

AN OUTBREAK OF PSITTACOSIS IN PIGEONS, INVOLVING THE
PRODUCTION OF INCLUSION BODIES, AND TRANSFER
OF THE DISEASE TO MAN

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PLATES 5 AND 6

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Spontaneous infection with the virus of psittacosis is known to be widespread among birds of the psittacine family and also to occur in members of other orders of the class, *Aves* (1). Coles (2) in 1940 described an epizootic of psittacosis among domestic pigeons (*Columba livia* var. *domestica*) in South Africa and this disease was later encountered among pigeons in many parts of the United States (3-5). Although no human infections were associated with the first epizootic, Meyer and his coworkers (3, 6) subsequently reported the transmission of psittacosis from pigeons to man. A recent article by Smadel (5) indicates that at least one-fourth of the individuals in a group of 45 persons with sporadic atypical pneumonia were infected with strains of the virus of psittacosis, and that the majority of the patients with psittacosis had direct or indirect contact with diseased pigeons. Such observations as these, as well as those of Eaton and Corey (7), demonstrate that psittacosis is a more common human disease than was previously recognized. In order to understand fully the epidemiology of this newly recognized form of psittacosis in man further information concerning the spontaneous disease in pigeons and other birds is needed.

Observations made on an outbreak of psittacosis in a flock of pigeons and on the disease encountered in two persons who handled the sick birds are the subject of this report. We shall speak of the psittacosis-lymphogranuloma venereum group of viruses when discussing this family of agents; furthermore, we shall consider the psittacosis virus encountered in pigeons and parrots as strains of the same agent. A number of articles can be consulted for a discussion of the relationship of the viruses of psittacosis, lymphogranuloma venereum, and other allied agents (5-9).

Materials and Methods

In the course of a study on the rôle of certain known viruses in the etiology of sporadic cases of atypical pneumonia (5) a physician with psittacosis was encountered. An epizootic disease had appeared in pigeons of this patient's flock about the time he became ill. During the epizootic 7 of the 50 birds in the flock died and 6 of these were submitted to us for examination. The pigeons were autopsied and blocks of tissue from various organs were taken for histological study. In addition, portions of liver

and spleen were removed aseptically, cultured for bacteria, and stored at -70°C . During the ensuing 2 months a number of surviving members of the flock were bled for serological tests and at the end of this time all of the birds were sacrificed.

Serological Methods.—Complement-fixation tests for demonstrating in the sera of pigeons, patients, and mice antibodies which were capable of reacting with psittacosis antigen were carried out in the manner previously described (5). Antigen for use in the diagnostic tests was prepared from agar slant tissue cultures (10) infected with the 6BC strain of the virus of psittacosis; this strain was isolated from a parrakeet in 1941 by Dr. K. F. Meyer and was obtained from him. In addition, inactivated suspensions of washed elementary bodies of the viruses isolated during the present studies were prepared by the same method and tested against sera from human beings, pigeons, and mice convalescent from psittacosis or sera from mice hyperimmunized with active virus. The tests were appropriately controlled by the use of normal antigen prepared from non-infected agar slant tissue cultures or by normal human, pigeon, or mouse serum; in addition, known positive psittacosis antigen and serum were included in each test.

Isolation of Virus from Pigeons.—When the results of histological studies on sections of pigeon organs became available, bacteriologically sterile tissues from each bird were removed from storage at -70°C . Suspensions of spleen or liver or pooled organ suspensions were then prepared and inoculated intracerebrally into mice and on chorio-allantoic membranes of 10 day old embryonated eggs. The infective agents isolated in mice were maintained by serial passages in the brains of mice, while strains of the same or other agents isolated directly in eggs were maintained in eggs.

Cross Immunity Studies.—All but one of the strains of virus isolated from pigeons were pathogenic for mice when inoculated intracerebrally. Large amounts of these agents when injected intraperitoneally failed to kill mice but they did immunize them against a subsequent intracerebral inoculation of 10 to 1000 M.L.D. of the homologous virus. Accordingly, groups of 25 to 50 mice were immunized against each agent pathogenic for mice and then tested for intracerebral resistance to the homologous strain. Subsequent cross immunity tests were done in the following manner:—Groups of 4 immune mice were injected intracerebrally with suspensions containing varying quantities of M.L.D. of the heterologous pigeon strains of virus and of the 6BC psittacine strain. Mice immune to the 6BC strain of psittacosis were obtained with some difficulty since this agent is highly pathogenic when administered by the peritoneal as well as the cerebral route. However, animals that survived the inoculation of a small amount of virus by the former route followed by repeated injections of increasing amounts of the agent then resisted the intracerebral inoculation of 10,000 M.L.D. of the 6BC strain. Such psittacosis immune mice were tested for their resistance to viruses isolated from pigeons.

Microscopic Studies.—Histopathological studies were made on paraffin sections of tissues from pigeons, mice, and chick embryos which were prepared in the usual manner following fixation in acetic Zenker's solution and which were stained by Giemsa's method. Impression smears made from tissues of infected mice, eggs, and cultured material were stained by the technique of Machiavello and examined microscopically.

History of the Pigeon Flock.—Pigeons of the flock under investigation were kept in three large connecting houses with adjoining enclosed flyways; the birds were not

permitted free flight. They were constantly under observation and individual pigeons were periodically examined during the course of studies on the development of plumage. The 12 original members of the colony were Homers purchased in 1938. New birds (Tiplers, Flights, and Kings) from several outside sources were added to the group in October and November of 1941 and also late in January of 1942. No unusual disease was noted among the group of approximately 50 birds until about 3 weeks after introduction of the latest purchases.

