STUDIES ON THE MECHANISM OF RECOVERY IN PNEUMONIA DUE TO FRIEDLÄNDER'S BACILLUS

I. The Pathogenesis of Experimental Friedländer's Bacillus Pneumonia*

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Plates 23 and 24

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Recent studies on the mechanism of recovery in pneumococcal pneumonia have served to define the manner in which antibacterial chemotherapy brings about a favorable termination of the pulmonary infection (1–3). Evidence has been presented that "surface phagocytosis" is instrumental in bringing about destruction of pneumococci in the lung in the absence of type-specific immune bodies (3). Since this particular phagocytic phenomenon has not previously been described and since its general biological significance is not known, an effort has been made to determine its relationship to the mechanism of recovery in other bacterial infections, particularly those caused by encapsulated microorganisms which are naturally resistant to phagocytosis. The present investigation deals with pneumonia due to Friedländer's bacillus.

The clinical and pathological features of acute Friedländer's bacillus pneumonia have been described in detail (4–11). Although the essential characteristics of the disease are in many ways similar to those of pneumococcal pneumonia, there are two notable differences: (1) Friedländer's pneumonia causes a higher case fatality rate in human patients than does pneumococcal pneumonia; (2) Friedländer's infection of the lungs frequently leads to suppuration and the formation of lung abscess, a complication rarely encountered in pneumococcal pneumonia, except in the case of type III infection. It should be emphasized also that although Friedländer's bacillus and the pneumococcus are both encapsulated organisms possessing many characteristics in common, the former is a Gram-negative bacillus which differs in certain fundamental biological properties from Gram-positive cocci.

Although the pathologic anatomy of Friedländer's pneumonia as observed in fatal cases of the disease has been carefully described, little is known about the pathogenesis of the pulmonary lesion. Experimental Friedländer's pneumonia has been produced in dogs (12–14) and mice (15) but only the end

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stages of the resulting lesion have been studied. Since basic information is lacking concerning the pathogenesis of Friedländer's pneumonia, a study of the evolution of the pneumonic lesion has been made in white rats and is described in the present paper. Subsequent reports (16, 17) deal with (*a*) the action of an effective chemotherapeutic agent upon the pulmonary lesion of Friedländer's pneumonia and (*b*) a detailed analysis of the mechanism involved in the destruction of Friedländer's bacilli in the lung.

Method

The method used to produce acute Friedländer's bacillus pneumonia in rats was essentially the same as that employed in experimental pneumococcal pneumonia. Details of the technique have been reported in a previous paper (18).

White rats, varying in weight from 175 to 300 gm. and free of chronic pulmonary infection, were inoculated intrabronchially under ether anesthesia with 0.15 ml. of a suspension of type A Friedländer's bacilli[1] in 6 per cent mucin. The virulence of the organism was maintained by frequent mouse passage and by storage at −70°C. in defibrinated rabbit blood under vaseline. The number of bacilli inoculated into the left main bronchus of each rat varied from 100 to 3000. Following inoculation, each animal was kept under light ether anesthesia for 30 minutes in a vertical position to insure penetration of the mucin into the terminal bronchi. Blood cultures were taken from the tail vein at frequent intervals during the course of the infection. All surviving rats were killed with ether, and only the lungs of freshly killed animals were used for microscopic study. The lungs, after gross examination, were fixed in Zenker-formol solution by the method of Loosli (19) and sections 7 microns thick were stained by a modification of the Gram-Weigert technique (18).

RESULTS

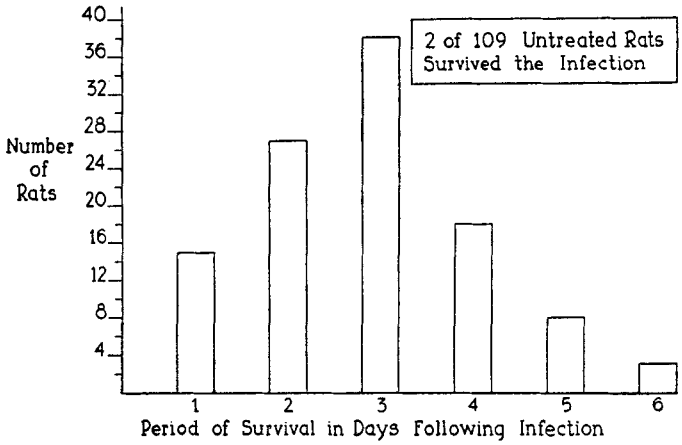
Fatality Rate.—The pneumonia produced by Friedländer's bacillus was fatal in all but two of 109 animals. The two surviving rats apparently developed chronic Friedländer's infection of the left lung. As is shown in Text-fig. 1, all animals of the 107 animals that died did so within 6 days, the majority succumbing on the 3rd day.

