A VIRUS CAUSING PNEUMONIA IN CATS AND PRODUCING ELEMENTARY BODIES

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PLATE 2

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A respiratory tract infection in cats—variously called nasal catarrh, influenza, or distemper—has been observed frequently within the past several years in the northeastern United States. The main characteristics of the disease are its highly infectious nature, debilitating effects, and long course, about a month. Its respiratory nature is manifested by sneezing and coughing, accompanied by a mucopurulent discharge from the eyes and nose. Unless the animal is markedly affected pneumonia cannot be demonstrated during life, but autopsy reveals grayish, densely consolidated areas in the anterior lobes of the lungs.

While studying a pneumonia in calves on a farm, our attention was called to the simultaneous occurrence of pneumonia in kittens. A study of the cat disease was made and the results obtained are presented here.

Transmission of the Cat Disease to Mice

Since many types of pathogenic agents have been demonstrated in mice by intranasal inoculation, a similar method was used to inoculate suspensions of the lungs from sick cats. The suspensions were prepared as follows:—

The pneumonic lung was removed, weighed, and a 10 per cent suspension in buffered salt solution made by the use of a glass grinder. This suspension was then centrifuged 5 minutes at 1200 R.P.M. and the supernatant used for inoculation.

This Department has a stock of mice that has been repeatedly tested and found negative for the respiratory viruses which have proved so complicating a factor in the work of Horsfall (1) and others on influenza and other diseases. In the experiments recorded here mice from this stock only were used. Animals 3 to 5 weeks old were inoculated intranasally with 0.05 cc. of the suspensions prepared as above, while under light ether anesthesia. They were autopsied 3 to 5 days after inoculation, and suspensions of their lungs prepared in the same way as the cat lung were inoculated intranasally into other mice.

Strains from 5 sick cats were established in mice by the method outlined but transfers from a 6th cat gave negative results. The mice became sick as a result of the initial inoculation and died in 3 to 5 days following the inoculation of two strains (A obtained from a cat in New Jersey and B from one in New

York State). The other strains (C, D, E, obtained from cats in different localities in New Jersey) produced disease but the mice survived. Those inoculated with each strain showed a definite pneumonia with more than half the lung substance consolidated. Serial passage of all strains increased their virulence, death occurring in 2 to 3 days after inoculation. Similar serial passages of lung suspensions from uninoculated mice gave entirely negative results. Strain A was used for all the work which follows except where otherwise noted.

On the 5th and 20th passages in mice the M.L.D. for mice inoculated intranasally was determined. Four groups of 5 animals each were then inoculated respectively with 10 M.L.D. intracerebrally and intranasally, 50 M.L.D. intraperitoneally, and 100 subcutaneously. The mice inoculated intranasally died in 2 to 3 days, while those inoculated by the other routes developed no visible signs of illness. Nevertheless the agent was present in suspensions of the brains of the mice inoculated intracerebrally and in the suspensions of spleens of those injected intraperitoneally, for the inoculation of them intranasally into mice produced pneumonia. The pneumonia was not extensive and the agent present was probably residual from that originally inoculated. Attempts to adapt the agent to tissues other than the lung by 3 intracerebral passages of infected brain and three intraperitoneal inoculations of spleen suspensions gave negative results.

To determine whether the disease could be transmitted to mice by contact, 6 uninoculated mice were placed in the same cage with a similar number inoculated with 10 M.L.D. of the agent, as given intranasally, and the inoculated animals were allowed to die before removal from the cage. After 5 to 7 days, half of the exposed mice were killed and examined for pneumonia, and suspensions of their lungs were passed to other mice for 3 subsequent serial passages. No pneumonia was found either in the contact mice or in those of the passage tests. The remainder of the exposed mice were held for 3 to 4 weeks and were then tested for immunity by the intranasal inoculation of 10 M.L.D. of the agent. All died. This experiment was repeated, and again the results were the same, indicating that the disease did not spread by contact in mice.

Groups of 5 mice each were inoculated intraperitoneally, intracerebrally, and subcutaneously with mouse lung suspensions containing active material. Two to 3 weeks later each mouse was given intranasally 0.05 cc. of 10 per cent suspensions of lungs from infected mice. This experiment was repeated using egg yolk sac suspensions of infective material as the initial injections. The results are shown in Table I.

