PATHOLOGIC FINDINGS IN THE LUNGS OF FIVE CASES FROM WHICH INFLUENZA VIRUS WAS ISOLATED *

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Reports in the literature of fatal cases from which influenza virus has been isolated are remarkably few, and still fewer are the descriptions of the pathologic changes in the lungs in such cases. We have been able to find only three such reports. The first case was described by Scadding [1] in 1937, the second by Stokes and Wolman [2] in 1940, and the third by Himmelweit [3] in 1943. The strain of influenza virus was not mentioned in Scadding's case. In Stokes and Wolman's case, it was influenza A (PR 8 strain). Himmelweit's case yielded an influenza virus closely related to, but not identical with, influenza B (Lee strain). All three cases were complicated by *Staphylococcus aureus* infection.

The pathologic change in Scadding's case [1] consisted of a necrotizing process involving the trachea, bronchi, bronchioles, and alveoli. The alveoli were filled with red blood cells, resembling an infarct. In limited areas there were small abscesses, 2 to 3 mm. in diameter.

In Stokes and Wolman's case [2] there was marked congestion of the alveolar capillaries. The alveoli were filled with edema fluid, red blood cells, and a few polymorphonuclear leukocytes. The septa were edematous and the lymphatics were filled with a serofibrinous exudate. Many lobules contained colonies of staphylococci with little or no leukocytic reaction about them. The bronchioles had lost their epithelium and their lumina contained mucus and leukocytic débris. The epithelium of the trachea and large bronchi had desquamated and had been replaced by a thick exudate of organizing fibrin and purulent material. The submucosa was thick, edematous, and congested, and was infiltrated with phagocytes, chiefly of the mononuclear variety.

In Himmelweit's case [3] the lungs showed bronchopneumonia, necrosis near the bronchioles, much hemorrhage, and many staphylococci. The epithelium of the trachea had been shed and there was a little fibrin on its surface with masses of cocci but only a few leukocytes. The bronchial epithelium had likewise desquamated.

In a fourth case reported by Wollenman and Finland [4] from the 1940–41 epidemic, the influenza virus was not isolated but evidence was given for its presence in the lung. A ferret inoculated intramuscu-

* Received for publication, August 4, 1945.

larly with a suspension of the lung of that case developed no signs of infection, but proved refractory to subsequent inoculation with influenza A (PR 8), and the ferret's serum taken prior to the second inoculation protected mice against infection with this strain of virus. This case, too, was complicated by infection with *Staphylococcus aureus* and the pathologic changes were very similar to those described by Stokes and Wolman.[2]

In addition to these four reports, Andrewes, Smith, and Stuart-Harris [5] recorded the isolation of influenza virus from the lungs of three cases of fulminating pneumonia which occurred during the 1936–37 epidemic in England and from which pure cultures of *Staphylococcus aureus* were obtained. One of these three cases is the same one that was reported by Scadding,[1] but the morbid anatomy of the lungs in the other two cases was not described.

We have had an opportunity to study the lungs from five cases from which an influenza virus was isolated. Two of the cases were unusual in that no significant pathogenic bacteria could be demonstrated, while of the other three, one was complicated by infection with *Staphylococcus aureus*, one by *Staphylococcus aureus* and *Streptococcus hemolyticus*, and one by Pneumococcus, type I.

The pertinent gross and microscopic findings, as well as the bacteriologic and viral studies, are given below.

Report of Cases

Case 1 *

A white woman, 26 years old, was admitted to the Faulkner Hospital on the afternoon of March 21, 1943. She had been in good health until the evening of March 18 when she had a rather abrupt onset of malaise, fever, restlessness, and insomnia. On the following day she had a slight sore throat with dysphagia and a harsh unproductive cough. On the afternoon of March 20 her temperature was 99.6° F. and her throat was very sore and slightly swollen but did not appear inflamed. At that time she began to have substernal distress with her cough. That night she became more restless. On the following morning the temperature was 100.6° F., pulse was 90, respirations were 28, and the patient was slightly cyanotic, somewhat stuporous, and complained of substernal oppression. That evening her temperature was 103.6° F.; pulse, 120; respirations, 42. The breath sounds in her lungs were diminished and a few râles were heard at both lung bases, but there were no signs of consolidation. She was sent to the hospital immediately.

On admission, there was slight injection of the pharynx, some hoarseness, and slight tenderness over the larynx. Examination of the lungs was negative except for a few scattered râles. The leukocyte count was 4,000 with a predominance of lymphocytes. Blood culture showed no growth. Urine was concentrated and showed a trace of albumin and a few white blood cells in the sediment.

The patient was given oxygen, sedation, and a total of 4 gm. of sufadiazine. She became increasingly cyanotic and her respirations more labored. She also com-

* Courtesy of Drs. A. A. Cushing and G. K. Mallory.

plained of more pain throughout the chest. The respirations sounded "moist" but there was very little cough or sputum. During the night, tracheal râles increased, and signs of consolidation and many râles were made out in the right lower chest. She died 12 hours after entry.

Post-Mortem Examination

Autopsy was performed 2 hours following death. The right pleural cavity contained approximately 100 cc., and the left approximately 300 cc. of serosanguineous fluid. The heart was negative.

The right lung weighed 740 gm. and the left, 1100 gm. The pleural surfaces of both lungs were smooth and glistening. The right lung anteriorly was salmon pink, soft and pillowy in consistence, and showed discernible alveolar detail associated with early emphysematous changes. There were three areas, 1 to 2 cm. in diameter, deep red and sharply demarcated from the surrounding lung tissue. Two of these were in the upper lobe and one in the middle lobe. These areas were subcrepitant and on section had a homogeneous red-purple color and exuded fluid blood on pressure. The posterior half of the right lung was boggy and subcrepitant to noncrepitant. The color was dark red to violaceous and was mottled by irregular areas of deeper purple-red. On section the cut surface was wet, oozed considerable blood, and presented irregular and rounded areas of deep purple, noncrepitant tissue from which dark, bloody fluid could be expressed. These areas bore no relationship to bronchi or bronchioles. The entire left lung had the same appearance externally and on section as the posterior half of the right lung. No pink, crepitant tissue was visible and both the upper and lower lobes had the consistence of liver tissue, although somewhat more friable and nodular. The trachea and major bronchi were filled with frothy fluid. The mucous membrane from the trachea to the tertiary bronchi was covered by a thin, yellow-gray, membranous exudate which could be peeled away only with difficulty to reveal an intensely congested, raw surface.

The spleen weighed 125 gm. and was not remarkable. The liver weighed 1700 gm. and appeared negative.

Microscopic Examination. In the lungs some alveoli were empty and appeared distended. Others contained edema fluid while still others contained red blood cells and varying numbers of polymorphonuclear leukocytes. In such alveoli there were masses of cocci. Others were filled with polymorphonuclear leukocytes and cocci. In addition, several small abscesses were present. Some of the alveolar capillaries were thrombosed. The bronchioles showed necrosis of their epithelium and of the underlying connective tissue which was infiltrated with numerous cells, some of which were polymorphonuclear

leukocytes, but many of which were so necrotic that their type could not be distinguished. The blood vessels of the bronchioles contained fibrin in their walls and some were thrombosed. The majority of the bronchioles were empty but a few contained red blood cells or polymorphonuclear leukocytes and cocci. There was a slight perivascular infiltration of some lymphocytes and plasma cells. Several medium-sized arteries contained thrombi. The pleura was edematous and the pleural lymphatics contained polymorphonuclear leukocytes and large mononuclear cells.