EXPERIMENTAL

Clinical and Pathological Observations on the Disease in Pigeons

Several pigeons of the flock under investigation became obviously sick during the last week in February, 1942. This was about the time that their owner developed a disease which was proved to be psittacosis and was some 3 weeks after he had purchased new birds which he added to the flock. The affected birds were inactive, they failed to eat, some had diarrhea, and at least one had an exudate about the nares and suffered difficulty in breathing. The first death, that of pigeon 1, occurred on March 1 and during the next 3 days a sick bird was sacrificed and another died, pigeons 2 and 3, respectively. Careful clinical observations were not made during the following 3 weeks, but no pigeons succumbed in this period of time. Another sick bird, 4, was encountered by us on April 4 while bleeding members of the flock for serological studies; it expired 5 days later. Within the next week 3 more pigeons died, *viz.*, 7, 12, and 30. On April 18 after a number of the surviving birds had been bled the entire flock was sacrificed.

Macroscopic abnormalities encountered at autopsy varied considerably in the 6 pigeons from which strains of virus were isolated. Three of the birds, *viz.*, Nos. 3, 12, and 30, presented relatively minor changes which consisted of fecal soiling of the tail feathers, dehydration, and moderate distention of the intestines with congestion of the serosal vessels. Hepatic lesions were inconspicuous and splenic enlargement was not prominent. Additional changes were observed macroscopically in pigeons 1, 4, and 7. Both P-1 and P-4 were malnourished, dehydrated, and dirty from fecal contamination. Their abdominal cavities contained small amounts of fluid and flecks of fibrinous exudate were present on the peritoneal surfaces. The most striking lesion was a focal pancreatitis with opaque, chalk-like areas which resembled fat necrosis of the pancreas in man. The liver and spleen of No. 1 were slightly enlarged and were definitely enlarged in No. 4; furthermore, the liver of the latter bird was deep red in color and friable. The disease process in No. 7 appeared more acute than in the other 2 birds; malnutrition was not evident, the skin had a faintly icteric tint and moderate subcutaneous edema was present over the abdomen. The peritoneal cavity was distended with about 5 cc. of serogelatinous exudate which in places adhered to the viscera and the bile-stained peritoneum to a height of 3 to 4 mm. The liver of pigeon 7 was enlarged with rounded edges and the friable tissue presented a mottled appearance with opaque yellow areas on a dark red background. The spleen which measured

1.5 × 0.5 × 0.5 cm. was also friable and mottled like the liver. The lungs of this pigeon were markedly congested and edematous.

The pathological changes encountered in pigeon tissues on microscopic examination were more uniform than those observed macroscopically. In general, the differences in individual birds were of degree rather than of kind. Sections of spleen and liver were available from all 6 of the birds, and, in addition, sections of pancreas, lung, and kidney from some of the pigeons were examined.

Intense vascular congestion was present in practically all organs. The livers of all 6 pigeons, *viz.*, Nos. 1, 3, 4, 7, 12, and 30, contained focal cellular collections of large mononuclear cells and lymphocytes. These occurred in and about the periportal spaces, and in some areas they extended into the parenchymatous tissue where they replaced hepatic cells which were undergoing necrobiotic changes. Focal necrosis involving small or large groups of liver cells in various parts of the liver lobules was likewise encountered in each of the birds, as shown in Figs. 1 and 2. Polymorphonuclear cells were rarely present either in the areas of cellular infiltration or in the areas of necrosis. A diffuse fatty change involving hepatic cells was seen in a few of the birds and a beginning cirrhotic process extending from the portal spaces occurred in several pigeons.

Congestion was so intense in the more severely involved spleens that hemorrhage occurred into the pulp. Furthermore, in one bird (No. 7) this was accompanied by deposits of coagulated protein and of strands of fibrin. Cellular elements of the pulp were increased by hyperplasia of endothelium and by diffuse infiltrations of large mononuclear cells and of lymphocytes. Cellular destruction was observed in all spleens except that of pigeon 30, but it varied in the size of the areas involved and in the number of foci (see Fig. 3).

Acute necrosis was present in the pancreatic tissue of pigeons 1 and 4. In the latter the process was so recent that cellular outlines were still discernible in the involved areas and infiltration of inflammatory cells into the necrotic tissue had not yet begun (see Fig. 5). The picture in the spleen of pigeon 1 was more complicated, with areas of complete destruction alternating with areas of advanced necrobiotic change. Furthermore, mononuclear and polymorphonuclear cells had infiltrated some of the older areas of destruction. In the pancreas of each pigeon many of the acini were dilated; the distended lumina contained precipitated protein and desquamated epithelial cells in various stages of degeneration.

Cellular inclusions were found in sections of one or more of the organs of each pigeon from which virus was isolated (see data summarized in Table I). Large acidophilic intranuclear inclusions of the herpetic type were so numerous in certain organs, *e.g.*, the spleen of pigeon 3, that practically every intact cell in some fields contained one (Fig. 3). Intranuclear inclusions were present in tissue of all 6 pigeons and were abundant in pigeons 1, 3, 4, and 7; however, these structures were occasionally absent from certain organs and sometimes occurred infrequently in the organs in which they were found. In the liver intranuclear inclusions occurred principally in parenchymatous cells but were occasionally found in epithelial cells lining small bile ducts and

rarely were they present in the large mononuclear cells in the areas of infiltration. In the spleen intranuclear inclusions were encountered both in cells of the pulp and of the Malpighian bodies, while in the pancreas these nuclear structures were more frequently observed in cells of the islets of Langerhans than in acinar cells. In sections of organs from this group of pigeons, an occasional large mononuclear or parenchymatous cell was found with its cytoplasm filled with granular bodies that took a purple-blue color with the Giemsa stain. These granules (see Fig. 5) closely resembled the elementary bodies seen in sections of tissue infected with psittacine strains of the virus of psittacosis. Rarely an individual cell was encountered which had an intranuclear inclusion and also elementary bodies. A summary of the data dealing with the distribution of intranuclear inclusions and of elementary bodies in the tissues of spontaneously infected pigeons is given in Table I.

