The Etiology and Pathology of Rabbit Influenzal Pneumonia
THE ETIOLOGY AND PATHOLOGY OF RABBIT INFLUENZAL PNEUMONIA AND ITS CORRELATION WITH HUMAN INFLUENZAL PNEUMONIA.

BY

CLARENCE CHARLES SAELOHOF

B. S. University of Illinois

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THESIS

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OF THE

UNIVERSITY OF ILLINOIS

1920
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Clarene Charles Seethoff
ENTITLED The Etiology & Pathology of Rabbit Influenza Pneumonia & its Correlation with Human Influenza Pneumonia
BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

David J. Davis
In Charge of Thesis
Head of Department

Recommendation concurred in

Committee on Final Examination

*Required for doctor's degree but not for master's
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INTRODUCTION.

During the past two years, pneumonia has superceded tuberculosis as a cause of death. This unusual prevalence has led to many intensive studies, especially upon the etiological classification of pneumonia, the correlation of the clinical features and pathology of the disease and prevention by the use of various vaccines and sera. In this paper, I wish to report certain studies on pneumonia in rabbits and incidently to compare the lesions there found with similar lesions found in man.

LITERATURE.

Little data has been reported upon the pathology of pneumonia occurring in rabbits. Experimental work has been performed with special reference to the initial point of infection, pathogenesis and the route of infection, particularly by Müller (1) and Rasquin (2).

Many attempts have been made to produce experimental pneumonia in animals (3). Among the more recent workers are Lamar and Meltzer,(4) Wollstein and Meltzer,(5) Winternitz and Hirschfelder,(6) Sisson and Walker (7) and Blake and Cecil (8). All have been more or less successful in the production of experimental pneumonia in animals, chiefly by various methods of intratracheal or intrabronchial insufflation.
EPIDEMIOLOGY.

An epidemic of rabbit influenza occurred among the laboratory animals in the fall and winter of 1918 and the spring and summer of 1919. While contemporaneous with the epidemic of human influenza, it had occurred many times before in the laboratory, and there was no good reason to surmise a common etiology.

The duration of the disease was variable, most animals succumbing at the end of five or seven days. In those dying during the natural course of the disease, weakness and depression were most evident in three to six days. There was subsequent loss of appetite. The nares were moist with a thin, scant and watery discharge, accompanied by frequent sneezing. The discharge descends from the nares to the breast and anterior extremities, and becomes, as the disease progresses, mucoid and purulent in character. Later it is more tenacious and is expelled from the nostrils by sneezing or by rubbing the nose with the fore leg.

Animals used for various experimental purposes succumb earlier—within six to 48 hours—after an experimental injection of different materials, bacteria, extracts, etc., and showed typical acute symptoms, the disease seemingly being brought from the latent to the acute stage by this procedure. Evidently the resistance of the animals is easily lowered by injections of various kinds.

The transmission of the disease may occur in one or more of the following ways: inhalation, contact, contaminated food, contaminated dust and carriers.
The quarters in which the animals were kept were divided into compartments approximately one and one half feet square, with wire mesh partitions. Two animals were, as a rule, kept in each compartment. It seems logical to assume that the droplet or inhalation method was the most important means of transmission of the exciting bacterium from an infected animal to a healthy one. Next in importance would be transmission by contact; food, dust and carriers probably occupy a more subordinate role in the transmission.

**ETIOLOGY.**

The exciting cause is the Bacillus bipolaris, an organism classed in the hemorrhagic septicemia group. It causes a generalized infection and hemorrhagic inflammation of the internal organs. The bacillus is short, about 1-3 microns in length, staining intensely at the poles and only slightly at the middle, and at times is pleomorphic. It is non-motile, non spore forming, aerobic and facultative anaerobic. It stains with the ordinary aniline dyes and is negative to the Gram stain. In fluid media the organism is often coccoid while on solid media it tends to retain its characteristic form. It has appeared in cultures as a coccoid bacillus, a diplo-bacillus, a streptobacillus and at times in thread like forms similar to

* This organism has been reported under various names: e.g. Bacillus bronchisepticus, Bacillus bovis septicus, Bacillus of pleuropneumonia, Bacillus of rabbit septicemia, etc. In this...
Bacillus influenzae. After cultivation on artificial media for a few generations, it tends to lose its characteristic bi-polar staining. The action of the organism on various medias is recorded in Table 1.

The organisms were recovered and cultured from the following sources: nasal discharge, naso-pharynx, pleural fluid, pericardial fluid and heart's blood of rabbits. Koch's postulates were fulfilled; the organism being cultivated, reinoculated causing the disease and isolated from the experimentally infected animal.

**EXPERIMENTAL.**

Experimental production of the disease was performed as follows: a 24 hour agar slant of bacteria was emulsified in 5 cc. sterile normal saline and sprayed into the nose and naso-pharynx of healthy animals. All succumbed to the disease in the acute stage. To prevent the possibility of contamination at the institution, two animals were obtained from a source other than that from which they were usually obtained and kept at a distance of some miles from the laboratory, undergoing the same experimental procedure, readily contracted and succumbed to the disease eight days after inoculation.

... article I will use the name Bacillus bipolaris, because of its characteristic phenomena.
<table>
<thead>
<tr>
<th>Media</th>
<th>Growth</th>
<th>pH</th>
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<td>Acid, no gas</td>
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**Table 1.**

<table>
<thead>
<tr>
<th>Suspensions</th>
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<th>72 hours</th>
<th>48 hours</th>
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<tr>
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<td>Gel</td>
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<tr>
<td>Pseudomass</td>
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<tr>
<td>Mass</td>
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**References:**

PATHOLOGY.