These experiments show that intraperitoneal, intracerebral, or subcutaneous inoculation of either lung or yolk sac suspensions produced a solid immunity in more than half of the mice; 1 or 2 in each group showed partial immunity and an occasional mouse none.

TABLE I

Immunity of Mice to an Intranasal Inoculation of Infective Material As Tested 14 to 21 Days after an Initial Parenteral Injection of Active Material

Material	Initial inoculation		No. of mice	No. of mice showing lung lesions			No. of deaths
	Amount	Route		None	Slight	Extensive	
	cc.						
10 per cent lung suspension of infected mice	0.1	Intracerebral	5	3	1	1	1
	0.25	Intraperitoneal	5	4	1	0	0
	0.5	Subcutaneous	5	3	2	0	0
Controls	—	Uninoculated	5	0	0	5	5
10 per cent infected yolk sac suspension	0.1	Intracerebral	5	2	2	1	0
	0.25	Intraperitoneal	5	3	1	1	1
	0.5	Subcutaneous	5	3	1	1	0
Controls	—	Uninoculated	5	0	0	5	5

TABLE II

Susceptibility of Laboratory Animals to Intranasal Inoculation of the Mouse-Adapted Agent from Cat Pneumonia

Animal	No. of intranasal mouse M.L.D.	Results			Dilution of pneumonic lung producing lesions in mice
		Fever	Pneumonia	Death	
				days	
Cats	200	+	+	−	10^{-5}
Mice	10	−	+	+ 2–3	10^{-5}
Hamsters	50	−	+	+ 3–4	10^{-5}
Guinea pigs					
Young	100	±	+	+ 5–7	10^{-5}
Adult	100	+	+	−	Not tested
Rabbits					
Young	100	+	+	−	10^{-5}
Adult	100	+	+	−	Not tested

Pathogenicity for Other Laboratory Animals

A 10 per cent suspension of infected lungs from mice in the 20th passage was diluted to contain at least 200 M.L.D. for mice per cc. Mice, guinea pigs, hamsters, and rabbits were inoculated intranasally, while under light ether anesthesia, with this diluted material. Various amounts were given, depending on the relative size of the animals. The results are presented in Table II. Cats are included in the table for comparative purposes.

It can be seen that the susceptibility of the various species differed. Rabbits and adult guinea pigs showed a mild fever and a short illness, with a loss in

weight during the height of the disease. A normal appearance was regained after a week. Young guinea pigs (weighing less than 200 gm.) died in a week, after losing about 25 per cent body weight. Cats, on the other hand, were ill for about a month but eventually recovered. Mice and hamsters appeared ill 24 to 48 hours after inoculation and died 1 to 2 days later. Prior to death they were inactive, huddled together, and showed ruffled fur. As death approached respiratory difficulty was evidenced by slower and deeper breathing.

Cats, rabbits, and adult guinea pigs autopsied 5 days after inoculation showed pneumonia in the anterior lobes of the lungs, whereas in mice, hamsters, and young guinea pigs the amount of lung substance involved was greater.

Properties of the Agent

The agent was easily transferred to eggs by the method Cox (2) employed for rickettsiae. Following inoculation into the yolk sac of fertile hens' eggs that had been incubated for 5 days, the embryos died in 2 to 3 days, and this effect remained constant in subsequent serial passages. Suspensions of the yolk sac from these eggs produced pneumonia in mice and cats when inoculated intranasally and immunized mice when injected parenterally. The chorioallantoic membranes of 10 day embryonated eggs were thickened after equivalent amounts of infectious material were dropped on them, and after 5 serial passages suspensions of the membranes from the last passage produced pneumonia when inoculated intranasally into mice. No effect on the embryos themselves was noted.

Cultures on blood agar from the lungs of naturally infected cats and of infected mice showed few bacteria and frequently no organisms at all were cultivated. All attempts failed to demonstrate a cultivable agent from infected eggs on the usual blood agar plate, sealed piece of meat medium, and nutrient agar containing 10 to 30 per cent serum. These findings suggested that the agent was a virus.