TABLE I
Intranuclear Inclusions and Elementary Bodies in Tissues of Spontaneously Infected Pigeons

Pigeon	Organ					
	Liver		Spleen		Pancreas	
	I.N.I.*	E.B.‡	I.N.I.	E.B.	I.N.I.	E.B.
1	++	++++	+	++++	++++	+++
3	++++	+++	++++	+++		
4	+	+	+	+	+++	+
7	+++	+++	++++	+		
12	++	+++	-	+		
30	-	-	++	++		

* I.N.I. = intranuclear inclusions.

‡ E.B. = elementary bodies.

The clinical and pathological features of the epizootic disease encountered in pigeons can be summarized as follows: Sick birds presented signs of enteritis and in some instances of respiratory involvement. The illness in most of the fatal cases was sufficiently prolonged for malnutrition and dehydration to develop. While gross pathological findings other than those attributable to enteritis were scant in 3 of the 6 autopsied birds, the remaining pigeons also presented evidence of fibrinous peritonitis and of an acute focal necrotizing process in the liver, spleen, or pancreas. Histological examination showed that necrosis of hepatic and splenic tissue occurred regularly in all birds but varied in intensity. Elementary body-like structures in the cytoplasm of certain cells of the liver, spleen, or pancreas appeared to be a characteristic finding in the disease. The disease picture just summarized corresponds in many respects with that of psittacosis as seen in parrots (11) and in pigeons (3, 12, 13), with the more fulminating cases bearing a closer resemblance to the psittacine type than to the milder type usually encountered in pigeons. Histological studies revealed another important characteristic of the disease in our group of pigeons,

namely, the presence of intranuclear inclusions of the herpetic type in cells of affected organs. Intranuclear inclusions are not found in classical psittacosis (11) nor have they been described in ornithosis of pigeons (3, 12, 13). The presence of these intranuclear structures in our material stimulated us to study the epizootic more intensively than had been originally planned.

Development of Psittacosis Antibodies in Naturally Infected Pigeons

Infection with the virus of psittacosis gives rise to antibodies that fix complement in the presence of psittacosis antigen (14). This serological reaction appears to be specific for the psittacosis-lymphogranuloma venereum group of viruses even though it fails to differentiate sharply between individual members of the family (3-8). Selected pigeons of the flock were bled at 4, 5, and 7 weeks after the appearance of the epizootic and their sera were tested for antibodies capable of reacting with an antigen prepared from a parrakeet strain of virus.

Three of the 5 birds bled on Mar. 28 had no demonstrable antibodies against the virus of psittacosis, but 2 possessed large amounts (see Table II). One week later serum was obtained from 9 other pigeons and only one of these specimens failed to fix complement with psittacosis antigen. The bird that supplied the single negative serum on this date was obviously sick when bled and died 5 days later; a virus was isolated from its tissues. Seven of the 10 specimens of serum collected on Apr. 18 were obtained from birds that had been bled previously. Two of the pigeons, *i.e.*, 2 and 24, that had originally no complement-fixing antibodies now possessed them; the titers of their sera were 1:512 and 1:64, respectively. The level of antibody in the serum of pigeon 5 rose from 1:16 to 1:128 during the 2 weeks from Apr. 4 to Apr. 18. Sera obtained from 2 birds at the time of the last bleeding gave negative results in the test, a sample from one of these pigeons having been negative 4 weeks earlier. Two pigeons showed no change in antibody level from that of the preceding bleedings while one displayed a moderate decrease in its level. Two birds bled for the first time on Apr. 18 were found to have complement-fixing antibodies in their sera.

It is evident from the serological results summarized in Table II that an infection caused by a member of the psittacosis group of viruses was spreading through the flock during the period of observation. An increase in the proportion of birds with positive sera occurred at the time when the highest death rate was noted in the flock. Furthermore, antibodies appeared in the sera of at least 2 birds that did not have these immune substances 3 weeks earlier. Since neither of these 2 pigeons was obviously sick at any time, one may assume that their disease was mild. In contrast, pigeon 4, from whose tissues virus was subsequently isolated, showed signs of illness and died before antibodies were demonstrable in its serum.

Strains of Virus Isolated from Pigeons

During the course of the epizootic 7 pigeons died and one apparently ill bird was sacrificed. A viral agent was isolated from hepatic or splenic tissue of 6

of the 7 birds which were examined; the single failure was encountered with the tissues from the pigeon that was killed. The infectious agents isolated from the tissues of 5 pigeons were readily shown to be members of the psittacosis-lymphogranuloma venereum group of viruses but the virus recovered from one of the birds, pigeon 3, has not yet been classified. A summary of the important data on the pathogenicity and on the immunological characteristics of these viruses, all of which produced elementary bodies in infected tissues, is presented in Table III.

TABLE II
Psittacosis Antibodies in Sera of Pigeons

Pigeon	Complement-fixing titer		
	Mar. 28	Apr. 4	Apr. 18
2	Negative		1:512
4		Negative*	
5		1:16	1:128
6			Negative
8			1:1024
16		1:512	
17		1:1024	1:1024
21		1:512	1:512
22	1:1024		
24	Negative		1:64
25		1:128	
27		1:128	
28		1:128	
32	1:256		
38			1:16
40		1:64	1:16
41	Negative		Negative

When two samples of serum were available from one pigeon, they were both run in the same test.