The epithelium of the trachea had been completely destroyed (Fig. 6). There was extensive necrosis involving the connective tissue of the wall of the trachea and of the leukocytes which had infiltrated it. Masses of cocci were present here. There were focal hemorrhages and deposits of fibrin. The majority of the blood vessels were partially or completely thrombosed. There was a marked infiltration of lymphocytes and plasma cells around the glands and between the smooth muscle fibers.

The heart was negative. In the spleen the histiocytes of the malpighian corpuscles were hyperplastic. The bone marrow showed maturation arrest of the granulocytic series, the majority of the cells being myelocytes with only an occasional adult polymorphonuclear leukocyte present.

Bacteriology. A hemolytic *Staphylococcus aureus* was obtained in pure culture from the heart's blood, lungs, right main bronchus, pleural cavities, and spleen.

Virus Studies. A filtrate of a 20 per cent suspension of the left upper lobe of the lung obtained at autopsy was used for virus studies. In the first attempt no lesions were obtained after ten intranasal passages in mice. Lungs of the third mouse passage, however, yielded virus when transmitted through eggs by allantoic inoculation. Allantoic fluid from subsequent egg passages produced fatal lesions in mice and agglutinated hen cells to a dilution of 1:256. This agglutination was inhibited by anti-PR 8 rabbit serum to a titer of 1:2048 and by anti-Lee serum to a titer of 1:8. Comparable inhibitions were obtained with PR 8 virus. Serum obtained from cardiac blood at autopsy gave no significant titer of antibodies for either PR 8 or Lee virus in complement fixation or agglutination inhibition tests.

Case 2

Case 2 has been reported elsewhere [6] because of the finding of acute nonbacterial myocarditis. The patient was a woman, 34 years of age, who had been in good health until the middle of December, 1942, when she had a mild attack of bronchitis or "flu." Cough and fatigue persisted thereafter but there were no positive

findings demonstrable by physical or roentgenological examination. On April 4, 1943, the patient noticed unusual fatigue and on the following morning she had generalized malaise and an increase in her cough which was unproductive. On April 6 she had chilly sensations, and on that evening her temperature was 103.4° F. but her lungs were still clear. On April 9 her condition became much worse; the temperature was 102.6° F.; pulse was 124, feeble and thready, and the blood pressure was 125/78 mm. Hg. There were suggestive signs of consolidation of the right lower lobe and a few scattered râles in both lung bases. Heart sounds were faint and distant. The white blood count was 20,000, of which 70 per cent were polymorphonuclear leukocytes. The urine showed 2 plus albumin and a few white blood cells. Sputum could not be obtained but a throat culture yielded a scant growth of *Neisseria catarrhalis* and a few colonies of *Staphylococcus aureus*.

The patient was given 4 gm. of sulfadiazine and 1 gm. every 4 hours thereafter. She received a total of 8 or 9 gm. The signs and symptoms thereafter were those of increasing cardiac embarrassment and she was admitted to the Peter Bent Brigham Hospital late in the afternoon of April 10. A bedside roentgenogram of her chest at that time showed irregular mottling extending out from both hilar regions and a small amount of fluid in the axillary region. An electrocardiogram showed complete heart block and bizarre ventricular complexes. In spite of oxygen therapy and digitalis, the patient continued a downhill course and died 7 hours after admission.

Post-Mortem Examination

Autopsy was performed 8 hours after death. The right pleural cavity contained 950 cc. of a pale yellow fluid with flecks of fibrin, and the left contained 600 cc. of a similar fluid. There were several old fibrous adhesions binding the anterior and lateral surfaces of the right lung to the chest wall. Elsewhere the pleural surfaces of both lungs were smooth and glistening. The heart weighed 480 gm. The myocardium was light pink. Both the right and left ventricles were increased in thickness, the right measuring 0.8 and the left 2.0 cm. The valves were negative.

The lungs were somewhat heavier than normal. They were fairly crepitant throughout save at the bases where diminution in crepitation was more marked. Bloody fluid could be expressed from the cut surfaces. No areas of consolidation were present. The bronchial mucosa was slightly reddened but was glistening and free from exudate. The trachea appeared normal. There was no secretion or exudate present.

The spleen weighed 120 gm. Its architecture was well preserved and only a small amount of pulp could be scraped away. The liver weighed 1700 gm. and was negative. Bone marrow expressed from a rib was copious and deep red.

Microscopic Examination. The lungs showed a considerable degree of congestion. The alveoli contained a moderate number of large mononuclear cells, many of which had phagocytized carbon. Sections from the right lower lobe showed a perivascular infiltration of a few lymphocytes, plasma cells, eosinophils, and an occasional mast cell

(Fig. 2). One focus of acute inflammation was found in a section from this lobe (Fig. 1). Here several alveoli contained fibrin, large mononuclear cells, lymphocytes, and polymorphonuclear leukocytes. The walls of these alveoli contained cells of the same type. There was also swelling of the alveolar lining cells. A section from the left upper lobe likewise showed an acute inflammatory lesion in which the alveoli contained polymorphonuclear leukocytes, fibrin, and some large mononuclear cells. Stains for bacteria failed to reveal any microorganisms in these acute lesions or elsewhere. The epithelium of the bronchioles was unaffected. Their walls were infiltrated with a few lymphocytes, plasma cells, and a rare polymorphonuclear leukocyte. The heart showed extensive necrosis of muscle fibers with interstitial infiltration of lymphocytes, plasma cells, some large mononuclear cells, and an occasional eosinophil and mast cell. The spleen showed hyperplasia of the histiocytes in the malpighian corpuscles. In some areas of the liver there was central necrosis. The hepatic cells throughout showed fatty as well as hydropic degeneration. The bone marrow was not remarkable.

Bacteriology. Cultures of the heart's blood, lungs, and serous cavities yielded no growth.

Virus Studies. A Berkefeld V filtrate of a 20 per cent suspension of part of the right lower lobe was used for virus studies. The first attempt in mice was abandoned after six intranasal passages failed to produce pulmonary lesions. In the second attempt a different portion of the same lung was used, lesions appeared in the second passage, and death occurred on the fourth and subsequent passages. Survivors of the third passage were later given a challenge dose of 100 lethal doses of PR 8 and survived. In eggs, the virus became well established by the sixth passage. The allantoic fluid of later passages agglutinated hen cells up to a dilution of 1:2048. This agglutination was inhibited by anti-PR 8 rabbit serum to a titer of 1:1024, and by anti-Lee rabbit serum to a titer of 1:32. The patient's own serum obtained before death failed to fix complement or inhibit hen cell agglutination with PR 8 virus.

Case 3 *

The patient was a white, single office worker, 18 years old, who was admitted to the Evans Memorial Hospital at 4:00 p.m. on December 13, 1943. Her illness had begun abruptly at 3:00 p.m. on December 11, when she noted a sore throat and dysphagia. On the following day a physician found her temperature to be 101° F. and told her she had a bad sore throat and prescribed cough medicine. She had anorexia and vomiting that day and felt somewhat drowsy. At 1:00 a.m. on the morning of admission she developed severe pains across her lower chest and a slight cough productive of a small amount of sputum. She was given four sulfonamide

* Courtesy of Drs. Chester S. Keefer and John J. Curry.

tablets but soon became delirious and her physician sent her into the hospital after making a diagnosis of pneumonia.

