STUDIES ON THE MECHANISM OF RECOVERY IN PNEUMOCOCCAL PNEUMONIA

I. The Action of Type Specific Antibody upon the Pulmonary Lesion of Experimental Pneumonia

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Plates 9 to 13

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In spite of the remarkable advances of the past decade in the treatment of pneumococcal pneumonia, very little has been learned of the manner in which pneumococci are destroyed in the pneumonic lung. Fully encapsulated living pneumococci are resistant to the attack of phagocytic cells unless previously sensitized by specific antibody (1–3). Since phagocytosis is "the only method so far determined by which the host is able to kill the pneumococcus" (4), the attention of most investigators has been centered upon the rôle of antibodies in recovery. Studies of serological reactions in patients and experimental animals have contributed greatly to the knowledge of pneumococcal infections in general, but they have failed to explain the mechanism of recovery in lobar pneumonia as shown by the following examples.

1. Untreated Patients.—Although type specific antibodies usually appear in the blood serum at the time of natural crisis, they do not invariably do so (5). Complete recovery may occur at a time when no circulating antibody can be demonstrated (6). If the phagocytosis of virulent pneumococci is dependent upon specific opsonins, how does the lung overcome the infection in the apparent absence of immune bodies?

2. Patients Treated with Antiserum.—Intravenously injected antibody often brings about rapid recovery in lobar pneumonia. Yet considerable evidence has been advanced that antibodies cannot penetrate the lung (7, 8), and the action of type specific antiserum upon the pulmonary lesion is therefore not understood. To complicate matters further, serological data have been published indicating that, were the antibody able to enter the alveoli, it could not accumulate in sufficient amounts to neutralize the antiphagocytic polysaccharide in the exudate (9).

3. Patients Treated by Chemotherapy.—Drugs of the sulfapyridine group are chiefly bacteriostatic rather than bactericidal in the concentrations usually attained in treating

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human patients (10–12). Nevertheless, following treatment with sulfapyridine, patients often recover without the aid of circulating antibodies (13, 14). Since phagocytosis is thought to depend upon specific opsonins, and the sulfapyridine itself kills relatively few organisms, it is not at all clear how chemotherapy brings about the final destruction of pneumococci in the lesion.

The purpose of the present series of studies has been to investigate the problem of recovery in lobar pneumonia by the more direct methods of pathology. The pathogenesis of experimental pneumonia has been studied in a suitable laboratory animal, and the lungs of animals treated with antiserum and sulfapyridine have been examined in detail at various stages of the disease. By correlating the observed histological changes with the progress of the infection and the immune reactions of the host, data have been obtained which appear to have a direct bearing upon the mechanism of recovery.

This paper deals with the histopathology and pathogenesis of a lobar pneumonia uniformly fatal in untreated rats and describes the effect of type specific antibody upon the pulmonary lesion following treatment with antipneumococcal serum.[1] Observations relating to the process of recovery in rats treated by chemotherapy will be reported in a subsequent paper.

Methods

In 1935, Nungester and Jourdonais (15) produced lobar pneumonia in white rats by inoculating them intrabronchially with pneumococci suspended in a viscous mixture of mucin. The disease produced was not uniformly fatal, and for that reason, preliminary experiments were undertaken at the start of the present investigation to increase the severity of the infection. A group of 100 rats was inoculated by various modifications of the Nungester technique, and although at first approximately one in every four rats survived, a more malignant pneumonia was finally produced which killed all of the untreated animals. Changes in the method which seemed to be of importance included: (*a*) inoculation with a younger (6 hour) culture of pneumococci, (*b*) the use of a more concentrated (6 per cent) mixture of mucin,[2] and (*c*) a prolonged, light anesthesia under ether during which the rats were maintained in a vertical position to allow the mucin to flow further into the terminal bronchi. Elimination of the chance of spontaneous recovery greatly clarified the results of subsequent experiments designed to demonstrate the effect of specific therapy.