A series of 17 animals was collected of which 12 succumbed to the disease in the natural manner and in five the disease was produced experimentally. Necropsies were performed within one to 14 hours after death. Sections for microscopical study were routinely taken from the lungs, trachea, heart, liver, kidney and spleen, fixed in formalin or Zenker's fluid, sectioned in parrafin and stained with the hematoxylin-eosin stain. The portions of the lungs from the apex, middle and lower lobes were in addition stained with the following: Van Gieson connective tissue stain, Wiegert's elastic fibre stain, Polychrome methylene blue, Pyronin methyl-green, Giemsa and Gram stains.

Macroscopically the lungs at autopsy presented the typical picture of confluent broncho-pneumonia. The lungs did not completely collapse on opening the chest and the pleural surfaces were frequently mottled with patchy areas of dark, bluish-red color; often in the acute stage the pleura was covered with a thin layer of fibrin. The pleural cavities usually contained some fluid. Small consolidated masses varying in size from a pin point to a pea, hard and firm, with crepitating lung tissue surrounding them can be felt, usually more prominent in the lower lobes and especially of the right lung. The distribution of the broncho-pneumonic areas is recorded in Table 2.

On cut section the lungs were moist and edem-
<table>
<thead>
<tr>
<th>Right Lung</th>
<th>Left Lung</th>
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</thead>
<tbody>
<tr>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>13</td>
<td>Middle Lobe</td>
</tr>
<tr>
<td>8</td>
<td>Upper Lobe</td>
</tr>
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</table>

Confluent Broncho-Pneumonia

<table>
<thead>
<tr>
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<th>Left Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Lower Lobe</td>
</tr>
<tr>
<td>2</td>
<td>Middle Lobe</td>
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<tr>
<td>2</td>
<td>Upper Lobe</td>
</tr>
</tbody>
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Patchy Broncho-Pneumonia

**DISTRIBUTION AND CHARACTER OF BRONCHO-PNEUMONIA**

**TABLE 2**
atous, with a red, frothy liquid, sometimes purulent in character, exuding from the cut ends of the bronchioles. On scraping, in some instances plugs of necrotic material was removed. The distribution of the consolidated areas was variable; in the more acute cases it was usually in small patches near the periphery of the lung; in the more chronic cases it often became confluent in character and was situated around a main or central bronchiole. The consolidated areas were usually surrounded by a zone of hyperemia. At times the centers of these consolidated areas appeared necrotic. Engorgement of all the pulmonary vessels was particularly evident.

Microscopically, the picture varied with the stage of the disease. In the acute stage, perivascular edema and leucocytic infiltration was the most prominent feature. In some instances, a clear edematous exudate containing few or no cells was prominent. In Rabbit 6, there was a proliferation of cells beneath the intimal coat of the blood vessels. As the stage of the disease became more advanced, it tended to resemble the stage of red hepatization. The alveolar epithelial cells were swollen, edematous and revealed degenerative changes. In some instances complete desquamation has taken place. The interstitial tissue was edematous and swollen, the interstitial vessels were hyperemic and distended, with perivascular infiltration, as a rule. The alveoli were packed with red cells and desquamated alveolar cells together with strands of fibrin interspersed. In the advanced stage, the in-
vasion of large numbers of white cells occurred, similar to the stage of gray hepatization. The fibrin increased in amount and later became organized. The outlines of the alveoli were indistinct and in some areas imperceptible, the process having become confluent with subsequent obliteration of the individual alveoli. Not infrequently the pneumonic process was complicated by a tendency to small abscess formation. In the small consolidated areas, central necrosis, quite intense in some instances, may be noted. Not infrequently the process involved over one-half of the consolidated area. In many instances the bronchioles contained an exudate, the constituents of which depended on the stage of the process. All stages of cellular degeneration could be observed. Phagocytosis was often seen; the phagocytized structures being red cells, polymorphonuclears and cellular debris. Definite focal hemorrhages were occasionally seen, often involving large areas.

In the very acute cases, less alteration was noticed, a slight transudation of serum and a few leukocytes plus slight hyperemia being the chief manifestations. In the more subacute and protracted cases, the picture varied from degenerative changes of the epithelial lining to complete desquamation and necrosis of the underlying tissue. The larger bronchi were involved in only about fifty percent of the cases, the involvement varying from slight to intense necrosis and sloughing. In most instances the peribronchial lymphatic spaces were distended and infiltrated with leukocytes.
The trachea contained a slimy, mucoid or frothy, slightly blood tinged fluid, especially near the bifurcation of the bronchi. On removal of this material intense hyperemia of the mucosa was evident. In some instances, on opening the trachea, the affected side revealed more of the frothy, blood tinged fluid and showed a sharp line of delimitation running down the middle, separating the diseased from the normal side. Microscopically in about fifty percent of the cases, evidences of an acute trachitis was present, with swelling, edema and degenerative changes in the epithelial lining varying in character from pyknosis to necrosis and sloughing; in the remaining no pathology except a slight hyperemia was evident.

The small bronchioles presented the same changes as the larger tubes. Desquamative bronchiolitis was most frequent, with complete or nearly total destruction of the mucosa. Strands of fibrin and polymorphonuclear cells were found interspersed in the various areas. Others appeared apparently normal.