Suspensions of lungs from infected mice and of egg yolk sac membranes were passed through Berkefeld N filters. Prior to use the filters were checked for defects by noting the amount of water that passed through in 5 minutes at 10 mm. Hg pressure (all passed 30 to 45 cc., the amount passed by N filters without defects). Attempts to pass the agent through these filters were essentially negative, for only 1 of 5 filtrates showed the agent and in this instance it was present in a very small amount.

An examination of sections of yolk sac membrane stained with Giemsa and films treated by Macchiavello's method (3) revealed numerous structures similar to the elementary bodies resulting from infection with psittacosis virus.

Films prepared from the lungs of mice and hamsters revealed structures which indicated that the agent had a cycle similar perhaps to that described for psittacosis by Bland and Canti (4). Dense structures, which might be

termed plaques, were found lying in the cytoplasm of monocytes. Other cells contained similar structures that were less dense and apparently breaking down into smaller elements, while still others contained clumps of definite small bodies (Fig. 2). Large numbers of these forms were noted lying free, either singly, in diploid formation, or in small aggregates. Single elements usually appeared to be round.

Centrifugation at 10,000 R.P.M. for 30 minutes of suspensions containing the infective agent concentrated the small bodies as observed by an examination of stained films (Fig. 4). While the original suspensions had produced deaths in mice inoculated intranasally, the supernatants after centrifugation produced only small pneumonic lesions, an indication that the infective agent had been sedimented by this procedure. Resuspending the sediments to their original volume resulted in preparations which again were lethal for mice. These findings are considered to furnish evidence that the infective agent as well as the small bodies was sedimented.

Impression films from lungs of cats, rabbits, and guinea pigs showed few small bodies. Mice and hamsters, the species most susceptible to intranasal inoculation, showed more small bodies in similar films. Yet infected lung tissue from all species in the same concentrations inoculated intranasally into mice produced pneumonia. This discrepancy could not be accounted for unless cells from the different species reacted differently to staining, or the agent in the cats, rabbits, and guinea pigs was in a stage in which the small bodies were not readily visible.

Other properties of the agent were determined. Suspensions of yolk sac from infected eggs were active after a storage period of 1 week at room temperature, and at 4°C. for 1 month. Suspensions of lungs from infected cats, containing 10 per cent sterile horse serum, were active when tested after storage for 3 months at −76°C. In the dried state the agent remained active for at least 6 months. When heated at 50°C. for 30 minutes or at 60°C. for 10 minutes the agent was inactivated. Heating at 45°C. for 30 minutes or at 50°C. for 10 minutes apparently destroyed some of the agent but did not cause complete inactivation. Lung suspensions in 50 per cent glycerin for 30 days were still active although much of the agent appeared to be destroyed.

The failure to pass the agent through Berkefeld N filters and its sedimentation at a speed too low to concentrate particles much smaller than those microscopically visible, strongly suggested that the agent was represented by the small bodies. Furthermore, these small bodies in their morphological features closely resembled the elementary bodies of psittacosis and related viruses, all of which are well established pathogenic forms. Cultures from the lungs of infected cats and mice showed no constant bacterial flora while those from inoculated eggs were negative. All the data indicated that the infective agent for eggs and mice and the cause of the cat pneumonia was a virus that formed elementary bodies.

Production of the Experimental Disease in Cats

Choice of the experimental animals required considerable care because of the occurrence of the infection in nearby localities. Therefore, most of the cats used were obtained before they were a month old and before weaning since the effects of any past infection would be evident in animals of this age and those which had been sick could be excluded. Mother cats and their young were placed in isolation and observed for a period of several weeks before they were used in experiments. The kittens were weaned during this period. A number of cats several months of age were used after assurance by the owners that they had never been ill. These also were held for a period of observation. Daily temperatures were taken for a week before inoculation in order to obtain an indication of each animal's normal range. All experiments were conducted upon single animals kept in strict isolation, unless contact experiments were planned.