Animals.—White rats, varying in weight from 180 to 250 gm., were obtained from several breeders. Chronic pulmonary lesions of undetermined etiology were occasionally encountered. Whenever a chronic lesion was found at autopsy and its presence interfered with the interpretation of the findings in the pneumonic lung, the rat was discarded and the experiment repeated. No spontaneous lesions resembling the acute experimental pneumonia were encountered in any of the 350 rats examined.

Pneumococcus Cultures.—A Type I pneumococcus of the A_5 strain, generously sup-

[1] A preliminary report of these studies was published in *Science*, 1940, **92**, 15.

[2] Kepl and Gunn (16) have recently obtained a high mortality using 5 per cent mucin.

plied by Dr. O. H. Robertson, was used in all experiments. Cultures of the heart's blood of rats dying with bacteriemia were seeded in defibrinated rabbit blood under vaseline, incubated 24 hours, and stored in the ice box. Under these conditions pneumococci have been found to retain their virulence for several months (17), but to insure maintenance of maximum virulence the organism was passed through rats at least once every 3 weeks.

For each experiment the inoculum was prepared as follows: From the stock culture in rabbit's blood, a transfer was made to blood broth and incubated 18 hours. 1 cc. of the blood broth culture was then transferred to approximately 10 cc. of 0.05 per cent dextrose beef infusion broth to which defibrinated rabbit blood had been added in a concentration of 1 per cent. After 6 hours of incubation at 37°C., 1 cc. of the dextrose blood broth culture was diluted 1:1000 in beef infusion broth and 1 cc. of the diluted culture was added to 9 cc. of a mucin-saline mixture. 0.1 cc. of the resulting suspension was used for intrabronchial inoculation.

Mucin.—Finely powdered, commercial mucin[3] was employed as routine. It was found unnecessary to sterilize the mucin or grind it further before adding it to normal salt solution. Most consistent results were obtained when a relatively concentrated mixture of mucin was used in suspending the pneumococci; the concentration finally adopted was 6 per cent.

Intrabronchial Inoculation.—The rats were inoculated according to the technique described by Jourdonais and Nungester (18).

Size of Inoculum.—Bacterial counts of the inoculum were carried out in each experiment by the pour plate method. The number of organisms in the 0.1 cc. of mucin mixture varied from approximately 4,000 to 7,000. In preliminary experiments it was found that if fewer pneumococci were used, rats would occasionally recover spontaneously.

Prolonged Anesthesia.—To insure the penetration of the inoculum into the terminal bronchi, the rats were hung (by the upper incisor teeth) in a vertical position for 30 minutes while still under light ether anesthesia. This procedure seemed to increase the severity of the pneumonia produced.

Blood Cultures.—Blood cultures were taken from the tail at frequent intervals during the course of the pneumonia, and in rats treated with antiserum, cultures were made just before treatment and 5 minutes after the injection was completed. A loopful of blood was streaked on a blood agar plate and incubated for 24 hours at 37°C. The identity of the organism cultured was frequently checked by the Neufeld *Quellung* reaction. Similar cultures were made as routine from the heart at autopsy and were also taken from the pleura, pericardium, and lungs when indicated.

Methods of Studying Pathology.—All surviving rats were killed with ether. Because of the possibility of post mortem growth of pneumococci in the lesion, the lungs of rats autopsied more than one hour after death were not considered suitable for microscopic study. The lungs were fixed in Zenker-formol solution (5 per cent formalin) by the method of Loosli (19). The thorax was opened and the aorta was immediately clamped. If the heart was still beating, it was allowed to do so for a moment, and then a tight ligature was placed about its base to isolate the pulmonary circulation. If the heart had already stopped beating, blood was forced on into the pulmonary vessels by clamping

[3] Granular mucin, type 1701-W, prepared by the Wilson Laboratories, Chicago.