The heart grossly was little altered. In most cases, a few cubic centimeters of clear, straw-colored fluid was contained within the pericardial sac. In no animal in this series was there evidence of a fibrinous pericarditis. Occasionally the heart was in the stage of acute dilatation with the right ventricle being especially enlarged. On opening the cavities they contained goose fat clots; the valves were normal in every case except one in which a
small vegetation occurred on the mitral valve and endocardium, from which a mixed culture of hemolytic streptococci and a Gram negative bacillus was obtained. The muscle was reddish brown and no evidence of infarcts or myocarditis was present. Microscopically the pericardium was unaltered. The heart muscle itself not infrequently showed a fragmentation and segmentation.

The kidney, grossly, appeared unaltered; congestion, however, being marked in most cases. On section, the capsule stripped readily, leaving a smooth, shining surface. The parenchyma appeared bloody and edematous; the glomeruli could be readily discerned. Microscopically, the glomeruli and tubules showed evidence of parenchymatous degeneration in the larger percentage of cases. In a few, evidences of foci of hemorrhages were present, with slight involvement of the interstitial tissue as a hypertrophy, showing a sub-acute nephritis.

Grossly the liver appeared enlarged, swollen and congested; on section the capsule retracted and the tissue bulged out, with a moist, bloody surface presenting. On scraping the fluid away, the lobules could be distinctly made out with dark brown centers and a pale gray periphery. Microscopically the capsule was normal, the central veins were engorged and congested as likewise the interlobular vessels; the liver parenchyma showed a parenchymatous degeneration and in some instances a slight fatty infiltration. No areas of focal necrosis were evident.

The spleen grossly was slightly engorged and
congested; the capsule appears shiny and tense, as though it were under increased pressure. On section, a moist, bloody surface presented, with the Malphigian bodies fairly discernable, in some cases, however, quite indistinct. Microscopically, prominent trabeculae were noticeable in two instances; parenchymatous degeneration and engorgement was demonstrable in all.

**COMPARATIVE PATHOLOGY.**

A description of the exciting bacterium and the gross and microscopical pathology of rabbit pneumonia having been described, I will now compare the characteristics of the organisms and the gross and microscopical features evident in the animal and human broncho-pneumonias.

Comparison of the Bacillus influenzae and the Bacillus bipolaris shows that the organisms should be classed in the same general group. They have many characteristics in common, including their size, shape, staining reactions and their reactions on various medias. They tend to die out after three or four transplants and are killed by exposure to extremes of cold, light, etc.. The Bacillus bipolaris tends to produce thread forms similar to the Bacillus influenzae. Both organisms may be grown with considerable ease on media containing animal fluids.

These points of difference, however, exist: the Bacillus bipolaris can be grown on ordinary plain agar without blood for a generation or so while the Bacillus influenzae must have hemoglobin in some form or other. Symbiosis does not
enhance the growth of the Bacillus bipolaris as it does the Bacillus influenzae.

While there seems to be no question as to the Bacillus bipolaris being the cause of rabbit influenzal pneumonia, there exists much doubt as to the true cause of human influenza. During the pandemic of 1889-90, the bacteriologic factors associated with the disease were far from being recognized. Pfeiffer's bacillus, the Bacillus influenzae, was not described until 1892. Perusal of the literature since that date indicates the uncertainty that has existed regarding the etiological significance of this organism. Recent studies continue to give indications of this uncertainty (9, 10, 11). Far from regarding the Bacillus influenzae as the primary infective agent, many investigators still believe that influenza is a disease (quoting Kinsella), caused by some as yet undiscovered agent which produces intense inflammation of the entire respiratory tract and effects a profound lowering of resistance. Under this circumstance, invasion follows by the various bacteria capable of becoming pathogenic, although harmless in the normal mouth. Clinical observations, however, of the disease in foreign quarters seems to agree with those of our recent pandemic. It is interesting to note the contrasting views. The bacteriologists of the Kitasato Institute (12) claim to have found the Bacillus influenzae in almost pure cultures in the nasal cavities of patients. Serologic and biologic studies led the Japanese investigators to the conclusion that this pandemic of influenza was
caused by the Pfeiffer's bacillus. Bloomfield and Harrop (13) came to the conclusion that proof is lacking that the Bacillus influenzae is the primary cause of influenza. A similar conclusion has been reached by Howard (14) that the Bacillus influenzae is merely a secondary invader, although in some cases it may be a frequent cause of terminal broncho-pneumonia. Pritchett and Stillman (15) have found the Bacillus influenzae in the mouths of 43 percent of normal individuals examined in the personnel of a large hospital. On the other hand, they isolated the organism from the mouths of 93 percent of cases of influenza and broncho-pneumonia. This high incidence of the Bacillus influenzae in the upper respiratory tract during the epidemic is, according to Pritchett and Stillman, a point in favor of the view that the microorganism may be of significance in the disease in question. Opie (16) in a group of twenty three patients from one to six days after the onset of the disease made cultures from each individual from the nose, from the throat and from the sputum on blood agar (5 percent of horse's blood in meat, infusion agar) and sputum injected into the peritoneal cavity of white mice. Their conclusions were that multiple cultures have demonstrated in almost pure culture the presence of the Bacillus influenzae in all these cases of early influenza. Passage of the sputum through white mice proved the most effective means of demonstrating the organism; cultures from the sputum or throat were nearly as effective. Lucke, Wight and Kime (17) recovered the organism by special media
(oleate) obtaining it in 61 percent of cases of influenza. These investigators seem to conclude that the Bacillus influenzae is the causative agent or is in some way etiologically related to the disease.