Gross Pathology of Pulmonary Lesions.—Representative pulmonary lesions in rats dying of Friedländer's pneumonia are diagrammed in Text-fig. 2. In over 50 per cent of the animals the lesion was confined to the left lung.

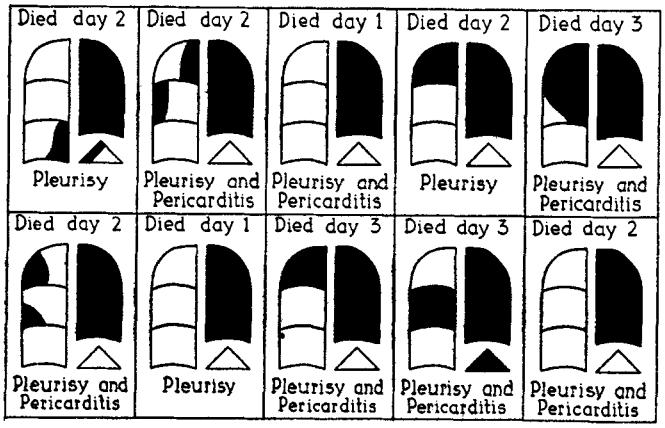
Stages in the development of the pneumonic lesion were studied in a series of twenty-six rats killed at 20 minutes, and at 2, 6, 12, 18, 24, and 36 hours after inoculation. Three to five rats were sacrificed at each interval. The lungs were examined and then fixed for histological study. The results are represented diagrammatically in Text-fig. 3.

[1] Strain "Chic" obtained through the courtesy of Dr. Frances Clapp of the Lederle Laboratories, Inc. Organisms from a 4 to 6 hour culture in tryptose phosphate broth were used in all experiments.

In rats killed 20 minutes after inoculation, the left lung showed a small hemorrhagic area at the site of inoculation, probably due to trauma. After 2 hours the lesion was more hyper-



TEXT-FIG. 1. Survival time of untreated rats dying of experimental Friedländer's pneu monia.

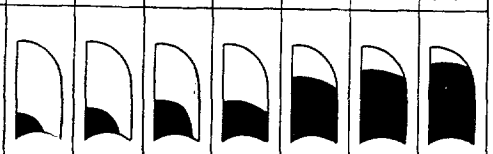


TEXT-FIG. 2. Representative pulmonary lesions in untreated rats dying of experimental Friedländer's pneumonia. All blood cultures positive at time of death.

emic and was clearly demonstrable. Rapid spread occurred after 6 hours with involvement of almost the entire left lung at 36 hours. In the first 36 hours, spread to other lobes of the lung was noted in only two of the twenty-one animals. The lesion was dark red in color during the first 24 hours, and there was little change in the size of the left lung. At 36 hours, however, the left lung was noted to be enlarged, firm, and of a gray-pink color, only the outer margin of

the lesion remaining hemorrhagic. Later lesions seen in animals dying after 48 or 72 hours were of a whitish yellow color and caused considerable increase in the size of the lung (see Fig. 1). Some lesions in animals dying after the 3rd day showed unmistakable evidence of abscess formation.

Incidence of Bacteremia.—The results of blood cultures taken at frequent intervals during the course of the disease are recorded in Text-fig. 3. Bacteremia did not occur during the first 6 hours of infection. After 18 hours, 36 per cent of the animals had positive blood cultures and 75 per cent were bacteremic at 36 hours. Blood cultures from all animals dying of the infection were positive.