* Bird was sick when bled; it died 5 days later and virus was isolated from its tissues.

On primary inoculation into mice by the intracerebral route the tissue suspensions from 5 of the pigeons produced an illness that generally terminated fatally. Impression smears prepared from the exposed meninges of these animals and of mice from subsequent passages, when stained by Machiavello's technique, contained mononuclear cells with many red granules in their cytoplasm. The morphological and tinctorial qualities of these granules were identical with those of elementary bodies seen in similar preparations of tissues infected with psittacosis virus. After a few intracerebral passages in mice the strains of virus were of such virulence that they killed mice in 2 to 4 days; the brains of animals that succumbed were infectious in dilutions of 10^{-6} to 10^{-8} when titered intracerebrally in mice. Although highly infectious passage material usually failed to produce a fatal disease in mice when injected intraperitoneally, multiplication of the agent occurred at this site because

typical L-C-L bodies were demonstrable in the peritoneal exudate which was present in variable quantities from 5 to 8 days after inoculation. Mice that received several intraperitoneal injections of active virus developed antibodies that fixed complement in the presence of psittacosis antigen. Finally, mice immunized in this manner were resistant to intracerebral inoculation of homologous and heterologous strains of pigeon virus (10 to 1000 M.L.D.) and to the 6BC strain of psittacosis virus (10 to 10,000 M.L.D.). Mice that survived immunization with the 6BC strain of psittacosis virus were resistant to the maximum amounts of the pigeon strains of virus that were given, *i.e.*, 10 to 1000 M.L.D., as shown in Table III.

TABLE III
Characteristics of Strains of Virus Isolated from Pigeons

Virus strain	Pathogenicity		Elementary bodies	Immunized mice						Tissue culture		
	Mice	Egg membranes		Resistance to viruses					C-F antibodies against 6BC psittacosis antigen	Multi-plication	C-F antigen with psittacosis antibodies	
				Psittacosis 6BC	Pigeon viruses							
					P-1	P-4	P-7	P-12				P-30
P-1	+	+	+	+	+	+			+	+	Not demonstrated	
P-3	-	+	+	-						+	Not demonstrated	
P-4	+	+	+	+	+	+			+	+	+	
P-7	+	+	+	+	+	+			+	+	+	
P-12	+	+	+	+	+		+		+			
P-30	+	+	+	+	+			+	+			
Psittacosis 6BC.	+	+	+	+	+	+	+		+	+	+	

Pigeon strains of virus, except P-3, killed mice when inoculated intracerebrally but not when administered intraperitoneally. Mice infected by the latter route developed peritoneal exudate containing L-C-L bodies and subsequently were resistant to intracerebral inoculation of psittacosis virus. Psittacosis virus, 6BC, was lethal for mice when inoculated either intracerebrally or intraperitoneally.

All 5 of the agents that were pathogenic for mice grew well on the chorio-allantoic membranes of developing chick embryos. Discrete pocks were formed when sufficiently dilute inocula were placed on the membranes of 10 day old embryos; these were visible as tiny dewdrop structures 2 to 3 days after inoculation but became larger and more opaque by the 5th or 6th day. The lesions contained many cells rich in elementary bodies. Three of the 5 strains of virus were also carried in agar slant tissue cultures for 4 to 6 passages. The tissue culture experiments with strains P-4 and P-7 were more successful than those with strain P-1 inasmuch as the first 2 strains provided material infectious in dilutions of 10^{-7} and 10^{-8} , respectively, when titered intracerebrally in mice, while the infectivity of the 6th culture passage of P-1 was only 10^{-6} . A suspension of washed inactivated virus prepared from the more infectious material of cultures P-4 and P-7 fixed complement in a titer of 1:16 and 1:2, respectively, in

the presence of both human and pigeon sera that were known to react with psittacosis antigen. Unsuccessful attempts to prepare a potent complement-fixing antigen from cultures of P-1 probably depended on the presence of an inadequate amount of virus.

The experimental observations just presented would permit one to conclude that the agents isolated from pigeons 1, 4, 7, 12, and 30 were similar to, if not identical with, the ornithosis virus isolated from pigeons by Meyer, Eddie, and Yanamura (3) and with the virus recovered by Eaton, Beck, and Pearson (9) from patients with atypical pneumonia. This point will be discussed more fully after the histopathology of the experimental infections has been presented.

Studies of the type which suggested the identity of the 5 viruses which were pathogenic for mice gave inconclusive results when applied to the agent isolated from pigeon 3. Suspensions prepared from the spleen and from the liver of pigeon 3, when inoculated on chorio-allantoic membranes of eggs, produced pock-like lesions; this disease process was readily transmissible in eggs. However, suspensions of the pigeon tissues and of egg passage materials were incapable of inducing a disease in mice which could be attributed to the agent present. This was true even though several blind passages were made in mice from animals which had been inoculated intracerebrally or intraperitoneally or by both routes. Mice which received a number of injections of membrane material containing the agent isolated from pigeon 3 neither resisted an intracerebral inoculation of 100 to 1000 M.L.D. of known psittacosis virus nor developed complement-fixing antibodies against psittacosis antigen.