Nicolle and LeBailey (18), Riviere (19) and Gibson and Connor (20) published results which would seem to suggest the possibility of a filterable virus, but their findings are not identical and are contradicted by the experiments of Rosenau and Keegan (21) at the Deer Island Naval Station, the experiments of the Naval Medical Corps at San Francisco (22) and the results of Nuzum (23) at the Cook County Hospital. The experiments of Julia Parker (24) would seem to indicate that the Bacillus influenzae excretes a filterable exotoxin, and if her work is confirmed, it will constitute a very valuable addition to our knowledge. Symmott and Clark (25) state that "the Bacillus of Pfeiffer has been encountered in the majority of cases when looked for".

From the animals dying of the so-called "rabbit influenza" or "snuffles" there was isolated from the nasal cavities, pharynx and purulent material of the lungs a small, Gram negative, non motile bacillus with tendency to chain and thread forms which has some of the properties of the Bacillus influenzae, but, however, in certain respects is distinctly different. In the literature are reports of a number of varieties of bacilli which have been isolated from rabbits dying of lung involvement. Beck (26) mentions a small Gram negative, non motile bacillus, pathogenic for
rabbits, guinea pigs and mice, having a marked tendency to form threads, as the cause of Breustseuche in rabbits. Laven (27) described a bacillus pathogenic for rabbits and guinea pigs; small, Gram negative, variable in size and a tendency to grow in thread and chain forms. It is strictly hemolytic and gives a peculiar sperm-like odor on blood agar. Kurita (28) described a small Gram negative polar-staining bacillus which killed animals when injected by producing Breustseuche. Undoubtedly the organism isolated from my series of cases belongs to the same group as those previously described, being a Gram negative, polar staining bacillus, growing feebly on first isolation, but after artificial cultivation for three or four transfers reproduces with evident ease, and produces a confluent lobular pneumonia on intratracheal insufflation. It differs, however, from the Bacillus influenzae by preferring media containing animal fluids, is not strictly hemophilic as is the Bacillus influenzae and its growth is not enhanced by symbiosis, a very distinctive property of the Bacillus influenzae. There can be no doubt that the exciting agent of rabbit pneumonia is the Bacillus bipolaris; but the etiological relation of the Bacillus influenzae to human influenza is doubtful to say the least.

I will now discuss the comparative morbid anatomy of human and rabbit influenza and in so doing it will be necessary first to analyze in some detail the pathology of human influenza. The question whether influenza is always accompanied by a pneumonitis, or whether it
is only one of the complications of the disease has been much discussed. The wide spread influenzal bronchitis prepares areas of lessened resistance for primary invasion by the Bacillus influenzae or for the secondary microorganisms of the mouth and throat which, in suitable environment, readily assume pathogenic properties and produce a pneumonitis. Of the types of pneumonias described in the literature as a sequelae or terminal infection of influenza classed in their order of importance are: broncho-pneumonia, lobar pneumonia and lobar pneumonia with purulent bronchitis. There is a great diversity of opinion among observers in regard to the exact type of pneumonia occurring during influenza. Some speak of the lesion as a broncho-pneumonia which in severe cases develops a conglomerate character simulating lobar pneumonia. MacCallum (29) suggests that while these pneumonias are characteristic interstitial broncho-pneumonias in type, the rapid growth of virulent organisms was such that it spread rapidly producing an extensive homogenous consolidation, owing to the filling of the alveoli with leucocytic exudate loaded with organisms. Nuzum (23) speaks of it as a massive, confluent pseudo-lobar pneumonia. The usual form of influenzal pneumonitis has been described as lobular, the consolidations varying in size from very minute areas to an entire lobe. Such types have been described by Kelch and Antony (30), Wallis (31), Marchand (32), Ribbert (33) and others. All describe the foci of infection more or less differently. At times
the cut surface was found smooth, at other times granular. The periphery was rarely sharply demarcated. The lung was generally hyperemic, but at times it may be definitely anemic. Purulent bronchitis has been observed by Kranrhals (34), Kundrat (35), Kuskow (36) and others. Noncroupous lobar pneumonia or pseudolobar lobular pneumonia has been so frequently encountered that it seems to be almost characteristic of the disease. The consolidated tissue here has an even, smooth cut surface and presents under the microscope an exudate, poor in fibrin, and rich in catarrhal and polymorphonuclear cells.(32, 37, 38)

Microscopically, the exudate in the various forms have been found to consist of every conceivable composition. Pfeiffer (39) found fibrin to vary greatly in amount, at times even totally absent or present in only small amounts. The cells were generally polymorphonuclear with frequent infiltration of the alveolar walls. He believed that influenzal pneumonia manifested itself first as a peribronchial inflammation, which by coalescence formed larger lobar consolidations. Ribbert (33) found fibrin to vary greatly in amount and the cells were chiefly polymorphonuclear, desquamated epithelium and red cells. Klebs (40) found fibrin constantly present together with large numbers of red cells; polymorphonuclear leucocytes being later invaders. Ziegler (quoted by Leichtenstern) likewise emphasized the hemorrhagic character.
During the present epidemic, the pneumonitis was chiefly lobular. Symmers (41) reports the exudate hemorrhagic, purulent and catarrhal, with fibrin relatively absent. Blanton and Irons (42) report the exudate fibrinous only once. Oberndorfer (43) found marked hemorrhagic infiltration and, in later stages, catarrhal and suppurative exudations. MacCallum (44) found the exudate to vary with the type of organisms present. All the above indicates that the pathological picture varies according to the time, location and duration of the disease.