Time after Inoculation	20 min.	2 hours	6 hours	12 hours	18 hours	24 hours	36 hours
Approximate Size of Pulmonary Lesion *							
Blood Culture Positive	0	0	0	3	7	10.	13
Blood Culture Negative	17	18	17	13	12	7	4
Incidence of Bacteremia	0%	0%	0%	18%	36%	59%	76%

* Based on autopsy findings in 3-5 rats killed at each interval

TEXT-FIG. 3. Extent of pulmonary lesion and incidence of bacteremia at various stages o experimental Friedländer's pneumonia.

Occurrence of Pleurisy and Pericarditis.—Fibrinous pleurisy, pericarditis, or both, occurred constantly as complications of the fatal infection. Pleural effusion was occasionally noted as early as 6 hours and was frequent at 18 hours, particularly in the bacteremic animals. The fluid was thin and serosanguineous in rats dying in less than 18 hours and was cloudy, but rarely frankly purulent, in those surviving longer. Pericarditis was occasionally encountered at 12 hours and was frequently seen in animals dying later with bacteremia. The pericardial fluid at the time of death was purulent and occasionally of a gelatinous character. Cultures of the cloudy pleural and pericardial fluids were uniformly positive for Friedländer's bacilli.

Histopathology of Pneumonic Lesion.—The histological features and pathogenesis of the pneumonic lesion produced by Friedländer's bacilli were revealed in the microscopic sections cut from rats killed at the intervals designated in

Text-fig. 3 and also from rats surviving as long as 72 hours. The histology of the lesion during the first 6 hours of the infection was essentially the same as that described for experimental pneumococcal pneumonia (18). At this early stage few organisms were visible in the alveoli and the lesion contained many mucin particles, hemorrhagic edema fluid, and a light polymorphonuclear exudate. After 12 hours the full blown pneumonic lesion was easily recognized and may be briefly described as follows:—

The outer margin of the spreading lesion was characterized by an edema zone in which the alveoli were filled with fluid, essentially free of leucocytes (Fig. 2). Floating freely in the edema fluid were many bacteria which were apparently multiplying rapidly (Fig. 3). Inside this outer edema zone there appeared a zone of early consolidation in which polymorphonuclear leucocytes, together with many organisms, could be seen in the alveoli. In this area of the lesion the leucocytic exudate was thin, and very little, if any, phagocytosis was noted (Fig. 4). In the inner zone of the lesion, characterized by advanced consolidation, two contrasting processes were noted. In some areas there was marked phagocytosis, practically all of the bacteria having been taken up by the alveolar phagocytes which were still predominantly polymorphonuclear leucocytes (Fig. 5). In other adjacent areas there was apparently no phagocytosis, and the alveoli were filled with a solid mass of organisms (Fig. 6). In the spreading pneumonic lesions bacteria and edema fluid were also frequently observed in the bronchi (Fig. 7). Infected edema fluid was likewise found in alveoli adjacent to the visceral pleura, suggesting the possibility of direct penetration of the bacteria into the pleural space (Fig. 8). Only rarely were bacteria seen in the peribronchial lymphatics (Fig. 9).

In addition to the histological features noted in the early spreading lesions, there were other important microscopic findings observed in animals surviving for 48 to 72 hours. In many areas of the more advanced pulmonary lesions there were heavy deposits of fibrin in the alveoli, as shown in Fig. 10. In the central portions of the advanced lesions areas of clearing and beginning resolution were noted even in animals dying from the infection. The alveoli in such areas contained macrophages predominantly, but occasionally fibroblasts were also seen, suggesting a tendency for the lesion to become organized (Fig. 11). Finally, in the older lesions, unmistakable evidence of abscess formation was frequently noted. Microscopic examination of such abscesses revealed complete destruction of the alveolar walls and a dense confluent exudate composed largely of polymorphonuclear leucocytes (Fig. 12).

DISCUSSION

Experimental Friedländer's pneumonia produced in rats appears to be more acute than the natural disease in man. The essential pathological features of the experimental infection, however, are the same as those observed in fatal human cases. The lobar distribution of the lesion, the firmness and enlargement of the affected lung, the existence of abscesses in the consolidated lobes,

the large number of bacilli in many of the alveoli, the active phagocytosis by polymorphonuclear leucocytes and mononuclear cells and the frequent occurrence of bacteremia, pleurisy, and pericarditis, constitute the principal pathological findings in the experimental disease and simulate closely those noted in fatal human cases examined at autopsy (5–11).

