell (arrow). Hematoxylin and eosin stain. × 853.

FIG. 7. Suprarenal cortex, oil immersion. Small cytoplasmic inclusions (arrows). Hematoxylin and eosin stain. × 853.

FIG. 8. Epithelium of cornea, oil immersion. Cytoplasmic inclusions (arrows). Hematoxylin and eosin stain. × 853.



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