The typical human influenza broncho-pneumonia as described by MacCallum is as follows: There is no exceptional pleural exudate although the pleural surfaces may be covered with fibrin. The bronchi contain a thick, yellow pus and the bronchial glands are moderately enlarged. On section, the lung is found to be in a large part air-containing, but is studded throughout with palpable shot like nodules or with some what larger patches of firm consistency. The cut surface of these nodules are smooth and grayish yellow in color. They are seen to be peribronchial so that when they stand alone the center is occupied by the lumen of a bronchiole. Microscopically it is found that the bronchi are filled with an exudate of leucocytes, among which numerous influenza bacilli may be seen lying, some times in clumps and scattered freely, but most often enclosed in phagocytic cells. The wall of the bronchus is greatly thickened by an infiltration of mononuclear cells with a
few leucocytes and by the new formation of connective tissue cells. The alveolar walls for a considerable distance about this are similarly thickened, infiltrated and indurated. The alveoli contain an exudate which is usually rich in leucocytes, but which is often predominantly composed of desquamated epithelial cells and dense fibrin. In this exudate it is rarely possible to find the influenza bacillus. Organization is advancing rapidly, and in many instances it has completely replaced the fibrinous exudate with fibrous tissue over which epithelial cells have grown.

The lymphatic channels in the bronchial walls and the widened interlobular septums are inconspicuous and none are found distended with exudate. There is no intense infection of the pleura or great outpouring of exudate.

LeCount (45) gives in addition the microscopical picture of a condition of disseminated necrosis of the pulmonary capillaries in influenzal pneumonia. He reports the case of a soldier, aged 23, who became acutely ill after having had a slight cold for six days and died four days later. The lung showed chiefly edema, with few red cells and small amounts of fibrin peripherally disposed about the air sacs, a little exudate of leucocytes but no highly cellular consolidation. The pathology consists of widely disseminated, sharply defined focal necrosis of the minute blood vessels and little alteration of the lung except edema. This condition he gives as an explanation for the large amount of hemorrhage and edema so often found
in influenzal lungs.

A comparison of the gross descriptions of rabbit influenzal broncho-pneumonia and human influenzal broncho-pneumonia reveals quite similar alterations if approximately the same stages of the disease are taken. Microscopically, however, the rabbit broncho-pneumonia showed a marked perivascular edema and leucocytic infiltration exactly in contrast to that of the human type. Areas of central necrosis were much in evidence in the rabbit lung while little or no mention is made of them in the human disease. The exudative material is perhaps more cellular than in human broncho-pneumonia, although certain uncontrollable factors here enter into consideration. Fibrin was not especially marked, similar to the human influenzal broncho-pneumonia. Bacteria were not detected in the exudate, but miliary abscesses were not infrequently found. Phagocytic activity was quite pronounced. No focal necrosis of the pulmonary blood vessels was evident, as described by LeCount, occurring in human influenzal broncho-pneumonia.

Although the gross pathology of pneumonia was accurately described years ago, little definite knowledge concerning the pathogenesis of the disease has been developed. Two principal theories with respect to the initial mode of infection have existed. Some writers (46) have advocated the theory that pneumonia, particularly the lobar type, is hematogenous in origin, basing their belief on the fact that
the causative organisms have been isolated from the blood prior to the development of the disease, either the symptoms or physical signs. This point of view, however, has received no experimental support. Wadsworth (3), Hasquin (2) and Armstrong (47) and others attempted to produce pneumonia in animals by intravenous inoculation of the organism and consistently failed.

On the other hand, the more commonly accepted view that the mode of infection is by the way of the air passages, bronchiogenic in character, has received a certain amount of confirmation in the experimental production of pneumonia in animals by various methods of intratracheal or intrabronchial insufflation. The foremost workers reporting such results are Armstrong (47), Lamar and Meltzer (4), Wollstein and Meltzer (5), Winternitz and Hirschfelder (6) and Blake and Cecil (8). The last two experimenters working with monkeys have attempted to explain the initial point of invasion of the lung by the organism, the character and location of the primary lesion and the method by which the infection spreads throughout the lung parenchyma. These points are very obscure, if one may judge by the indefiniteness with which these subjects are presented in standard works of pathology.

A brief review of the literature reveals a striking paucity of attempts to answer these questions by experimental procedures. Muller (1) undertook a study of the pathogenesis of aspiration pneumonia experimentally
produced in rabbits by vagotomy. He showed that bacteria-bearing material aspirated from the mouth had penetrated as far as the terminal bronchioles at the end of six hours. At the end of eight hours evidence of bacterial infection was present. The walls of the alveoli, lying adjacent to the alveolar ducts in which the affected bronchioles terminated, were swollen and infiltrated with leucocytes; the epithelium was desquamated and beginning exudation was apparent in these alveoli, but the alveolar ducts, atria, and alveoli to which the affected bronchioles led were free from exudate. Bacteria were seen in the infiltrated alveolar walls and the adjacent alveoli, but not in those with which the affected terminal bronchioles communicated. Still later with wider extension of the process, the bacteria were found spreading in the lymph channels of the interstitial framework of the lung and in the alveolar walls; exudation and passage of bacteria into the alveolar spaces occurring only after bacterial invasion of the alveolar walls had occurred. From these observations he inferred that the bacteria gained entrance into the pulmonary tissue at the point where the cuboidal epithelium of the terminal bronchiole gave place to the flattened epithelium of the alveolar duct and atrium and that the invasion was facilitated by the mechanical injury caused by the aspirated foreign material. He established the fact that further spread of the infection was by way of the interstitial tissue of the lung framework and by way of the alveolar walls.
Kasquin (2) showed that the pneumonia produced in rabbits by the intratracheal injection of attenuated pneumococci, consolidation invariably occurred in the portions of the lung proximal to the hilum and usually in the posterior half, the distal portions being free from consolidations. Since the rabbits died with a pneumococcus septicemia in a comparatively short time, complete lobar consolidation was not found and he considered the process comparable with catarrhal rather than with lobar pneumonia.