On admission she appeared critically ill, toxic, cyanotic, delirious, dyspneic, and semicomatose. She was unresponsive, and incontinent of urine and feces. Temperature was 106.6° F. (rectal); pulse, 175; respirations, 53; blood pressure, 60/40 mm. Hg. She was well developed but poorly nourished. Her skin was hot and dry and she was markedly dehydrated. The mouth and tongue were very dry with thick, yellow, ropey material at the base of her tongue. Her pharynx was deeply injected. There was slight dullness at the base of the right lung posteriorly where the breath sounds were suppressed. A few fine, crepitant râles were heard there and in the right posterior axillary region. The lungs were otherwise clear. The heart sounds were rapid, faint, and distant. The hemoglobin was 10 gm.; red blood count, 4.86 million; white blood count, 1,350, with 24 per cent polymorphonuclear leukocytes, 72 per cent lymphocytes, and 4 per cent monocytes. A throat smear showed gram-positive diplococci and culture of the sputum yielded beta hemolytic streptococci and hemolytic *Staphylococcus aureus*. The latter also was grown from the blood culture. A portable roentgenogram of the chest showed large patches of dense infiltration in all but the upper part of the right lung field and a few similar patches were noted in the left mid-lung field.

Oxygen therapy was started at the time of entry and the patient was given an intravenous infusion of 1200 cc. of 5 per cent glucose in saline solution to which 5 gm. of sodium sulfadiazine had been added. The cyanosis improved somewhat and the blood pressure rose temporarily to 90/60 mm. Hg. The patient remained stuporous and combative. The râles in her chest increased and loud tracheal rhonchi were heard. The blood pressure soon dropped again and the pulse became thready and imperceptible. She became extremely restless and had a slight convulsive seizure with muscular twitchings of her face. Bloody foam exuded from her mouth and she expired about 3 hours after admission.

Post-Mortem Examination

Autopsy was performed 3 hours following death. The mucosa of the oropharynx and larynx was markedly reddened. Each pleural cavity contained approximately 500 cc. of slightly turbid, yellow, watery fluid. The pleural surfaces were smooth, dull, and bright red-gray. The heart weighed 225 gm. and was negative.

The right lung weighed 900 gm. and the left, 730 gm. The lungs were soft, boggy, and subcrepitant with only a few slightly firmer areas suggesting consolidation. On section, the alveolar architecture was obscured by a dark crimson background from which a large amount of frothy, serosanguineous fluid could be expressed. No definite consolidation could be found. The trachea and bronchi, which were filled with frothy, serosanguineous fluid, were lined with a dull, dark crimson mucosa which appeared to be extensively and superficially eroded. The hilar lymph nodes were enlarged and soft.

The spleen weighed 140 gm. The malpighian corpuscles were well defined against a dark crimson background. A large amount of soft pulp could be scraped away. The liver weighed 1280 gm. and was not remarkable.

Microscopic Examination. Sections from the lungs showed a variety of changes. In some there was intense congestion and the alveoli contained edema fluid, red blood cells, a few large mononuclear cells, and a rare polymorphonuclear leukocyte. In addition, there was a hyaline membrane, often appearing somewhat fragmented, lining some of the alveolar ducts and alveoli. The bronchioles in these areas contained edema fluid, numerous red blood cells, and large mononuclear cells. In addition, there was abscess formation. At the periphery of the abscesses, the intra-alveolar hemorrhages were more extensive and there was also a deposit of fibrin. The blood vessels here contained thrombi. Some bronchioles showed necrosis of their epithelium and contained in their lumina polymorphonuclear leukocytes, large mononuclear cells, red blood cells, and cocci. The walls of the bronchioles were infiltrated with large mononuclear cells and polymorphonuclear leukocytes. Gram-positive cocci, growing both in clusters and in long chains, occurred in all sections but were especially numerous in the abscesses and in the bronchioles that showed necrosis of their mucosa. In some sections the pleura was covered with a thin layer of fibrin.

The heart showed a few scattered, large mononuclear cells and lymphocytes in the interstitial tissue of the myocardium. In the adrenal cortex there were several foci of necrosis in which the necrotic cells had been invaded by polymorphonuclear leukocytes. The bone marrow showed numerous myelocytes but only rare adult granulocytes.

Bacteriology. Hemolytic *Staphylococcus aureus* and *Streptococcus hemolyticus* were cultured from the pericardial cavity, the upper and lower lobes of the right lung, the lower lobe of the left lung, the right and left bronchi and both pleural cavities.

Virus Studies. A 20 per cent suspension of a portion of the hemorrhagic lung was passed through a Berkefeld V filter and the filtrate used for inoculation of mice and of chick embryos. No transmissible lesions were found in the mice after six intranasal passages, and further attempts were abandoned. The first intra-allantoic inoculation, however, yielded virus recognizable by moderate agglutination of the erythrocytes of the embryo as the allantoic fluid was withdrawn. Fifth passage allantoic fluid, diluted 1:10, regularly produced deaths with typical lesions in mice, and these were completely prevented by simultaneous inoculation of anti-influenza A (PR 8) ferret serum but were unaffected by similar amounts of anti-influenza B (Lee) ferret serum. This virus behaved in an atypical manner in agglutination-inhibition tests. With anti-PR 8 rabbit serum inhibition occurred in a titer of 1:64, and with anti-Lee serum in a titer of 1:32. Further studies of this virus are in progress.

Case 4

Case 4 was also included in a previous report[6] because of small myocardial lesions found at autopsy. The patient was an Italian iron worker, 39 years of age, who was admitted to the Boston City Hospital on January 31, 1944, complaining of dyspnea and hemoptysis of 2 days' duration. He denied having had cardiorespiratory symptoms until January 23 when he had a slight "head cold" which was followed by malaise, anorexia, and prostration. On January 26, he had severe shaking chills followed by fever. Three days later he began to have marked dyspnea, cyanosis, and cough, and raised grossly bloody sputum. He also had two attacks of substernal pain.

When he arrived at the hospital the patient was markedly cyanotic and dyspneic and had audible tracheal râles. He was coughing and expectorating dark red blood. The temperature was 98.4° F., pulse was 100 and regular, and respirations were 28 and labored and had a prolonged expiratory phase. The blood pressure was 138/88 mm. Hg. In both lungs there were numerous medium and coarse, moist râles but no definite signs of consolidation. The leukocyte count was 19,000 of which 88 per cent were polymorphonuclear leukocytes, and the hemoglobin was 96 per cent. The nonprotein nitrogen of the blood was 135 mg. per 100 cc. A smear of sputum showed gram-positive cocci and bacilli. An electrocardiogram showed left axis deviation but no other significant abnormality. A bedside roentgenogram of the chest showed diffuse clouding of both lung fields with irregular, fluffy areas of density. The patient failed to respond to therapy with oxygen and other supportive measures and died 14 hours after entry.