The pathogenesis of the pulmonary lesion in experimental Friedländer's pneumonia would appear to be essentially the same as that previously described in pneumococcal pneumonia (18). The outer margin of the spreading lesion is characterized by an edema zone in which the alveoli are filled with watery edema fluid containing many bacteria. Not only do the organisms apparently multiply freely in the edema fluid but they seem to be carried mechanically by the fluid into adjacent alveoli through the pores of Cohn and the communicating alveolar ducts and bronchioles.[2] Early studies on the pathogenesis of pneumococcal pneumonia led to the hypothesis that the infection spread by way of the pulmonary lymphatics (21). This suggestion was later refuted by the careful experiments of Loosli (22) which conclusively proved that the spread occurred mainly by way of infected edema fluid. The rarity with which Friedländer's bacilli were found in the lymphatics in the present study would appear to indicate that the same mechanism of spread operates in both pneumococcal and Friedländer's pneumonia.

As in pneumococcal pneumonia, heavily infected edema fluid is seen in many of the large bronchi, suggesting an obvious mechanism of spread of the pneumonia to other lobes. Convincing evidence that such spread in pneumococcal pneumonia is due to infected edema fluid in the large bronchi has been presented by Robertson and Hamburger (23). The bronchial findings in the present study indicate that the same mechanism obtains in pneumonia caused by Friedländer's bacillus.

The histological features of the more central portions of Friedländer's bacillus lesions are also similar in many respects to those of the pneumococcal lesions (18). In the zone of early consolidation adjacent to the edema zone, leucocytes and bacteria are present in the alveoli, but there is little or no phagocytosis. Only in the more central zone of advanced consolidation where the alveoli are packed with leucocytes is phagocytosis noticeable. Here both polymorphonuclear leucocytes and macrophages have ingested the bacteria. That the phagocytes ingest the bacteria and ultimately destroy them, even in animals dying of fatal infections, is evidenced by the fact that in adjacent areas no bacteria remain in the alveoli and there are signs of resolution. Fibrin is prominent in the alveolar exudate of the central portion of the lesions.

Although the reaction of the murine lung to infection with Friedländer's

[2] The spreading process in undoubtedly accentuated by the respiratory movements of the lungs (20).

bacillus is apparently similar to its reaction to pneumococcal infection, there are several important differences in the pathogenesis of the two pneumonias. The first and most important difference concerns the number of organisms noted in the areas of consolidation. In pneumococcal pneumonia, the number of bacteria in a given alveolus is never very great, and the number appears to diminish rapidly as consolidation proceeds, so that very few organisms can be found in the central zone of advanced consolidation (18). Such rapid destruction of the offending bacteria does not always occur in Friedländer's pneumonia. Although the destructive mechanism appears to function efficiently in most parts of the lesion, in some areas Friedländer's bacilli accumulate in tremendous numbers. Here the alveoli may be filled by masses of matted organisms. The ability of Friedländer's bacillus to multiply thus freely in the face of an inflammatory exudate, distinguishes the organism from the type I pneumococcus. Since the pneumonic exudate is said to create a somewhat acid environment in the alveoli and since Friedländer's bacillus can multiply at a lower pH than pneumococcus, it is conceivable that the differences noted in the respective pneumonic lesions are due, in part at least, to differences in the cultural requirements of the two organisms.

The tendency of Friedländer's bacillus pneumonia to lead to abscess formation constitutes a second characteristic that differentiates it from pneumococcal pneumonia. Although most types of pneumococci[3] that invade the lung call forth a pronounced inflammatory reaction in the affected alveoli, they rarely cause destruction of either alveolar or bronchial tissues. When pneumococcal pneumonia resolves, the lung is eventually restored to normal. Friedländer's bacilli, on the other hand, not only accumulate in large numbers in certain areas of the pneumonic lesion, but destroy the alveolar walls and thus cause abscesses. In the areas of abscess formation, where there occurs a breakdown of the normal alveolar architecture, the phagocytic cells function less efficiently in destroying the invading bacteria. The cause of the decreased efficiency of the phagocytes in such areas is discussed elsewhere in connection with the mechanism of destruction of Friedländer's bacilli in the lung (17).