Blake and Cecil (8) state that pneumonia was consistently produced in normal monkeys by intratracheal injections of pneumonococci and showed that the pneumonia produced ran a clinical course identical with that of man. They furthermore state that attempts to produce pneumonia by subcutaneous or intravenous inoculation have consistently failed. This was equally true of normal monkeys and of monkeys whose resistance to pneumococcic infection had been increased by preliminary inoculations by means of pneumococcus vaccine. It was also demonstrated that the organism may enter the bloodstream following intratracheal injections, in some instances before the symptoms or physical signs of pneumonia had developed. They state that it therefore seems safe to conclude that pneumonia is a bronchiogenic and not a hematogenous affair.

Blake and Cecil, however, obtained their experimental results by intratracheal inoculation of the animals through needle puncture. They apparently assumed that no
change was produced by this procedure; hence the pneumonia seems to have started despite the unharmed and normally adequate protective mucosa. Winternitz, Smith and Robinson (48) have pointed out, however, that in such inoculations, the needle, though sterile on entry, is unquestionably infected when it is withdrawn and consequently a possible path of infection to the lung may be found elsewhere than through the lumen of the trachea. They also have demonstrated that the submucosa of the trachea and bronchi furnishes a pathway of infection to the lung. It contains a rich plexus of lymphatics prominent everywhere and devoid of valves. There is a continuity throughout this lymphatic system so that bacteria which once find their way into it may easily spread.

In my series of rabbit broncho-pneumonias produced experimentally by intratracheal injections through a soft rubber catheter, the process produced was certainly not bronchiogenic in character, for the patchy, confluent consolidations were not situated near the large bronchi or the hilum of the lung; the larger bronchi in a large proportion of cases were uninvolved and no evidence of bacterial passage through the mucosa was discernable. These consolidations were situated indiscretely over the lung, both near the periphery and center, and numbering as high as 15 to 20 to a lobe. Microscopically the lymphatics, especially the perivascular, were dilated and infiltrated with leucocytes. Bacteria were not seen in them. From this it seems reasonable to assume that the rabbit influ-
enzal broncho-pneumonia is a hematogenous or lymphogenous affair in contradistinction to human influenzal broncho-pneumonia.

Blake and Cecil in order to determine the site of primary invasion of the lung tissue injected a monkey with 10 cubic centimeters of an 18 hour broth culture of the organism thrown down by centrifuge and resuspended in 1.5 cubic centimeters of the supernatant broth. Three hours after injection the monkey was killed and autopsy immediately performed. They conclude with reasonable positiveness that the initial invasion occurs somewhere near the hilum. Additional evidence was, in this case at least, the demonstration of direct penetration of the organism into the walls of the larger bronchi.

In my series of rabbits no direct evidence was obtainable as to the site of the primary invasion, due to the fact that they all succumbed through pulmonary consolidation and toxemia; the process had advanced beyond the stage of invasion. It seems reasonable to assume that the bacteria certainly must have passed through the mucosa somewhere near the hilum of the lung and entered the lymphatic system for two reasons: first, the catheter was inserted down to the bifurcation of the trachea and second, the peribronchial lymphatics were involved in most instances.

Histological sections studied by Blake and Cecil showed clearly that the primary lesions were of the interstitial framework of the lung with accompanying involvement
of the lymphatic system. The earliest alveolar lesions besides capillary engorgement were likewise interstitial in character, consisting in leucocytic infiltration of the alveolar walls before exudation into the alveoli occurred. This is similar in every detail to that observed in the rabbit broncho-pneumonia.

As to the spread of the organisms from the initial lesion, they state that it is by way of the peri-vascular, peribronchial and septal tissue and lymphatics. This is in harmony with the evidence derived from my studies of rabbit influenza; i.e. the process is spread mainly by the hematogenous or lymphogenous route from the initial point of invasion to the adjacent lobules of the lungs.

In no case have observations been recorded of a tendency to abscess formation in human influenzal broncho-pneumonia. In rabbit pneumonia consolidated areas were often seen undergoing an intense central necrosis and without delimitation of the process by capsule formation. But definite lung abscesses were never observed.

CONCLUSIONS.

A study of the etiology of rabbit broncho-pneumonia shows that the exciting organism is the Bacillus bipolaris and belongs in the hemorrhagic septicemia group. The organism is a small, Gram negative bacillus, pleomorphic in character especially on artificial cultivation and grows without difficulty on ordinary medias. It reproduces the
disease experimentally.

Under natural and experimental conditions a broncho-pneumonia appears which is usually equally distributed throughout all portions of the lung, both peripherally and centrally. Microscopically a broncho-pneumonia was observed, the pathological picture being dependent on the stage of the disease. Peribronchial and perivascular infiltration of the lymph spaces and regions of central necrosis were most constant.