the right ventricle. In either case the pulmonary capillaries were sufficiently distended with blood to facilitate identification of the alveolar walls in the microscopic sections (see Figs. 2, 3, 4, 6, etc.). After a blood culture had been taken from the heart, the lungs were fixed *in situ* by injecting the trachea with Zenker-formol solution. The fixative was introduced slowly under a pressure of not more than 15 cm. of water, and was allowed to re-expand the lungs until they again filled the thorax. The trachea was then tied off and the thoracic organs removed and placed in Zenker-formol solution for 12 to 18 hours. The fixed specimens were washed for 24 hours in tap water and dehydrated in 80 per cent alcohol. Sagittal blocks were cut from the single-lobed left lung, passing through the pneumonic lesion, and each block was further dehydrated in alcohol, imbedded in paraffin, sectioned, and stained by the Gram-Weigert technique.[4] Direct injection of the lung was found to fix the exudate in place, and by redistending the alveoli to their normal size, greatly clarify the histology of the pneumonic lesion (Fig. 6).

Treatment.—The rats were treated with Type I antipneumococcal serum obtained through the courtesy of Dr. W. G. Malcolm of Lederle Laboratories. Refined rabbit serum was used throughout these studies, except in one experiment where horse serum was employed. The rabbit serum contained 6,000 units of antibody per cc.; the horse serum 2,500 units per cc. All rats were treated with a single intravenous injection of antiserum. A dose of 1 cc. (6,000 units) was administered as routine, but in a few experiments smaller doses were given, and as little as 0.02 cc. (120 units) was found to be effective.

The technique employed in injecting serum intravenously was as follows: The groin was shaved and the skin was cleaned with iodine and alcohol while the rat was under light ether anesthesia. An incision about 3 cm. long was made over the femoral vessels, and the vein was exposed by blunt dissection. A No. 26 needle attached to a 1 cc. tuberculin syringe containing the antiserum was inserted into the femoral vein, and the serum was injected slowly over a period of approximately 5 minutes. Following the intravenous injection the incision was closed with a continuous suture of silk. Although superficial infection occurred frequently about the skin sutures, no serious deep infections were encountered when the wound was properly closed.

If the antiserum was injected rapidly, the rats sometimes died of sudden respiratory failure. The slow injection of serum caused an increase in respiratory rate, but no other reactions were noted except in extremely ill rats treated late in the course of the disease. The reaction observed in this latter group is described below.

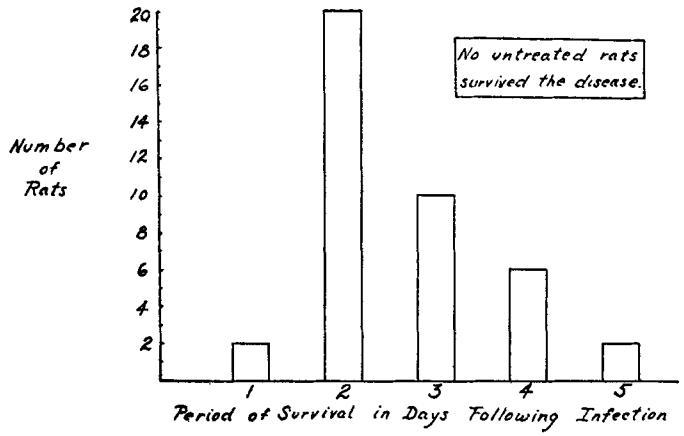
RESULTS

Fatality Rate among Untreated Rats.—The pneumococcal pneumonia produced experimentally by the method outlined was uniformly fatal. All of 40 untreated rats succumbed to the disease in less than 5 days, the majority dying within 48 hours (see Text-fig. 1).