The infectious agent isolated from pigeon 3, designated P-3, induced a more proliferative type of response in egg membranes than did the other strains of virus. Nevertheless, smears prepared from membrane lesions or from agar slant tissue cultures of P-3 and stained by Machiavello's method contained many cells with red granules in their cytoplasm that appeared typical of the elementary bodies of psittacosis. Infected chorio-allantoic membranes or tissue culture material when diluted and inoculated on egg membranes for titration purposes produced discrete pocks in dilutions as high as 10^{-4} or 10^{-5} and confluent lesions with more concentrated suspensions. Preparations of elementary bodies from membranes and from agar slant cultures infected with P-3 failed to fix complement in the presence of psittacosis antibodies, as shown in Table III. It will be recalled that similarly negative results were obtained with the antigen prepared from the suspension of P-1 which was also of rather low infectivity. Ordinary bacteriological cultures of tissue from pigeon 3 and of the passage materials containing the agent, P-3, did not reveal the presence of a bacterial organism that could be held responsible for the experimental disease, and no such agent was recovered from the agar slant tissue cultures of P-3.

Meyer and his associates (3) isolated from pigeons during epizootics strains of psittacosis virus which varied considerably in their pathogenicity for mice. We would probably have accepted without further question the P-3 virus as another example of a psittacosis strain of low virulence for mice had it not been

for the unusual histopathological findings in our spontaneously ill pigeons. The presence of intranuclear inclusions in tissues of these birds caused us to search for these structures in the experimental material in the hope that information thus obtained would permit a separation of P-3 from the other 5 strains of virus. The significant lesions observed on microscopic examination of sections of chorio-allantoic membranes of eggs and of brains of mice infected with the agents isolated from pigeons are summarized in the following paragraphs.

Mention has been made of pock-like lesions observed macroscopically on chorio-allantoic membranes of eggs infected with psittacosis virus and with all 6 strains of virus isolated from the dead pigeons. Histologically the membranes showed focal areas of hyperplasia of the ectoderm underneath which the mesoderm was heavily

TABLE IV
Intranuclear Inclusions and Elementary Bodies in Tissues Infected with Pigeon Strains of Virus

Tissue	Type of inclusion	Pigeon strain of virus						Psittacosis 6BC
		P-1	P-3	P-4	P-7	P-12	P-30	
Chorio-allantoic membrane	Intranuclear	++	++++	+	+++	+	+	-
	Elementary body	++++	+++	+++	+++	++++	++++	++++
Mouse brain	Intranuclear	-	-	-	-	-	-	-
	Elementary body	+	-	+	++	+	+	+

For comparison with tissues of original pigeons see Table I.

infiltrated with polymorphonuclear and mononuclear cells. Large intranuclear inclusions of the herpetic type were found in membranes inoculated with tissue from pigeons 1, 3, 4, 7, 12, and 30 and in membranes infected with egg passage material from each strain of virus thus isolated in eggs (Figs. 4, 6, and 10). Intranuclear inclusions were most numerous in membranes infected with the virus designated P-3, but were almost as frequently encountered in membranes infected with strain P-7 (see Table IV). Usually the intranuclear inclusions occurred in ectodermal cells but occasionally they were found in mononuclear cells in the mesoderm (Fig. 6). Structures similar to elementary bodies of psittacosis were found in ectodermal cells infected with all 6 strains of virus (Table IV and Figs. 4 and 9). Sometimes an ectodermal cell was observed which contained in its cytoplasm both an intranuclear inclusion and elementary bodies. It should be pointed out that intranuclear inclusions were difficult to find in membranes inoculated with mouse tissue infected with the strains of virus pathogenic for mice.

Histological changes encountered in the brains of mice inoculated intracerebrally with the 6BC strain of psittacosis virus were essentially identical with those described as characteristic of psittacosis infection (11, 12). Lesions similar to these were ob-

served in the brains of mice infected with each of the strains of pigeon virus pathogenic for mice (Figs. 7 and 8). Intranuclear inclusions were not seen in any of the sections of mouse tissue but cells containing elementary bodies were occasionally found in the meningeal exudate of mice infected with each of the strains (see Table IV). No significant lesions were observed in mice inoculated with the P-3 agent.

It is evident from the data summarized in Table IV that histological lesions produced on egg membranes by injection with each strain of virus had an important finding in common with the lesions of the original pigeons, namely, the presence of intranuclear inclusions. These nuclear structures were never encountered in the brains of mice infected with the 5 strains which were pathogenic for mice or in the mice inoculated with the P-3 agent. It may be mentioned that after strains P-1 and P-7 had been carried in mouse brains for 3 and 5 serial passages, respectively, they were returned to chorio-allantoic membranes for one or more passages. Membranes infected with these mouse-adapted strains contained rare intranuclear inclusions; the significance that can be attached to this observation is problematic since occasional structures of this type may occur in non-specific reactions in chorio-allantoic membranes (15). In brief, results of histopathological studies on experimentally infected tissues confused rather than simplified the issue. Even the strains of virus which had been shown to be immunologically related to psittacosis were found capable of inducing the formation of intranuclear inclusions under certain conditions. Neither classical psittacosis (11) nor pigeon psittacosis (12, 13) is characterized by the occurrence of inclusion bodies of the intranuclear type.

Disease in Two Persons Exposed to the Pigeons

Two persons who took care of the pigeon flock developed pneumonia. In view of the difficulties encountered in establishing the absolute identity of the viral agents which were isolated from the sick pigeons, a brief summary of the illness of each patient¹ and of the laboratory studies that were performed are presented.

A physician, aged 51, who had kept pigeons as a hobby for several years purchased additional birds from a dealer in New York City on Jan. 31, 1942. He became ill on Feb. 22 and during the next few days developed a fever of 103.8°F., headache, photophobia, drowsiness, and a mild, non-productive cough. When admitted to the hospital on Feb. 28, the essential findings on physical examination were limited to fever and signs of pulmonary consolidation. Roentgenography revealed an area of increased density at the left hilum and about the main bronchus of the left lower lobe. Sputum was produced on two occasions; pneumococci were not demonstrable on mouse inoculation but *Streptococci* of the *viridans* group were obtained on culture. 22 gm. of

¹ We are indebted to Dr. Dana Atchley and the Presbyterian Hospital of New York for permission to include certain of this information.

sulfadiazine administered during the first 2 days of hospitalization had no apparent effect on the disease; the temperature ranged between 103.0 and 104.6°F. until Mar. 4 and then dropped by lysis to normal on Mar. 8. Convalescence was uneventful; x-rays taken on Mar. 17 showed almost complete resolution of the pneumonic consolidation.