In attempts to produce the disease in cats, 1 cc. of a 10 per cent suspension of lungs from naturally infected cats (strains A and B) or infected mouse lungs (strains A and B), or of egg yolk sac suspensions containing strain A, was inoculated intranasally. Two animals were inoculated with material from each source, and a normal cat was placed in contact with each inoculated animal. One of the cats inoculated with each material and one of the contact animals were killed during the acute stage of the disease. The remaining cats were held for immunity tests and other studies. The results are presented in Table III. In addition, cats were injected intracerebrally with 0.1 cc. and intraperitoneally with 1 cc. of egg yolk sac suspensions containing strain A.

It can be seen that evidence of infection in cats regularly followed upon the intranasal inoculation of any active material, whether from the original host, mice, or eggs. The disease thus produced was transmitted to other cats by contact. The experimental disease was similar to that seen in naturally infected animals. All cats showed marked eye signs and evidence of nasal involvement. Pneumonia was found in 4 of 6 cats autopsied during the acute illness. Those killed after recovery showed no pneumonia, except one cat which had a small fibrotic nodule in the lung.

Lung suspensions from the cats killed during the acute phase of illness produced pneumonia in mice when given intranasally. It therefore appeared that the agent readily passed to cats placed in contact with those inoculated with active material.

The cats injected intracerebrally and intraperitoneally were devoid of external signs of the disease. The only evidence that infection may have occurred was a mild fever with the temperature reaching 40°C. By inoculating mice intranasally with suspensions of brain from the cat inoculated intracerebrally and of spleen from the one inoculated intraperitoneally the agent was shown to be present in these organs. However, the extent of the pneumonia thus produced was limited and may have been caused by a survival of the agent in the material originally inoculated into the cats.

Features of the Illness.—Some of the signs of illness in a cat placed in contact with one inoculated intranasally with a yolk sac suspension containing the infective agent are recorded in Text-fig. 1. The occurrences in this animal represent the reaction considered typical of the experimental infection. The first evidence of infection was a rise in temperature, but this was usually slight

TABLE III

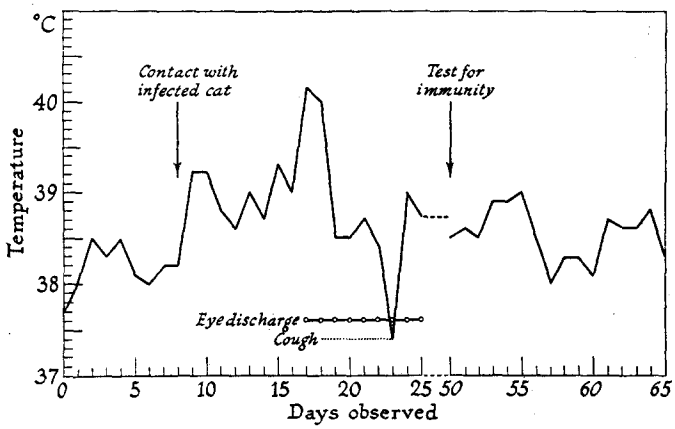
Infectivity for Cats of Various Preparations Containing the Virus

Source of virus	Cat No.	Method of infection	Signs of disease	Autopsy	Virus present at autopsy in	Immunity to intranasal inoculation
Suspensions of naturally infected cat lungs	11	Intranasal	Fever and respiratory	Pneumonia present 9 days after injection	Lungs	Not tested
	13	"	" "	No pneumonia present 46 days after 1st and 19 days after 2nd inoculation	Nasal turbinates, lungs	Immune
	20	Contact with cat 11	" "	No pneumonia found 21 days after exposure	Not tested	Not tested
	26	Contact with cat 13	" "	No pneumonia found 12 days after exposure	Nasal turbinates, lungs	" "
Suspensions of infected mouse lungs	8	Intranasal	Fever and respiratory	Pneumonia present 7 days after injection	Lungs	Not tested
	23	"	" "	Small area of fibrosis found 46 days after 1st and 19 days after 2nd inoculation	Eyes, nasal turbinates, lungs	" immune
	9	Contact with cat 8	" "	No pneumonia found 61 days after exposure	Nasal turbinates, lungs	Not tested
	27	Contact with cat 23	" "	Pneumonia present 13 days after exposure	Eyes, nasal turbinates, lungs	" "
Suspensions of infected yolk sac membrane	12	Intranasal	Fever and respiratory	No pneumonia found 70 days after 1st and 25 days after 2nd inoculation	Nasal turbinates, lungs	Immune
	18	"	" "	Pneumonia present 9 days after injection	" "	Not tested
	14	Contact with cat 12	" "	No pneumonia found 67 days after exposure	" "	" "
	15	Contact with cat 18	" "	No pneumonia found 12 days after exposure	" "	" "