Post-Mortem Examination

Autopsy was performed 14 hours after death. The surfaces of the pleural cavities were smooth and glistening. The right cavity contained 600 cc. of a reddish, straw-colored fluid; the left, 500 cc. of a similar fluid. The heart weighed 330 gm. The coronary arteries showed atheromatous changes but were not occluded. There was a fibrous scar about 2 cm. in diameter in the interventricular septum.

The right lung weighed 1250 gm. and the left, 1100 gm. Their surfaces were glistening and transparent. Both lungs were subcrepitant throughout. On section, no discrete areas of consolidation were present but all lobes felt much firmer than normal. The trachea and bronchi contained a slightly mucoid, serosanguineous fluid.

The spleen weighed 105 gm. The cut surface was purplish red and the malpighian corpuscles were poorly delineated. The liver weighed 1690 gm. and cut with some increased resistance. The kidneys each weighed 205 gm. and were not remarkable save for a moderate degree of congestion.

Microscopic Examination. The heart showed large areas of scarring of the myocardium. In addition, there was necrosis of scattered muscle fibers and an interstitial infiltration of large mononuclear cells, lymphocytes, plasma cells, eosinophils, and polymorphonuclear leukocytes.

All lobes of the lungs showed essentially the same histologic picture. Some alveoli contained large mononuclear cells, many of which had

phagocytized carbon. In other alveoli there was edema fluid, a few red blood cells, delicate strands of fibrin, and varying numbers of polymorphonuclear leukocytes (Fig. 4). Numerous alveolar ducts and alveoli were lined with a dense, acidophilic, hyaline membrane in which were often embedded particles of carbon, polymorphonuclear leukocytes, and large mononuclear cells (Fig. 3). Some of the ducts and alveoli lined with this membrane were empty; others contained edema fluid and strands of fibrin. There were a moderate number of alveoli in foci which contained polymorphonuclear leukocytes, large mononuclear cells, and fibrin. The alveolar capillaries contained an increased number of polymorphonuclear leukocytes, especially those adjacent to the hyaline membrane. Thrombosis of the capillaries was seen occasionally. The epithelium of the bronchioles was intact. Some of the bronchioles were empty; others contained mucus, polymorphonuclear leukocytes, red blood cells, and fibrin. Their walls were infiltrated with fairly numerous polymorphonuclear leukocytes, lymphocytes, a few plasma cells, and a rare mast cell. The septa were markedly edematous and their lymphatics were dilated. Some of the septa were infiltrated with large mononuclear cells, lymphocytes, plasma cells, and polymorphonuclear leukocytes. Stains for bacteria failed to reveal microorganisms in any section.

The spleen showed in the larger veins a subendothelial infiltration of a few lymphocytes and plasma cells. The liver cells in the centers of the lobules had been replaced by large mononuclear cells, lymphocytes, and a few eosinophils. In the kidneys, some tubules contained necrotic epithelial cells in their lumina and such tubules were lined with flattened epithelium in which an occasional mitotic figure was present. There was an interstitial infiltration of lymphocytes, plasma cells, and eosinophils. The bone marrow was essentially normal.

Bacteriology. Cultures of the various lobes of the lungs showed no growth. An unidentified gram-negative diplobacillus was cultured from the heart's blood and a gram-negative diplococcus from the spleen. Both of these organisms were considered to be contaminants.

Virus Studies. A sterile filtrate of a 20 per cent suspension of some hemorrhagic lung tissue was used for virus studies. Typical lesions appeared in mice on the sixth intranasal passage and deaths occurred regularly beginning with the seventh. Neutralization tests with the mouse lung suspensions and immune ferret serums showed definite protection by anti-PR 8 serum and none by anti-Lee serum. Virus was recognized in the allantoic fluid of the first eggs inoculated with the filtered lung suspension. The fluid from the ninth passage agglutinated hen cells up to a dilution of 1:512 and this agglutination was inhibited

by anti-PR 8 ferret serum to a titer of 1:1024 and by anti-Lee serum to a titer of 1:64. Serum obtained from the patient before death had no significant antibodies for the PR 8 or Lee viruses.

Case 5

A white painter, 61 years old, was admitted to the Boston City Hospital on March 6, 1944, too ill to answer questions. From his wife and sister it was learned that he had always been in good health except for childhood diseases and an attack of rheumatic fever in 1902, from which he recovered without recurrences or sequellae. For 2 months preceding the present illness he had seemed unusually tired but had improved and felt perfectly well during the week prior to entry. On March 3, at 3:00 p.m., while at work he had a shaking chill and went home feeling feverish and weak. He went to bed and on the following day had chilly sensations, pleuritic pain in the right lower chest, and cough productive of large amounts of dark, rusty sputum. A physician found his temperature on that afternoon to be 103° F. and diagnosed "grippe." His cough and chest pain increased in severity and on the morning of admission he was having drenching sweats.

On admission he appeared severely ill, markedly dyspneic, cyanotic, and dehydrated. His temperature was 103° F.; pulse, 136; respirations, 42; blood pressure, 158/78 mm. Hg. He was coughing and raising rusty sputum and obviously having pain with respiration. The throat was injected and covered with a mucoid exudate. Respiratory movements were limited, particularly on the right. There was dullness to flatness over the right lower lung posteriorly and crepitant râles were heard over this area and in the right axilla, but the rest of the lungs seemed clear. The heart sounds were rapid but regular. Hemoglobin was 95 per cent; white blood count, 2,000, with 60 per cent polymorphonuclear leukocytes, many of them band forms. Type I pneumococci were identified in his sputum by the Neufeld method and the same organism was obtained from the blood cultures. The urine was concentrated and showed 4 plus albumin, occasional white blood cells, and numerous granular casts. The nonprotein nitrogen of the blood was 45 mg. per 100 cc. and the Hinton test on the blood was negative.

Oxygen therapy was begun on admission and an intravenous infusion of 15,000 cc. of saline solution containing 5 gm. of sodium sulfapyrazine was given. Antipneumococcus serum was also given intravenously in amounts of 1, 5, and 14 cc. at approximately 2-hour intervals, a total of 200,000 units being given between 5 and 11 p.m. There were no immediate untoward effects and there was a slight decline in the temperature and pulse rate during this treatment. About 1 hour after the last dose, however, the patient's condition became very poor, the blood pressure dropped rapidly despite coramine and caffeine, and he died about 40 minutes later.

Post-Mortem Examination

Autopsy was performed 9 hours following death. The right pleural cavity contained approximately 500 cc. of cloudy, yellow fluid. There were friable, fibrinous adhesions to the whole lower lobe and to the diaphragm. The left pleural cavity contained no excess fluid and the pleural surfaces were smooth and glistening.

The heart weighed 400 gm. and showed changes consistent with arteriosclerotic heart disease.

The right lung weighed 1910 gm. The upper and middle lobes were deep red while the lower lobe was yellow. The consistence of the lower

lobe was firm and noncrepitant. On section, a yellow-pink material gushed forth. The cut surface was roughened and showed no discrete, firm, or raised areas. The bronchioles exuded a yellow, purulent material on pressure. The upper and middle lobes were crepitant. On section, pink, watery fluid exuded. The cut surface was uniformly soft and wet. The bronchioles contained pink, watery fluid. The left lung weighed 820 gm. It resembled the upper and middle lobes of the right lung, both externally and on section. The trachea and major bronchi contained a watery, pink, frothy fluid. The mucosa of the trachea and bronchi was injected but was intact. The spleen weighed 265 gm. and was soft. The liver was slightly enlarged, weighing 2100 gm.