The 52 year old wife of the patient undertook the care of her husband's pigeons from Feb. 26 to Mar. 17. She handled a sick bird on Mar. 2 and 4 but had no other intimate contact with the pigeons. Her illness began on Mar. 30 with headache, malaise, low grade fever, anorexia, and some photophobia. On Apr. 8 a non-productive cough accompanied by substernal pain appeared. When hospitalized 2 days later she presented little evidence of pneumonia on physical examination; however, the results of x-ray studies demonstrated the existence of pulmonary consolidation in the left lower lobe. No sputum specimen was obtained. The patient was never gravely ill but recovery was slow; low grade fever persisted for several weeks and chest films did not show entirely clear lung fields until Oct. 5. Following a stay of 50 days in the hospital the patient remained in bed at home for 2 weeks. She did not fully regain normal health until 3 months later.

The incubation period of the disease in the second patient was apparently 26 to 28 days; it seemed more reasonable to consider her exposure as occurring on Mar. 2 and 4 when she had close contact with sick birds, rather than at the onset of her husband's illness. Circumstances suggest that the physician acquired his infection while inspecting the birds he purchased 3 weeks prior to his illness. Furthermore, it would appear that the pigeons bought at this time introduced the disease into the original flock, since the epizootic began at about the same time the first patient developed fever.

The results of laboratory studies indicate that both patients suffered with psittacosis. Specimens of serum taken from the husband at 2, 3, and 5 weeks after onset gave complement-fixation titers of 1:32, 1:64, and 1:256, respectively, when tested with psittacosis antigen. Serum obtained from his wife at the end of the 1st week of fever contained no detectable psittacosis antibodies but by the 4th week these immune substances were present in a titer of 1:128.

Mucopurulent material coughed up by the physician shortly after admission to the hospital was obtained promptly, emulsified with diluent, and injected intraperitoneally into mice. None of the animals became obviously sick but 2 that were sacrificed 14 days after inoculation had enlarged spleens which were bacteriologically sterile. A suspension of these spleens injected intracerebrally into mice induced illness or death in 4 to 8 days. Impression smears prepared from the brains of the latter mice and stained by Machiavello's method contained cells with red elementary body-like structures in their cytoplasm. In view of the serological data on the patient, which at this time indicated a diagnosis of psittacosis, further work with the strain of virus isolated from sputum was abandoned. Subsequently, when it became desirable to study this virus more thoroughly the strain could not be reisolated from stored material.

The disease of the two patients was similar clinically and serologically to that of a number of patients with atypical pneumonia caused by a member of the psittacosis-lymphogranuloma venereum group of viruses (5, 6).

DISCUSSION

The epizootic disease encountered in the flock of pigeons under investigation was undoubtedly caused by an agent of the psittacosis-lymphogranuloma venereum group of viruses. Complement-fixing antibodies which reacted with psittacosis antigen appeared in the sera from the majority of these pigeons. In addition, a number of agents that were isolated from birds dying during the epizootic were capable of immunizing mice against a psittacine strain of the virus of psittacosis and of inducing in this species the development of complement-fixing antibodies which reacted with psittacosis antigen. Furthermore, all strains of virus isolated except one were lethal for mice when injected intracerebrally but generally failed to cause death when inoculated intraperitoneally. All strains produced pock-like lesions on chorio-allantoic membranes, and elementary bodies were found in impression smears prepared from tissues infected with each of the agents. Finally, human infections indistinguishable from psittacosis developed in two persons who tended the birds. Thus, the disease in the pigeon flock, the agents isolated from the birds, and the occurrence of disease in individuals who had contact with the sick birds seemed to differ in no significant way from the experience of Meyer and his associates (3) with spontaneous psittacosis in pigeons.

Even though extensive evidence points toward the epizootic being one of psittacosis which affected approximately 90 per cent of the birds studied, we hesitate to state that the disease in our pigeons was caused entirely by a known member of the psittacosis-lymphogranuloma venereum group of agents. This uncertainty on our part is induced by the histopathological studies which revealed the presence of intranuclear inclusions of the herpetic type in tissues of spontaneously infected birds and in chorio-allantoic membranes inoculated with suspensions of pigeon organs. Classical strains of psittacosis do not incite the development of intranuclear inclusions in infected tissues (11) and mention has not been previously made of the occurrence of these intranuclear structures in sick pigeons infected with strains of psittacosis isolated from this species (12, 13).

The present observations lead one to formulate two mutually exclusive hypotheses; namely, that psittacosis of pigeons or certain strains of psittacosis virus are characterized in appropriate circumstances by the presence of intranuclear inclusion bodies in infected tissue, or that such is not the case, and that our pigeons were simultaneously infected with two viruses, one of which was associated with intranuclear inclusions. There are a number of possible objections that might be raised against the second hypothesis. However, no immunological relationship was demonstrated between the P-3 virus and the agent of classical psittacosis and this must be done before the first hypothesis becomes tenable. In considering the possibility that one member of the psittacosis

group of viruses may produce intranuclear inclusions in infected cells while another may not it is appropriate to recall the cellular changes in vaccinia and variola. Both of these closely related pock viruses are characterized by the presence of cytoplasmic inclusions and elementary bodies but intranuclear inclusions are found only in variola (16). Additional experiments designed to throw light on the identity of the P-3 agent have not yet elucidated the problem; these are being continued (17). If the P-3 virus should prove to be unrelated to psittacosis then one would like to know if it possesses characteristics in common with the agent which produces intranuclear inclusions in parrots (18, 19) or the virus that causes "blue comb" in chickens (20).