and of such short duration that the disease might almost be considered afebrile. The first recognizable manifestations of illness developed 6 to 10 days after exposure and were increased lacrimation, followed after a day by conjunctivitis and a mucopurulent discharge from the eyes (Fig. 1). A photophobia was apparently present. A nasal discharge accompanied the eye changes. Prolonged attacks of sneezing, especially when the cats were handled, frequently

occurred. A cough usually developed, indicating infection of the lower respiratory tract, which was usually a pneumonia. All these signs persisted for 1 to 2 weeks. Shortly after the onset of the illness an obvious loss in weight occurred. All inoculated cats that were not killed recovered from the disease, but it was a month from the time of onset before they appeared normal.

Pathology.—Early in the course of the disease the conjunctiva became reddened and the discharge of exudate from the eyes indicated a conjunctivitis. At the same time a similar inflammation of the nasal mucosa occurred. Progression of the disease usually, although not always, led to an inflammatory reaction of the lower respiratory tract. The larynx and trachea were reddened and the accumulation of small amounts of thick, cloudy mucus was noted.



TEXT-FIG. 1. Some of the signs of illness in a cat, following exposure to the experimental disease.

The pneumonia was generally characterized by dense consolidations of a pinkish gray color in the anterior lobes and occasionally in the diaphragmatic lobes. Small consolidations near the hilus were sometimes visible. The bronchial lymph nodes were not noticeably enlarged. An occasional animal showed slight enlargement of the spleen, but otherwise nothing was found when the other organs were examined.

Sections of the lung showed an exudate containing polynuclear and monocytic cells in the bronchi (Fig. 3). The bronchial epithelium was apparently undamaged. A serous and cellular exudate filled the alveoli and the predominating cell was the monocyte. Occasional areas of necrosis were seen. Giemsa-stained sections showed elementary bodies to be present, apparently in the cytoplasm of mononuclear cells.

Distribution and Persistence of the Virus in Cats

Tests of 5 cats, one of which was naturally infected, for the presence of the virus in suspensions from various organs of the body and certain secretions were conducted. The suspensions were inoculated intranasally into mice, and the establishment of a pneumonia constituted proof of the presence of virus although in such instances elementary bodies were found whenever looked for. The results are shown in Table IV.

Virus was shown to be present in eye discharges, nasal secretions, and lungs of both naturally diseased and experimentally infected cats. Suspensions of the liver, spleen, and kidneys, either in a pool or separately, were inactive, except in one severely affected kitten. The presence of a few consolidations in the lungs of the test mice indicated that a small amount of virus was generally distributed in this one animal.

TABLE IV

Distribution of the Virus in Various Organs and Secretions of the Cat As Determined by Mouse Inoculations and Elementary Body Demonstrations

Animal No.	Mode of infection	Organs and secretions tested					
		Eye discharge	Nasal discharge	Lung	Spleen	Liver	Kidney
3	Natural	+	+	+	−	−	−
23	Intranasal	+	+	+	−	−	−
27	Contact	+	+	+	−	−	−
30	Intranasal	+	+	+	+	+	+
31	"	+	+	−	−	−	−

Tests of 5 cats 1 to 2 months after recovery showed that virus was still present in the nose as demonstrated by inoculating suspensions of the ground nasal turbinates intranasally into mice. Such cats were presumably immune, for animals similarly inoculated and recovered showed no signs when given infective doses of virus.