Microscopic Examination. In the heart there were some focal collections of large mononuclear cells in the interstitial tissue of the myocardium.

In the lungs, sections of the right upper lobe showed the majority of the alveoli to contain edema fluid and a varying number of carbon-filled, large mononuclear cells. Some alveoli were considerably distended. The epithelium of the bronchioles was intact (Fig. 5). Some bronchioles were empty; others contained edema fluid. In sections of the right lower lobe the alveoli contained some large mononuclear cells and numerous polymorphonuclear leukocytes, many of which were necrotic. Many alveoli also contained fibrin adjacent to their walls, while there was little or none in the central portions of their lumina. In places, the alveolar capillaries were congested; in others they were thrombosed. The pleura was covered with a layer of fibrin beneath which were fairly numerous polymorphonuclear leukocytes. The left upper lobe was essentially negative save for some distended alveoli. The left lower lobe was similar to the right upper lobe. In addition, several alveoli contained a few polymorphonuclear leukocytes, large mononuclear cells, and a small amount of fibrin. The epithelium of the bronchioles was intact. Their walls were infiltrated with numerous lymphocytes and plasma cells. Their lumina contained edema fluid and a few polymorphonuclear leukocytes. Numerous gram-positive diplococci were present in the alveoli of the upper and lower lobe of the right lung and in the lower lobe of the left. In the upper lobe of the left lung similar microorganisms were seen only in the alveolar capillaries.

The bone marrow revealed numerous myelocytes but only rare adult leukocytes.

Bacteriology. Pneumococcus, type I, was grown from the heart's blood and from all lobes of the right lung, and the lower left lobe.

Virus Studies. A portion of the right upper lobe was taken under

sterile precautions and preserved at —70° C. for virus studies. A 20 per cent suspension of sterile filtrate was used for intranasal inoculation of mice and intra-allantoic inoculation of chick embryos. Lesions appeared in the lungs of mice on the second passage and deaths occurred in the third and subsequent passages. There was slight agglutination of the embryonic erythrocytes in the allantoic fluid of the first egg passage and strong agglutination in subsequent passages. In a preliminary neutralization test the virus produced fatal lesions regularly in mice and these were prevented by the use of anti-influenza A ferret serum diluted 1:50 but not by similar amounts of anti-influenza B serum. Serum obtained from the patient on admission was used in agglutination-inhibition tests and gave a titer of 1:64 with influenza A (PR 8), 1:256 with influenza B (Lee), and 1:256 with the patient's own virus.

Summary of Pathologic Findings

It will be noted from the above data that two of the cases (2 and 4) were uncomplicated by bacterial infections. In case 2, death resulted from cardiac failure associated with an extensive acute myocarditis. The changes in the lungs were surprisingly slight and consisted of acute lesions involving a few alveoli. The bronchioles were unaffected. In case 4, death was due to the pulmonary involvement. The lungs in this case showed edema, some alveolar hemorrhages, fibrin, and extensive formation of a hyaline membrane. The epithelium of the bronchioles was intact but their walls were infiltrated with cells of various types. Their lumina were empty or contained mucus, leukocytes, red blood cells, and fibrin. There was marked edema of the septa.

Case 1 was complicated by a fulminating *Staphylococcus aureus* infection, causing an extensive necrotizing process involving the trachea, bronchi, and bronchioles. The alveoli showed edema, hemorrhages, an exudate of polymorphonuclear leukocytes, and abscess formation.

In case 3 an attack of influenza was complicated by a secondary infection with a beta hemolytic streptococcus and a hemolytic *Staphylococcus aureus*. The lungs showed alveolar hemorrhages, edema, and hyaline membrane formation in some sections. In others there was abscess formation. The epithelium of the bronchioles was intact in the former areas and necrotic in the latter. There was in addition an acute fibrinous pleuritis.

Case 5 was complicated by a pneumococcal pneumonia of the right lower lobe and a pneumococcal bacteremia. The right lower lobe showed a resolving lobar pneumonia. The right upper and left lower lobes showed edema and, in addition, in the left lower lobe several alveoli contained polymorphonuclear leukocytes, large mononuclear

cells, and fibrin. The bronchiolar epithelium in all lobes was intact. Bronchiolar walls were infiltrated with lymphocytes and plasma cells. There was an acute fibrinous pleuritis of the right lower lobe.

Correlation of Pathologic Changes and Virus Studies

In each case, material from only one lobe was utilized for the isolation of the virus. In case 1, unfortunately no note was made as to which lobes the microscopic sections represented. In case 2, the virus was isolated from the right upper lobe and histologically the only lesions present were focal lesions involving a few alveoli and consisting of an exudate of polymorphonuclear leukocytes, fibrin, and some large mononuclear cells. In case 3, no record was kept as to which lobe was studied for the presence of a virus. In case 4, likewise, no such record was kept, but the process was uniform throughout all lobes and it would seem justifiable to assume that the changes described, namely, edema, alveolar hemorrhages, fibrin, and hyaline membrane formation, represent the reaction to the virus. It should be noted that the bronchiolar epithelium was intact in this case as it was in case 2. In case 5, virus was isolated from the right upper lobe and sections from this lobe showed edema of the alveoli with an exudate of a moderate number of large mononuclear cells. As in cases 2 and 4, the bronchiolar epithelium was unaffected.

The fact that a virus was found in a single lobe in each instance is, of course, no indication that it was not present in some, if not all, of the other lobes. However, due to practical difficulties it was impossible to utilize more than one lobe from each case for virus studies.

Comment

As was indicated earlier in this paper, the number of fatal cases in which influenza virus has been demonstrated and the pathologic changes described is remarkably small. Only four cases with pathologic descriptions of the lungs have been found by us in the literature. We have had an opportunity to examine five additional cases and these form the basis of this report.

It appears of no value to discuss the pathologic changes which have been described in previous pandemics and epidemics of influenza for nothing is known as to the etiologic agent. With a very rare exception, all such cases were complicated by secondary bacterial infections and the pathologic lesions described were caused for the most part, if not entirely, by such secondary invaders. Goodpasture [7] described two cases which were bacteria free. His first patient died 7 days after the

initial symptoms and 2 days after signs of consolidation appeared in the lungs. Microscopic examination of the lungs showed injury and destruction of the alveolar walls with hemorrhage, edema, a little fibrin, and scant cellular exudate. The alveolar ducts were dilated and some of them showed a hyaline membrane on their walls. His second case was of a subacute type with a terminal exacerbation. Microscopically, the lungs showed alveolar hemorrhages, innumerable foci of polymorphonuclear leukocytes, fibrin, large mononuclear cells, disintegrating hyaline material, and small areas of necrosis of the alveolar walls. In some areas there was a thick layer of hyaline material on the walls of dilated ducts and alveoli. The epithelial lining of the large and small bronchi was intact. In certain respects these two cases resemble histologically our case 4 which was likewise bacteria free.

In our series, two cases were bacteria free and three were complicated by secondary bacterial infections.