The third difference observed concerns the occurrence of organization in the late stages of the pneumonia. Rarely was evidence of organization noted in experimental pneumococcal pneumonia (18), whereas the fibroblastic activity characteristic of organization of the alveolar exudate was commonly encountered in Friedländer's infection. This difference can probably be attributed to a greater damage to the alveolar tissues caused by the large accumulations of Friedländer's bacilli referred to above.

[3] There is some evidence that pneumococcus Type III may behave more like Friedländer's bacillus in its relation to the animal host than do other types of pneumococcus (24).

SUMMARY

Experimental pneumonia due to Friedländer's bacillus was produced in white rats by the intrabronchial inoculation of the bacilli suspended in mucin. The pneumonia was lobar in type, was almost uniformly fatal, and simulated the acute form of the natural disease in human beings.

The pathogenesis of the pneumonic lesion was studied by examination of microscopic sections of the lungs of animals killed at frequent intervals during the course of the infection. The histologic characteristics of the various stages of the pneumonia were essentially the same as those previously described in experimental pneumococcal (Type I) pneumonia except for the following differences: (1) In isolated areas of the lung in Friedländer's pneumonia many more bacteria were encountered in the alveoli than were ever noted in experimental pneumococcal pneumonia. (2) Abscess formation was common in the late stages of Friedländer's infection, whereas it was not noted in the pneumococcal lesion. (3) Organization of the alveolar exudate, rarely observed in experimental pneumococcal pneumonia, was a prominent feature of the pneumonia due to Friedländer's bacillus.

The mechanism of spread of Friedländer's lesion appeared to be the same as that of pneumococcal pneumonia. Likewise there was noted the same phagocytosis of organisms in the lungs of even bacteremic animals dying of the infection.

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EXPLANATION OF PLATES

Microscopic sections from the lungs of rats with experimental Friedländer's bacillus pneumonia. The photomicrographs were taken by Mr. Milton K. Echtold, and all sections were stained by the Gram-Weigert technique.

PLATE 23

FIG. 1. Gross lesion in rat dying of pneumonia 72 hours after inoculation. Complete consolidation of unilobar left lung. ×2.