Immunity.—Four cats, one recovered from the natural and the others from experimental disease, were given intranasal inoculations of actively pathogenic lung suspensions from infected cats. Three of the cats showed no signs of illness but some evidence of reinfection was obtained in a kitten previously inoculated intranasally with a lung suspension from infected mice, namely an eye discharge. Autopsy showed only lesions resulting from the initial inoculation; the control showed recent extensive pneumonia.

Neutralization tests were attempted using mixtures of sera from recovered or normal cats and various dilutions of active mouse lung suspensions. When inoculated intranasally into mice, no clear-cut evidence of neutralization was obtained.

Complement Fixation Tests.—Complement fixation tests were made, using antigens of partially purified and concentrated elementary bodies prepared from infected mice.

Lungs from infected mice were ground with sterile sand and made up to a 10 per cent suspension. This suspension was centrifuged for 30 minutes at 1200 R.P.M. and the sediment discarded. The supernatant was then centrifuged at 3500 R.P.M. for 5 minutes. Again the supernatant was poured off and recentrifuged at 7500 to 10,000 R.P.M. for 30 minutes. The sediment was resuspended in saline to 1/5th the original volume. This diluted 1/5th or 1/10th constituted the antigen and it was not anti-

TABLE V

Complement Fixation Tests, Using Antigens Prepared from Lungs of Infected Mice, with Cat Sera Obtained before Infection and after Recovery

Cat No.	Least amount of serum fixing complement		
	Before infection	After infection	
	cc.	*days*	*cc.*
2*		Acute stage	>0.1‡
3*		30+	0.01
9	>0.1	17	0.01
		61	0.01
12	>0.1	24	0.05
13	0.1	25	0.05
14	0.1	22	0.02
		67	0.0025
18	>0.1	9	0.1
20	>0.1	20	0.02

* Litter mates.

‡ >0.1 indicates no fixation with this amount of serum which was the largest tested.

complementary. The concentration of elementary bodies, judged by microscopic examination of films made from the sediment, was approximately the same in the 4 antigens that were prepared.

Sera were collected from cats before inoculation and again from 9 to 67 days after infection. In addition, serum was taken from a cat during the early stages of the natural infection and from its litter mate after recovery.

Each tube contained antigen, serum, complement (2 units in 0.5 cc.), and salt solution to bring the total volume to 2 cc. A constant amount (0.5 cc.) of antigen and decreasing amounts of sera were used. The tubes were incubated overnight in the refrigerator. 1 cc. of a mixture of equal parts of 2 per cent washed sheep cells and hemolytic amboceptor diluted to contain 2 units per 0.5 cc. was added and readings were made after 30 minutes' incubation at 37°C. Adequate controls were included. The results obtained are given in Table V.

It can be seen that complement fixing bodies appeared in the sera of cats recovered from the natural disease or from the experimental infection. In the sera from 2 of the cats shown above, antibodies were found to be present more than 2 months after recovery, and in one of them 0.0025 cc. of serum fixed complement while the other showed no increase. Subsequently it was found that the sera from cats 13 and 14 which figured in a preliminary note on the disease now under discussion (5) yielded sera which fixed complement in 0.1 cc. amounts prior to infection, but serum specimens from the others gave negative results.[1]

DISCUSSION

The evidence produced permits the conclusion that the respiratory disease studied is due to a virus that forms elementary bodies. This virus appears to confine itself to the eyes and respiratory tract. Usually it can be isolated only from these sources and typical manifestations of the disease can be produced only through introduction of the virus into the nasal passages. A possible untested route is by inoculation on the conjunctiva. The disease was transmitted by contact to normal cats.

A study of the literature fails to show that the virus has been previously described. Rake, Eaton, and Shaffer (7) have shown that the viruses of psittacosis, meningopneumonitis, and lymphogranuloma venereum have a similar morphology and complement fixation tests have shown them to possess common or closely related antigenic constituents. Unfortunately the work had to be discontinued before an inquiry could be made into a possible relationship to these viruses. Lawrence and Syverton (8) and Hammon and Enders (9) simultaneously reported a filterable virus from a disease of cats characterized by a fulminating leucopenia. This virus does not infect ordinary laboratory animals, and cats are susceptible when the virus is injected parenterally. They called this disease panleucopenia, although it is also known under the name of cat enteritis. Verge and Christoforoni (10) and Hindle and Findlay (11) had previously demonstrated a filterable virus in cases of this latter condition, but it was thought to be capable of many manifestations and one form of pneumonia