A comparative study of the exciting cause of infectious rabbit pneumonia and human influenzal pneumonia shows that the two organisms have only certain characteristics in common. The Bacillus bipolaris is not hemophilic and does not reveal the phenomenon of symbiosis as does the Bacillus influenzae. For certain animals, especially rabbits, the former is far more pathogenic.

A comparative study of the pneumonias produced by these two organisms shows that there are certain points of similarity. Grossly and microscopically both are broncho-pneumonias, often confluent in type. They differ, however, in that in rabbit pneumonia the perivascular and peribronchial leucocytic infiltration of the lymph spaces and the regions of necrosis in the consolidated portions are quite constant and striking features.
DESCRIPTIVE PROTOCOLS.

Rabbit #3: Weight approximately 1270 grams, posted nine hours after death.

Anatomical diagnosis:

Broncho-pneumonia (confluent) of right lung.
Congestion of the left lung.
Acute bronchitis.
Hypertrophy of the spleen.

Hyperemia and petechial hemorrhages of all serous membranes.

Congestion of liver and kidney.
Acute lymphadenitis.

Grossly the right lung presented a few, fairly firm consolidations with the surrounding tissue emphysematous and extremely congested. On section, it was moist, soggy and highly edematous. A bloody, frothy fluid exuded from the cut capillaries. The consolidated areas were raised above the surrounding tissue and were surrounded by a zone of hyperemia. Microscopically the epithelial cells lining the alveoli were swollen and edematous, some cells being in the stage of degeneration with only nuclear material remaining as evidence of their existence. Leucocytic infiltration of the alveolar walls was strikingly evident in these areas of consolidation. A fibrinous exudate consisting chiefly of white cells, red cells and desquamated epithelium interspersed with debris was seen. Congestion and vascular
distension was present. Peribronchial and perivascular lymph spaces were extremely distended and surrounded by a zone of infiltrating leucocytes. A fairly typical picture of confluent broncho-pneumonia was evident.

The trachea was filled with a purulent fluid, slightly blood tinged and frothy. Congestion of the mucosa was extreme. On microscopic section, the mucosa was apparently intact; vascular distension being the only pathological alteration.

The heart grossly appeared normal, no evidences of a pericarditis or a myocarditis being present. On opening, the cavities contained goose fat clots; the valves and musculature were apparently normal. Microscopically, fragmentation and segmentation was found.

The liver grossly appeared congested, the capsule stripped readily leaving a smooth, shiny surface; on cut section, it cut with normal resistance and a bloody, frothy fluid exuded from the cut surface. On scraping away the fluid, the lobules could be distinctly seen, the central veins were congested and appeared as dark, brown spot surrounded by a lighter gray periphery. Microscopically, congestion and slight parenchymatous degeneration was evident. No areas of focal necrosis were seen.

The kidney grossly appeared slightly congested, with a few minute petechial hemorrhages scattered over its capsule. The capsule stripped readily leaving a smooth surface. On cut section, it appeared moist and bloody with
the glomeruli fairly distinct. Microscopically congestion and slight parenchymatous degeneration were evident.

The spleen grossly appeared normal; on cut section the parenchyma tended to bulge out from the capsule; a small amount of blood was present on the cut surface. Microscopically slight increase in the connective tissue was evident. Some congestion and slight degenerative changes were noticeable in the parenchymal tissue.

Bacteriological samples from the nasal discharge, pleural fluid, pericardial fluid, bronchial exudate and heart's blood were plated on blood agar and incubated at 37 degrees Centigrade for 24 hours.

Results:

Nasal discharge: Gram negative bacilli.  
Gram positive cocci.

Pleural fluid: Sterile.

Pericardial fluid: Sterile.

Bronchial exudate: Gram negative bacilli.

Heart's blood: Sterile.

Smears from the nasal discharge and bronchial exudate stained by the Gram method revealed in both instances a Gram negative bacillus.

Rabbit #7: Weight, 1200 grams; died acutely in four days. Posted 14 hours after death.

Anatomical diagnosis:

Broncho-pneumonia (confluent) both lower lobes.
Acute bronchitis.
Hydrothorax with slight fibrinous exudation.
Subserous hemorrhages.
Passive congestion of liver, spleen and kidney.

The lung grossly appeared soggy and edematous with slight fibrinous exudation over the areas of consolidation. Crepitus was absent except in the upper lobes. On cut section, a large amount of frothy, blood tinged fluid and mucus exuded from the ends of the cut bronchioles. The consolidated areas were elevated above the general surface of the lung and were surrounded by zones of hyperemia. Microscopically the bronchi were filled with a purulent exudate containing a small amount of fibrin, erythrocytes and leucocytes, the surrounding mucosa being edematous and in some instances necrotic. The peribronchial lymph spaces were distended and infiltrated with leucocytes. In the sub-mucosal tissue, collections of polymorphonuclear cells were evident, a seeming tendency to localized abscess formation. The alveolar wall was hemorrhagic and edematous, with the epithelial cells undergoing various stages of degeneration, dependent on the stage of the disease. The alveoli were filled with a large number of erythrocytes with a small amount of fibrin interspersed. The parenchymal tissue was greatly infiltrated with polymorphonuclear cells. Vascular distension of an extreme degree was present. Distension of the perivascular lymph spaces with a leucocytic infiltration was quite marked. The adventitial coat of the blood vessels
in some instances seemed to be somewhat increased in thickness. In the process localized to the lowermost part of the lobes, central necrosis was quite marked and advanced to a considerable degree in most instances. Tissue other than that involved appeared apparently normal.