SUMMARY

An epizootic disease in pigeons associated with atypical pneumonia in two persons handling the birds has been studied. Most of the observations made during the work were consistent with the idea that we were dealing with an infection caused by a member of the psittacosis-lymphogranuloma venereum group of viruses. The outbreak was peculiar, however, in that tissues of the diseased pigeons contained many intranuclear inclusions and that the viruses isolated from these birds produced both intranuclear inclusions and elementary bodies in the cytoplasm of cells of chorio-allantoic membranes of the developing egg. Whether the pigeons were simultaneously infected with two viruses or whether the virus of pigeon psittacosis can produce intranuclear inclusions under certain conditions remains to be determined.

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EXPLANATION OF PLATES

These photographs were made by Mr. Joseph B. Haulenbeek.

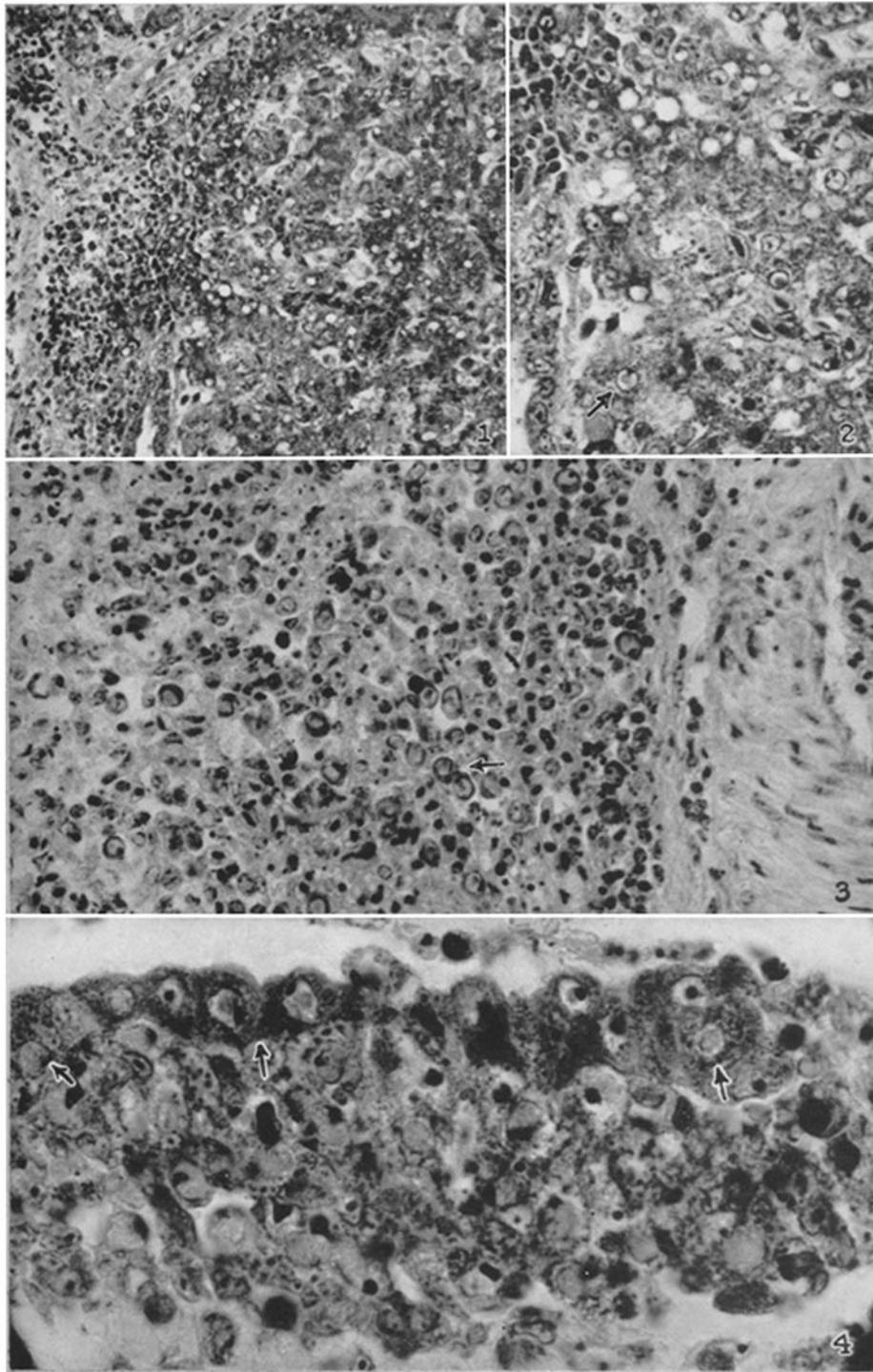
PLATE 5

FIG. 1. Pigeon 3. Photomicrograph of a section of liver showing an infiltration of mononuclear cells in and about a periportal space and some destruction of parenchymatous cells in the adjacent lobule. Giemsa stain. $\times 250$.

FIG. 2. Higher power of a portion of the field illustrated in Fig. 1 revealing the focal destruction of liver cells more clearly. Several acidophilic intranuclear inclusions of the herpetic type are present in parenchymatous cells (arrow). Deep staining granules are found in the cytoplasm of certain cells undergoing necrosis. $\times 500$.