[1] In the preliminary communication referred to (5) it was pointed out that complement-fixing bodies for the cat antigen appeared to increase in sera from some human patients with atypical pneumonia. Subsequently, Thomas and his coworkers (6) have found that sera of patients convalescent from primary atypical pneumonia have the power of fixing complement with a number of different antigens. In view of their report, complement-fixing tests for such cases can have little significance when employed to determine specific infection. It has been necessary to discontinue our experiments, but the virus has been given to a number of workers in this field and the possible relationship of the cat virus to human infections will doubtless be clarified.

without enteritis was described. The properties, pathogenicity, and pathology definitely differentiate the virus described here from that of panleucopenia. However, other viruses capable of producing pneumonia in cats may exist. Only recently Blake and his coworkers (12) reported upon a virus that produces a pneumonia in cats, especially kittens. This virus failed to infect mice and elementary bodies were not demonstrated. For this reason they concluded that it differs from the one described by us.

SUMMARY

From a contagious respiratory disease of cats an agent has been transmitted to mice, rabbits, guinea pigs, hamsters, and embryonated eggs. When inoculated intranasally into cats, it produces a disease like that seen in the naturally infected animals. Parenteral injection causes only a mild fever. From cats killed during acute illness, the agent was demonstrated regularly in the discharges from the eyes and nose and in the pneumonic lung but not in other organs. The agent can be demonstrated in the nasal mucosa of cats 1 to 2 months after injection.

Cultures of egg yolk sac containing the agent showed no growth, and cultures of active lung suspensions were usually negative, such positive findings as were obtained being due to respiratory contaminants. Attempts to pass the agent through Berkefeld N filters were generally unsuccessful. Stained preparations of egg yolk sac membranes and of infected mouse or hamster lungs showed typical elementary bodies. Centrifuge experiments showed that the agent and the bodies sedimented at the same rate. Complement fixation experiments using a suspension of partially purified bodies as antigen were negative with control sera and positive with sera from recovered cats. It is concluded that the respiratory disease of cats is due to a virus that forms elementary bodies.

BIBLIOGRAPHY

1. Horsfall, F. L., Jr., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.
2. Cox, H. R., *Pub. Health Rep., U. S. P. H. S.*, 1938, **53**, 2241.
3. Macchiavello, A., cited by Zinsser, H., in Virus and rickettsial diseases, Harvard School of Public Health Symposium, June 12–17, 1939, Cambridge, Harvard University Press, 1940, 896.
4. Bland, J. O. W., and Canti, R. G., *J. Path. and Bact.*, 1935, **40**, 231.
5. Baker, J. A., *Science*, 1942, **96**, 475.
6. Thomas, L., Curnen, E. C., Mirick, G. S., Ziegler, J. E., Jr., and Horsfall, F. L., Jr., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 121.
7. Rake, G., Eaton, M. D., and Shaffer, M. F., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 528.
8. Lawrence, J. S., and Syverton, J. T., *Proc. Soc. Exp. Biol. and Med.*, 1939, **38**, 914.
9. Hammon, W. D., and Enders, J. F., *J. Exp. Med.*, 1939, **69**, 327.

10. Verge, J., and Christoforoni, N., *Compt. rend. Soc. biol.*, 1928, **99**, 312.
11. Hindle, E., and Findlay, G. M., *Proc. Roy. Soc. Med.*, 1933, **26**, 197.
12. Blake, F. G., Howard, M. E., and Tatlock, H., *Yale J. Biol. and Med.*, 1942, **15**, 139.