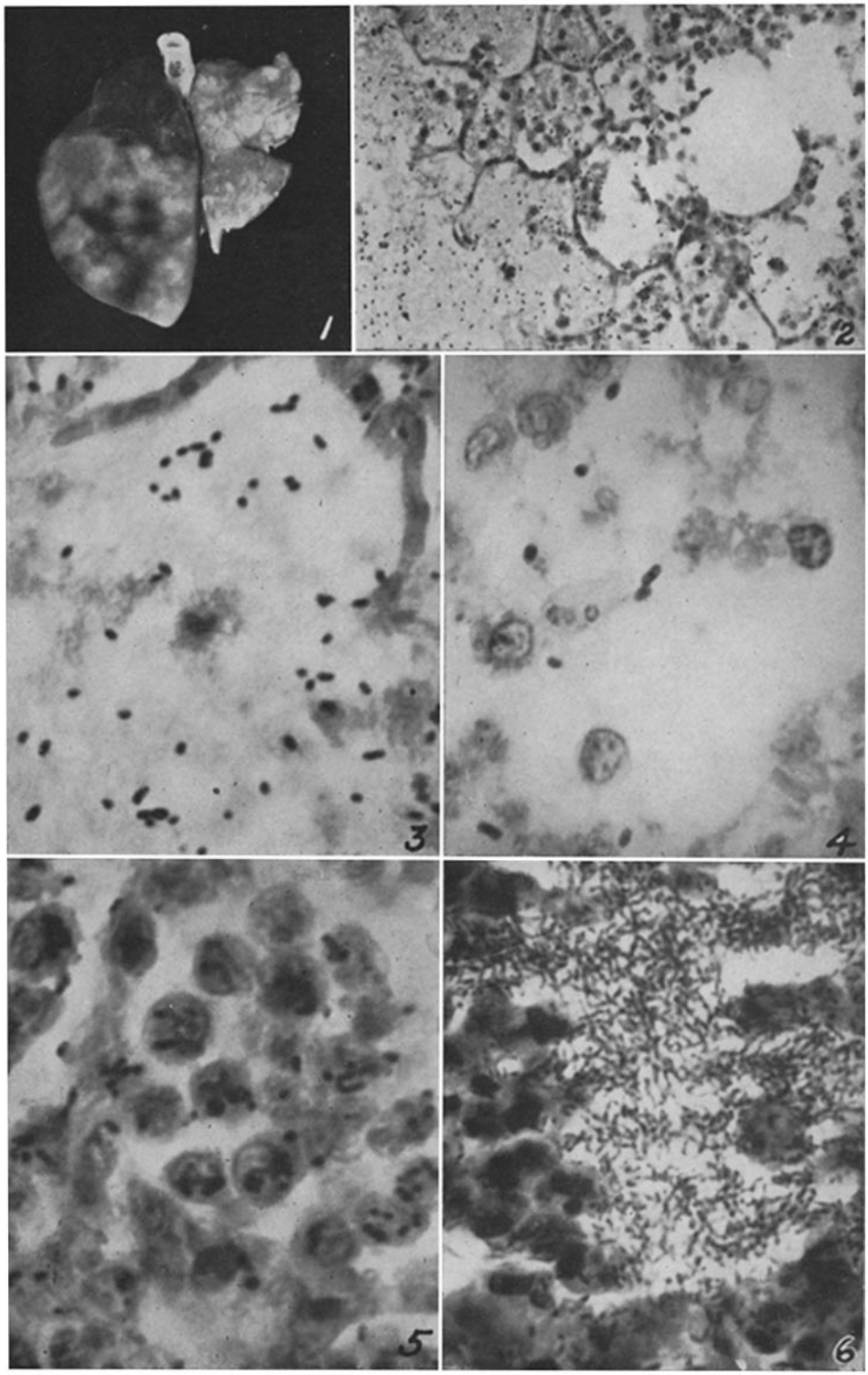
FIG. 2. Edema zone at margin of lesion. Alveoli are filled with bacteria-laden edema fluid, containing few leucocytes. Lesion is progressing from right to left. Animal sacrificed at 12 hours; no bacteremia. ×200.

FIG. 3. Friedländer's bacilli in alveolar edema fluid at margin of lesion. Few leucocytes have yet appeared in alveoli; and the organisms appear to be multiplying freely. Animal sacrificed at 12 hours; no bacteremia. ×900.

FIG. 4. Early consolidation showing Friedländer's bacilli and leucocytes in alveolus. Rat sacrificed at 12 hours; blood culture sterile. ×625.

FIG. 5. Marked phagocytosis in area of consolidation. Many organisms are seen within the cells, which are predominantly polymorphonuclear. This animal had bacteremia when sacrificed at 24 hours. ×1000.

FIG. 6. Masses of bacteria in area of advanced consolidation. No phagocytosis can be demonstrated in such areas. Rat sacrificed at 24 hours, with bacteremia. × 855.



(Sale and Wood: Mechanism of recovery in pneumonia. I)

PLATE 24

FIG. 7. Edema fluid laden with Friedländer's bacilli within the lumen of a large bronchus. Rat sacrificed at 12 hours. Blood culture positive. ×625.