The trachea showed a mucoid, slimy material covering the mucosa with an intense hyperemia of the submucosal vessels. Microscopically the only pathology evident was the extreme vascular distension.

The heart grossly was firm and contracted, with a smooth, normal musculature; no evidence of a pericarditis or myocarditis. On opening, the cavities contained goose fat clots; the valves and musculature were normal. Microscopically slight fragmentation of the fibres was noticed.

The liver grossly appeared congested, with a smooth, glistening surface; capsule stripped readily leaving a smooth surface. On cut section the surface was bloody and moist and small amounts of blood oozed from the cut lobules. The lobules were distinct, the central vein being congested and appearing as a dark, brown center, the rest of the lobule being a light gray in color. Microscopically congestion with slight parenchymatous degeneration was evident. No areas of focal necrosis were seen.

The kidney grossly appeared slightly congested, capsule stripped readily leaving a smooth, glistening surface; on cut section, the surface appeared to be covered
with a small amount of dark, fluid blood, the glomeruli were fairly distinct. Microscopically the glomeruli were slightly swollen, the tubules were filled in localized areas with red cells. Vascular distension was prominent.

The spleen grossly appeared congested, otherwise normal. On cut section, it seemed moist and bloody. Microscopically it was normal except for slight congestion. There were no areas of focal necrosis.

Bacteriological samples of the nasal discharge, pleural fluid, pericardial fluid, bronchial exudate and heart's blood were plated on blood agar and incubated for 24 hours at 37 degrees Centigrade.

Results:

Nasal discharge: Gram positive bacilli.  
Gram negative bacilli.  
Gram positive cocci (in chain form).

Pleural fluid: Gram positive cocci (in chain form).

Pericardial fluid: Sterile.

Bronchial exudate: Gram negative bacilli.  
Gram positive cocci (in chain form).

Heart's blood: Gram positive cocci (in chain form).

Smears from the nasal discharge stained with the Gram stain revealed Gram negative bacilli, Gram positive
bacilli and Gram positive cocci in chain form. Smears from the bronchial exudate treated in the same manner revealed Gram negative bacilli.

**Rabbit # 14:** Weight 980 grams, died from acute snuffles by experimental bronchial insufflation of 4 cubic centimeters of a saline emulsion. Posted 10 hours after death.

Anatomical diagnosis:

- Broncho-pneumonia (confluent) right lower lobe.
- Acute bronchitis.
- Fibrinous exudation over consolidated portion of the lung.
- Hydropericardium.
- Acute dilatation of the heart.
- Subserous hemorrhages.
- Passive congestion of liver, kidney and spleen.

The lung grossly sodden and edematous with little and very faint crepitus with numerous, shotty-hard nodules palpable in the lower lobe. On cut section, it was bloody and moist; a frothy, mucoid blood tinged fluid exuded from the cut ends of the bronchioles of pressure. The consolidated areas were firm and hard and were slightly raised above the general surface of the lung and were surrounded by zones of hyperemia. Microscopically the bronchi were slightly thickened with edema of the tissues and leucocytic infiltration. The mucosa was apparently intact; the
sub-mucosal blood vessels were extremely distended and the peribronchial lymph spaces were infiltrated and distended with leucocytes. The alveoli were filled with a few red cells, leucocytes and a small amount of fibrin and debris. Phagocytosis was very prominent in some instances. The consolidations showed areas of central necrosis, markedly advanced in some instances. Vascular distension was prominent with dilatation of the perivascular lymphatics and leucocytic infiltration. In a few instances, a marked proliferation of cells beneath the intimal coat, the endothelial lining of the blood vessels, was apparent. Uninvolved lung appeared apparently normal.

The trachea was covered with a mucoid, blood tinged secretion. On scraping away the secretion, the mucosa appeared normal, with intense injection of the sub-mucosal vessels. Microscopically, in some areas the mucosa was eroded away; the sub-mucosa was edematous and infiltrated with red and polymorphonuclear cells.

The heart grossly was in a stage of acute dilatation, with normal appearance. On opening, the cavities contained post-mortem clots; the valves were normal. Microscopically, slight segmentation was evident. No pericarditis or myocarditis was apparent.

The liver grossly appeared engorged and congested; the capsule stripped readily leaving a smooth, shiny surface; on cut section, the surface was moist and bloody. On scraping away the fluid, the lobules were seen with fair ease,
having dark, brown centers and a paler gray periphery. Microscopically, passive congestion and slight parenchymatous degeneration was evident. No areas of focal necrosis were present.

The kidney grossly appeared engorged, the capillaries stripped readily leaving a smooth, shiny surface. On cut section, the surface appeared normal with the glomeruli fairly distinct. Microscopically, the vessels were distended and slight parenchymatous degeneration was evident.

The spleen grossly showed engorgement. On cut section, the surface was slightly bloody, otherwise no evident change. Microscopically, no pathological alterations except for vascular distension. No areas of focal necrosis were seen.

Bacteriological study of specimens of the nasal discharge, pleural fluid, pericardial fluid, bronchial exudate and heart's blood were plated on blood agar and incubated for 24 hours at 37 degrees Centigrade.

Results:

Nasal discharge: Gram negative bacilli. Gram positive cocci (in grape form).

Pleural fluid: Gram negative bacilli.

Pericardial fluid: Sterile.

Bronchial exudate: Gram negative bacilli. Gram positive cocci (in grape form).
Heart's blood: Sterile.

Smears from the nasal discharge and bronchial exudate stained with the Gram stain revealed in both instances a Gram negative bacillus and a Gram positive coccus.
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