EXPLANATION OF PLATE 2

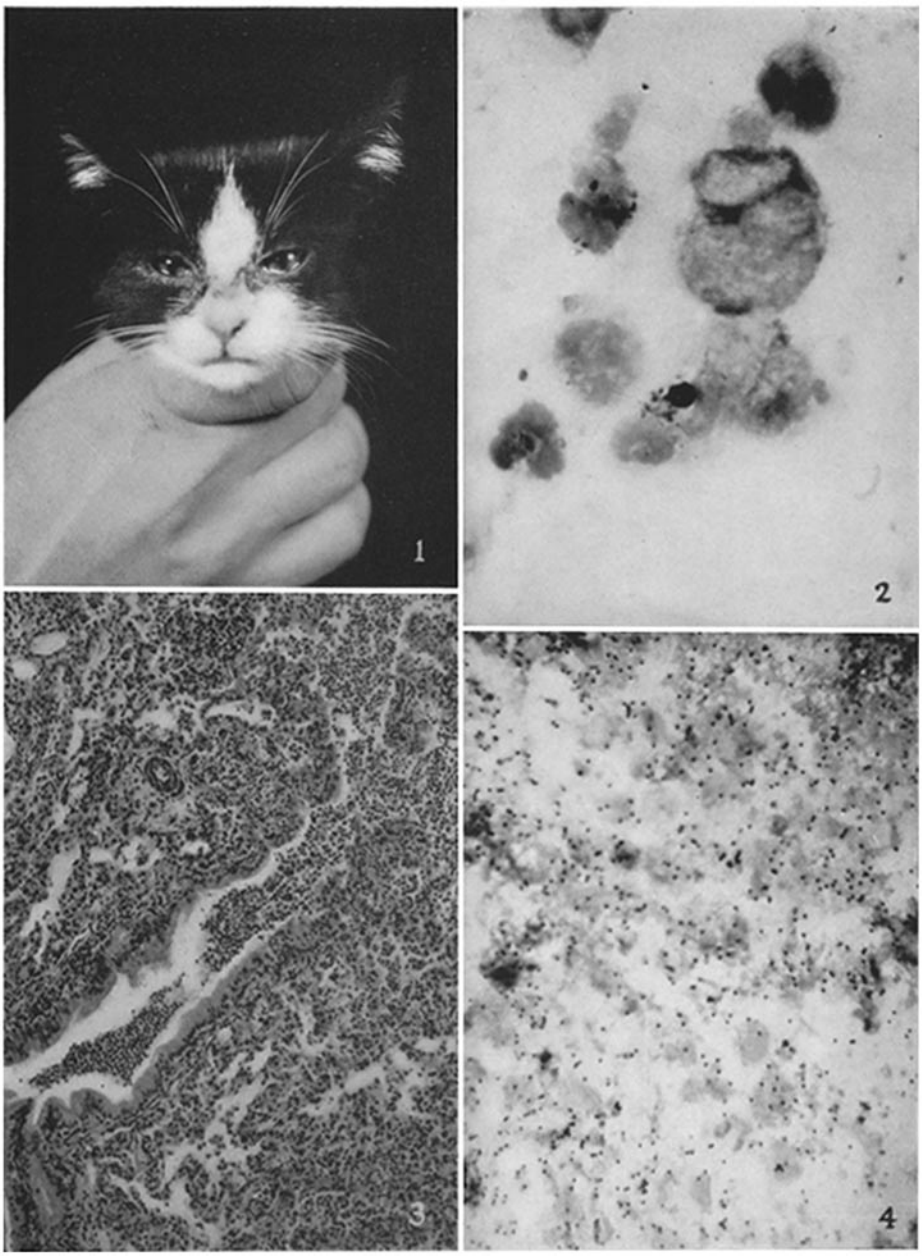
The photographs were made by Mr. J. A. Carlile.

FIG. 1. An infected cat showing typical eye signs. Note exudate draining over the nose. × 0.49.

FIG. 2. Impression film from lung of infected hamster showing elementary bodies. The dense rounded structure is a plaque such as breaks up into elementary bodies. Macchiavello's stain. × 1060.

FIG. 3. Section of lung from a cat. The exudate in the bronchus is a mixture of mononuclear and polynuclear cells, while the cellular infiltrations into the alveoli are mainly mononuclear cells. Hematoxylin-eosin stain. × 88.

FIG. 4. Films from sediment of centrifuged suspensions of infected mouse lungs showing numerous elementary bodies. Macchiavello's stain. × 1060.



(Baker: Virus causing pneumonia in cats)