FIG. 3. Pigeon 3. Section of spleen showing necrobiotic changes in many cells of the Malpighian body. Intranuclear inclusions (arrow) similar to those shown in Fig. 2 occur in most of the intact cells. Giemsa stain. $\times 500$.

FIG. 4. Chorio-allantoic membrane infected with the P-3 strain of virus. Many ectodermal cells (arrows) contain large intranuclear inclusion bodies; these are lighter in shade than the nucleoli which have been pushed toward the nuclear membrane. Giemsa stain. $\times 1000$.



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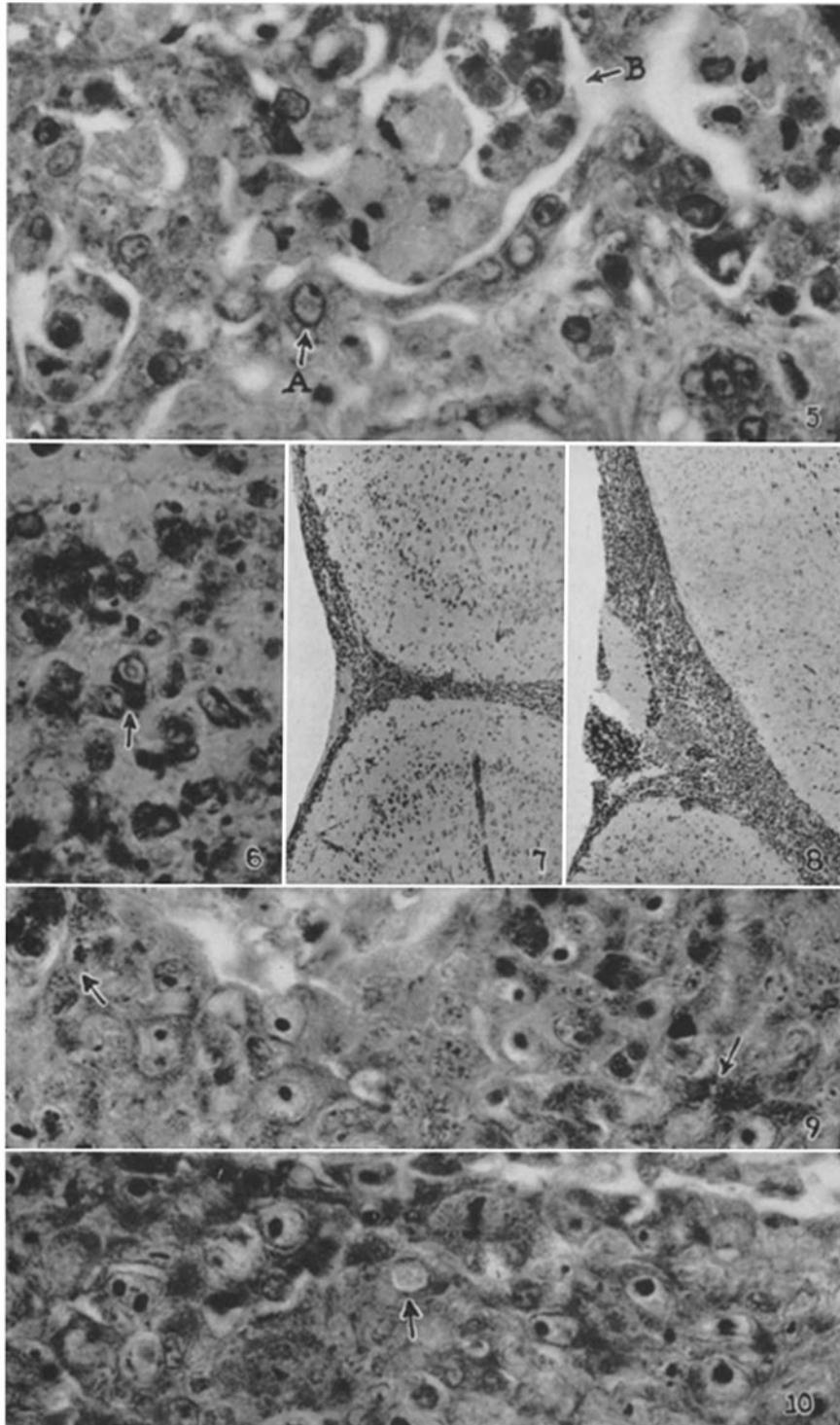
PLATE 6

FIG. 5. Pigeon 4. Section of pancreas showing necrosis of glandular cells. The acini which are dilated contain cellular debris and the interacinar tissue is edematous. Intranuclear inclusions are present in some cells (arrow A) while others have their cytoplasm stuffed with deep staining basophilic granules (arrow B). Giemsa stain. $\times 1000$.

FIG. 6. Chorio-allantoic membrane infected with the P-1 strain of virus. Amidst the cellular infiltration in the mesoderm is a cell with an intranuclear inclusion (arrow). Giemsa stain. $\times 1000$.

FIGS. 7 and 8. Mouse brains infected with the P-7 and 6BC psittacosis strains, respectively, showing a marked infiltration in the subarachnoid space in each reproduction. The inflammatory cells are principally lymphocytes. Giemsa stain. $\times 50$.

FIGS. 9 and 10. Chorio-allantoic membrane infected with the P-30 strain of virus. Two photomicrographs from different portions of the hyperplastic ectoderm contain cells with deep staining elementary body structures (arrows in Fig. 9) in the cytoplasm and cells with pale staining intranuclear inclusion bodies (arrow in Fig. 10). Giemsa stain. $\times 1000$.



(Smadel *et al.*: Psittacosis in pigeons and man)