Much emphasis has been placed in the past on necrotizing bronchiolitis as a feature of influenza. Such a process also has been found in experimental infections with influenza virus in mice and ferrets. However, in our two cases which were not complicated by bacteria the epithelium of the bronchioles was unaffected. This was also true of Goodpasture's case [7] in which he described the bronchioles. Furthermore, in our cases complicated by secondary bacterial invaders, the bronchioles in the portions of the lung which were not involved by the bacterial infection but which contained the virus were unaffected.

From our series of cases it would seem that it would be difficult to recognize changes produced by the virus in the presence of bacterial infections. It is possible that more definite lesions due to the virus had not been produced because of the short duration of the disease in these cases—2 to 3 days. It will be noted from the descriptions of the histologic changes in our cases that the lesions in four of the five cases were minimal. However, it is entirely possible that if it had been practicable to make multiple sections of each lobe, more severe lesions might have been found. A similar situation was true with the virus studies. In each case, tissue from only one lobe was tested for the presence of virus. Another explanation of the lack of severity of the lesions is the short course of the disease in the three cases complicated by secondary bacterial invaders. Death in these cases may well have been due primarily to the bacterial infections. In case 2, in which the pulmonary lesions were minimal, the duration of the disease was probably 7 days and death was due to acute myocarditis. The lesions in this case may represent a minimal infection with virus or possibly a late stage. The

TABLE I

Certain Relevant Data in Five Cases in Which Influenza Virus Was Recovered from the Lungs

Case number	1	2	3	4	5
Sex and age (years)	Female, 26	Female, 34	Female, 18	Male, 39	Male, 61
Dates: Admission	3/21/43; 9 P.M.	4/10/43; 6 P.M.	12/13/43; 4 P.M.	1/31/44; 4 P.M.	3/6/44; 3 P.M.
Onset of influenza	3/18; P.M.	4/4; P.M.	12/11; 3 P.M.	1/23	3/3; P.M.
Onset of pneumonia	3/20; P.M.	4/6 or 4/9(?)	12/13(?); 1 A.M.	1/26 or 1/29	3/4
Death	3/22; 8 A.M.	4/11; 1 A.M.	12/13; 7 P.M.	2/1; 1 A.M.	3/7
Pulmonary involvement					
Clinical: Consolidation	R.l.	R.l.(?)	Patchy R. and L.	o	R.l.
Râles	Bilateral	R.l., L.l.	Bilateral	Bilateral	R.l., m.
X-ray: Consolidation		o	o	o	
Mottled density		Mid-lungs	Bilateral	Bilateral	
White blood cell count					
Number per cmm.	4,000	20,000	1,350	19,000	2,000
% polymorphonuclear cells	40	70	24	88	60
Bacteriology					
Sputum			S. au.; Str. B	Negative	Pn. I
Blood (ante-mortem)	No growth	No growth	S. au.	No growth	Pn. I
Cardiac blood (autopsy)	S. au.	No growth	No growth	G—Bact. (contam.)	Pn. I
Lungs (autopsy)	S. au.	No growth	Str. B; S. au.	No growth	Pn. I
Others (autopsy) .	P.F.; S. au. Bronchus; S. au.	P.F.; no growth	P.F.; Str. B. and S. au. Bronchus; St. B. and S. au.		P.F.; Pn. I
Virus isolation					
Source	Lung (Lu)	Lung (R.l.)	Lung	Lung	Lung (R.u.)
Result in mice	Influenza A	Influenza A	Negative	Influenza A	Influenza A
Result in chick embryos	Influenza A*	Influenza A	Influenza A(?)	Influenza A	Influenza A

Abbreviations: R. = right; L. = left; l. = lower; m. = middle; u. = upper lobe.
S. au. = Hemolytic *Staphylococcus aureus;* Str. B. = Beta hemolytic streptococci.
Pn. I = Type I pneumococcus; P.F. = pleural fluid; G— = gram negative.

* Obtained by allantoic inoculation of a suspension of lung from the third mouse passage in this case. Others obtained from direct inoculation of the eggs with original lung suspensions.

fourth patient (case 4) lived 9 days and died of pulmonary involvement due to virus alone. The histologic changes, in our opinion, represent the typical picture of a pure influenza virus pneumonia.

Some of the relevant data in the five cases are summarized in Table I. It is seen that the three cases infected with bacteria showed a marked leukopenia. In these cases the bone marrows show maturation arrest of the granulocytic series. In the two bacteria-free cases, leukocytosis was present and the bone marrows were normal. The leukopenia may be attributed to the short course of the disease or to a depressant action of the bacteria. However, it appears that the uncomplicated influenza virus infections were accompanied by leukocytosis rather than leukopenia.

Because of the difficulties usually encountered in isolating an influenza virus from fatal cases as compared with the relative ease with which they were obtained by mouse and chick embryo inoculation and by passage from these cases and from other non-fatal cases,[8] the possibility must be considered that the viruses isolated from the present cases may not have originated from these lungs but have occurred as laboratory contaminants.[9] Such a possibility is extremely unlikely. The viruses in the first two cases were each isolated at a time when no other influenza virus was available in the same laboratory. In the case of the other strains, evidence for the presence of the virus was obtained after the original allantoic inoculation in each instance, and then increased with further passages. Mouse inoculation and passage of the same lung suspensions were successful in only two of the three cases. Furthermore, the virus in one of these cases was serologically distinct from the others. In addition, unsuccessful attempts were made by similar passages in mice and eggs obtained from the same sources to isolate viruses from nine other fatal cases of influenza and atypical pneumonia.

Summary

1. The pathologic changes have been described in five cases in which influenza virus was obtained from the lungs. Only three earlier reports in which the structural changes were described have been found in the literature.

2. Two of our cases were bacteria free; the other three had secondary bacterial invaders.

3. One of the bacteria-free cases showed pathologic changes which were considered typical of influenzal pneumonitis. These consisted of edema, alveolar hemorrhages, fibrin, and the formation of a hyaline membrane.

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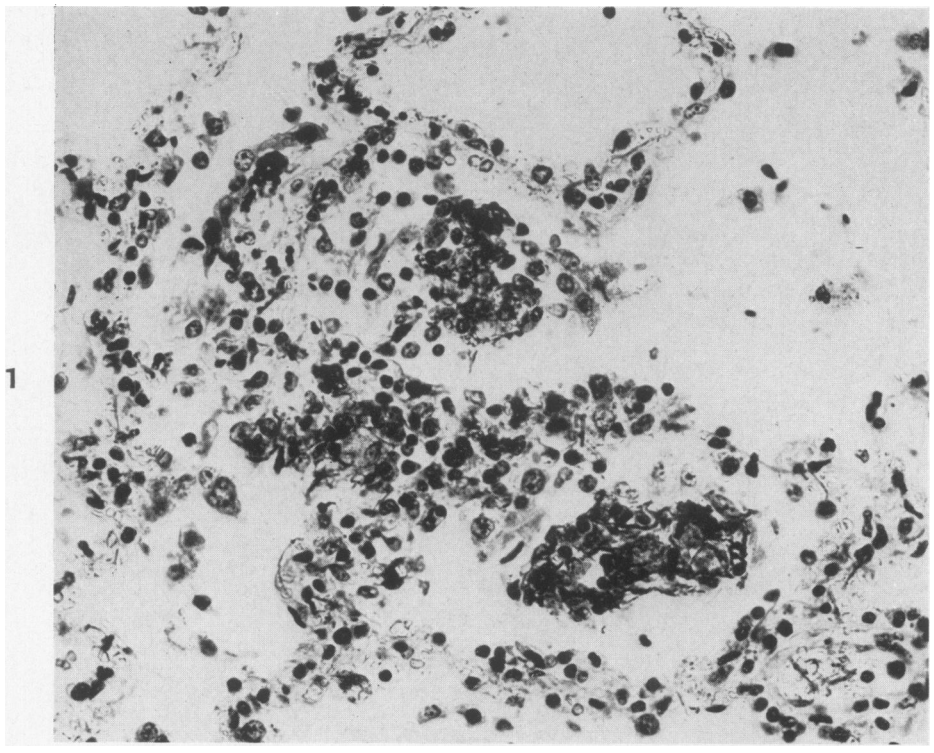
DESCRIPTION OF PLATES

PLATE 153

FIG. 1. Case 2. Acute focal lesion in the right lower lobe. The alveoli contain fibrin, large mononuclear cells, lymphocytes, and polymorphonuclear leukocytes. There is also swelling of the cells lining the alveoli. Phloxine-methylene blue stain. × 150.