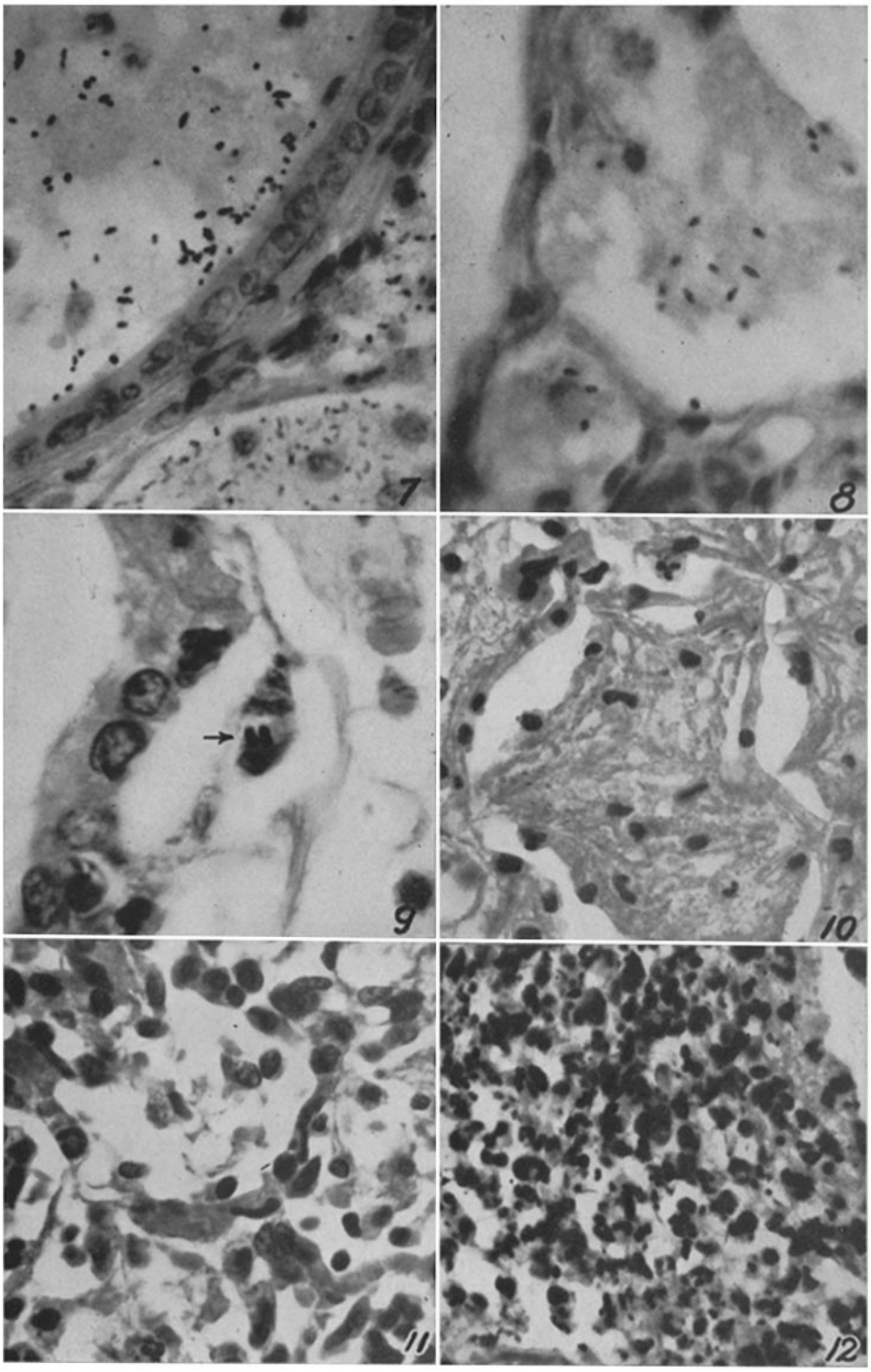
FIG. 8. Edema fluid containing Friedländer's bacilli in alveoli beneath visceral pleura. Animal killed at 12 hours; no bacteremia. ×850.

FIG. 9. Friedländer's bacilli (see arrow) in peribronchial lymphatic. Rat with bacteremia sacrificed at 24 hours. ×1000.

FIG. 10. Fibrin deposits in alveoli in area of consolidation. Strands of fibrin can be seen passing through pores of Cohn. Rat killed at 72 hours; blood culture positive. ×570.

FIG. 11. Clearing and early organization of lesion. Some of the cells in the alveoli are fibroblasts. Animal sacrificed at 72 hours with bacteremia. ×570.

FIG. 12. Abscess formation in area of advanced consolidation. Leucocytes are undergoing necrosis and the alveolar walls have been destroyed. Blood culture positive after 72 hours of infection. ×570.



(Sale and Wood: Mechanism of recovery in pneumoni a. I)