FIG. 2. Case 2. Right lower lobe. Perivascular infiltration of lymphocytes and plasma cells. Phloxine-methylene blue stain. × 150.



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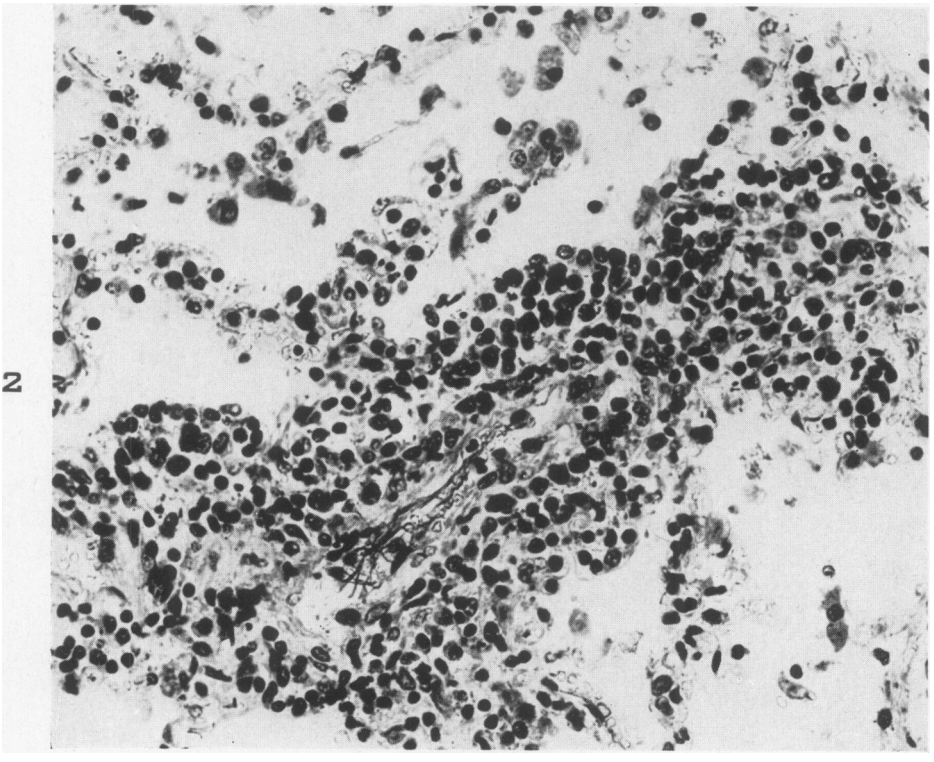

2

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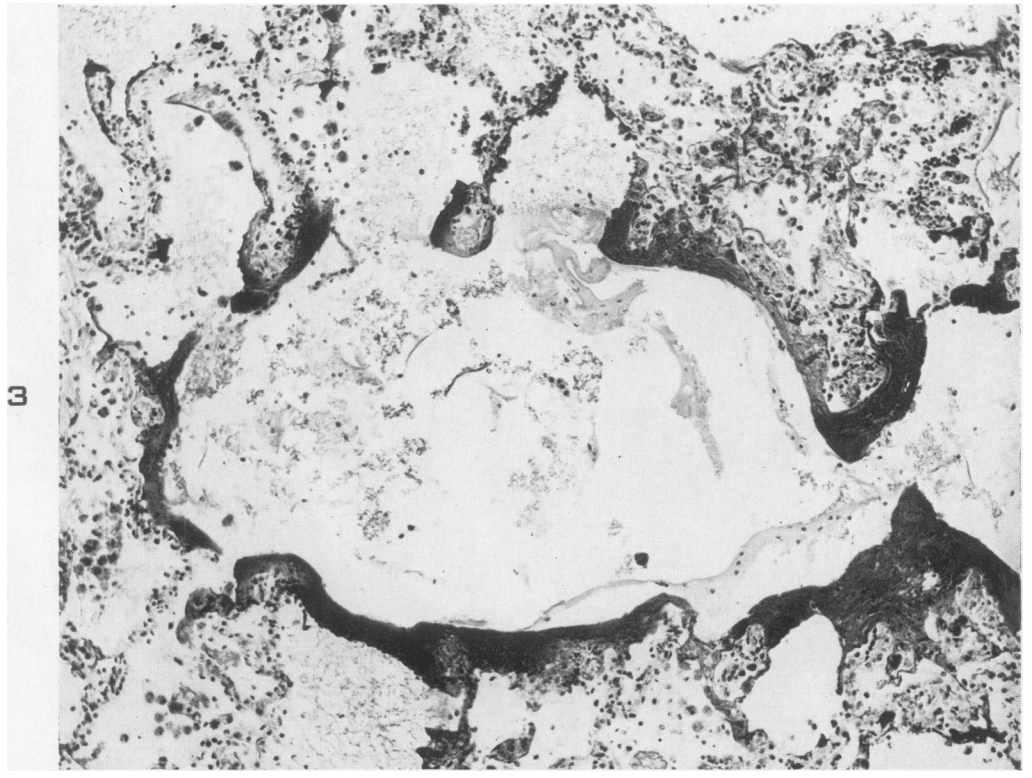
PLATE 154

FIG. 3. Case 4. Dilated alveolar duct lined with dense, hyaline membrane. Phloxine-methylene blue stain. × 125.

FIG. 4. Case 4. Alveoli show edema and an exudate of large mononuclear cells. Phloxine-methylene blue stain. × 125.



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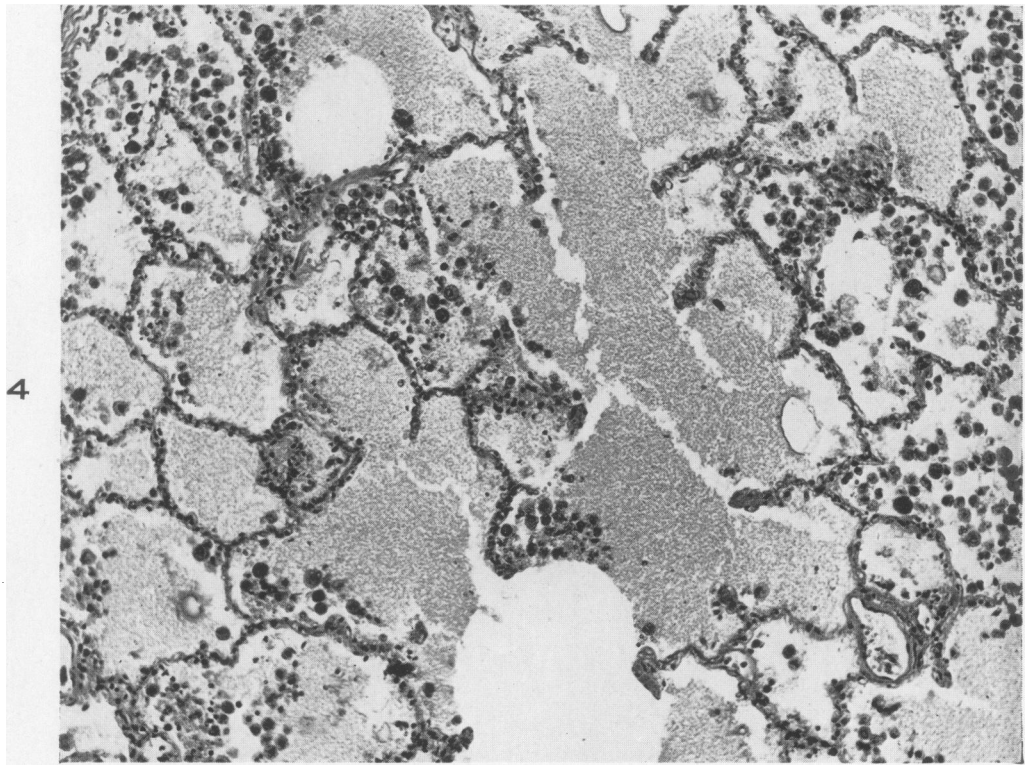

4

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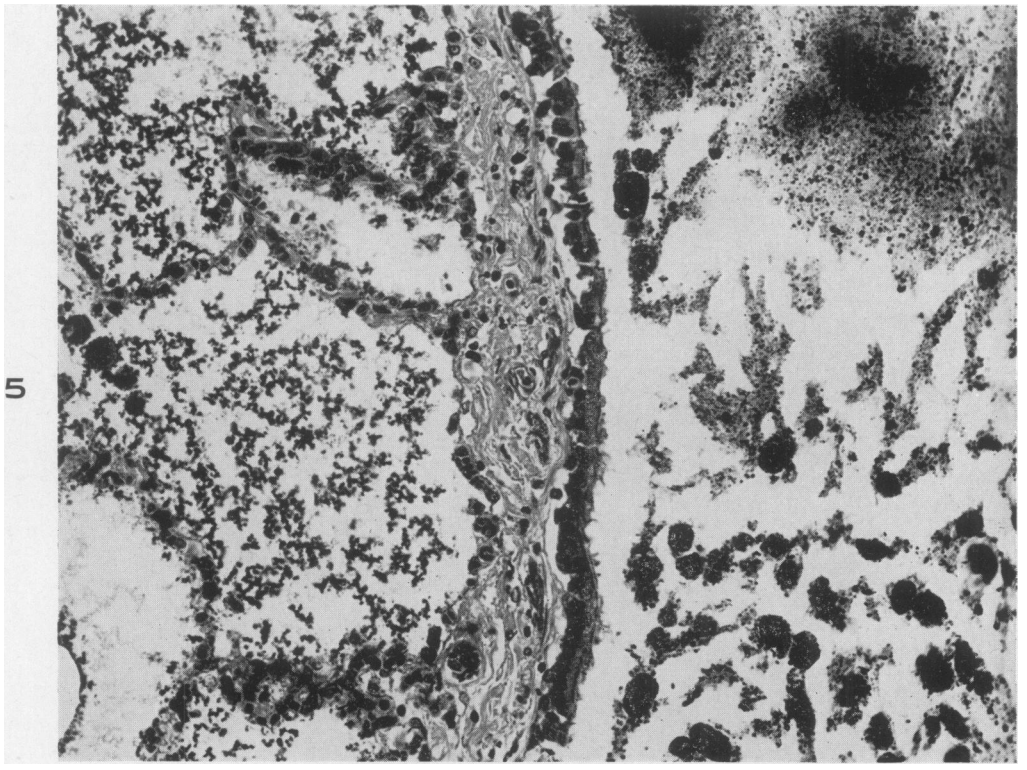
PLATE 155

FIG. 5. Case 5. Right upper lobe. Epithelium of bronchiole is intact. Lumen contains mucus and large mononuclear cells. Alveoli show edema. Phloxine-methylene blue stain. × 150.

FIG. 6. Case 1. Necrotizing tracheitis. Phloxine-methylene blue stain. × 80.



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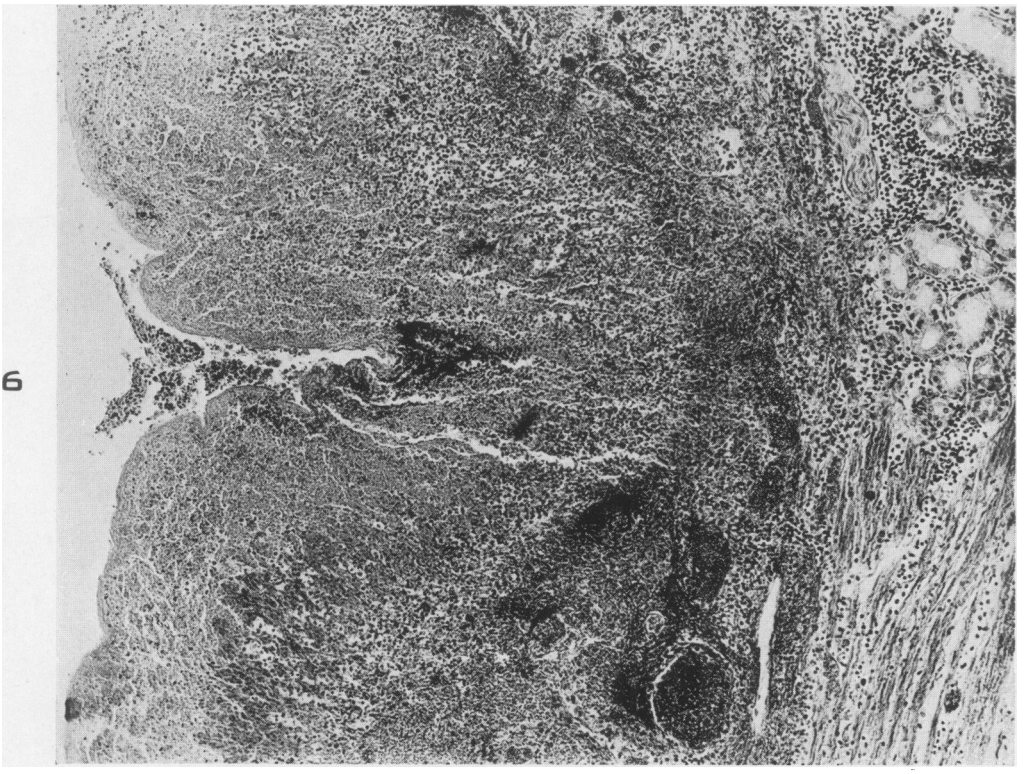

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