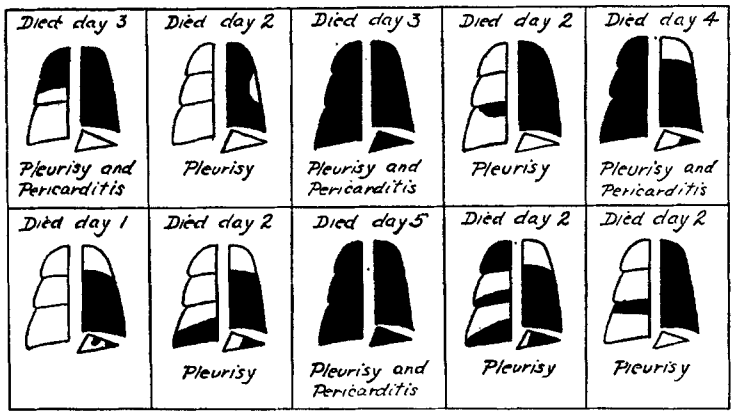
Extent of Lesion in Rats Dying of Pneumonia.—In only 5 rats was the pneumonia confined to the left lung. Representative pulmonary lesions

[4] In order to accentuate the cellular elements of the exudate, the Gram-Weigert method was slightly modified by overstaining the sections with hematoxylin and decolorizing in acid alcohol before staining with eosin.

are pictured diagrammatically in Text-fig. 2. All of the rats had bacteriemia at the time of death, and all but one developed pleurisy, pericarditis, or both. The pleural and pericardial exudates were thickest in the animals



TEXT-FIG. 1. Survival time of untreated rats dying of experimental pneumococcal pneumonia.



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

which survived longest; a condition simulating true empyema was seen in rats dying on the 4th or 5th day. The only sign of pleurisy found in rats dying within 36 hours was an accumulation of cloudy pleural fluid. When cloudy fluid was present, it consistently contained pneumococci.

Evolution of Pulmonary Lesion in Untreated Animals.—The various stages in the development of the lesion were studied in a series of 35 rats killed at

20 minutes and at 2, 6, 12, 18, 24, and 36 hours after inoculation.[5] 5 rats were sacrificed at each interval and the lungs were carefully examined in the gross before being fixed for histological study. The results are summarized in Text-fig. 3.[6] 20 minutes after inoculation a narrow hemorrhagic margin marked the site of injection of the mucin suspension. This very slight hemorrhagic reaction was thought to be due to trauma. At 2 hours the entire lesion had become hyperemic and could be easily distinguished from the surrounding normal lung. The pneumonia was seen to spread rapidly after 6 hours, and by 36 hours it had involved nearly the entire left lung. Spread to other lobes had not occurred in any of the rats within 36

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	5	18	24	25	10
Blood culture negative	10	24	20	15	4	2	0
Incidence of Bacteriemia	0%	0%	20%	55%	86%	93%	100%

TEXT-FIG. 3. Extent of pulmonary lesion and incidence of bacteriemia at various stages of experimental pneumococcal pneumonia.

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

hours. The consolidated area was found to be of firm consistency, and during the first day maintained its dark red, hemorrhagic appearance. At 36 hours the center of the consolidated area had become gray, only the periphery remaining dark red.

The margin of the spreading pneumonic lesion presented a very characteristic appearance. Its edges were hyperemic and extremely irregular, and the outer zone of the lesion as well as the surrounding normal lung were often edematous. The irregular hyperemic margin of an advancing pneu-

[5] Later lesions were not systematically studied because of the failure of the majority of untreated rats to survive for more than 48 hours.

[6] No attempt has been made to depict in Text-fig. 3 the exact location of the pulmonary lesions. The site of inoculation was found to vary somewhat in different animals, but was always in the left lung and was usually at its base.

monia could easily be distinguished from the clearly demarcated edge of arrested lesions seen in treated rats recovering from the disease. The site of inoculation of the mucin remained visible in most cases as a sharply circumscribed dark red patch. In rats inoculated with mucin alone the injected area was found to maintain this same characteristic appearance for nearly a week and, of course, differed from the pneumococcal lesion in that it failed to spread.

Invasion of the Blood Stream and Pleural Cavity.—Frequent blood cultures were taken during the course of pneumonia in untreated rats and representative results are recorded in Text-fig. 3. Bacteriemia was not encountered during the first 2 hours, but 5 out of 25 blood cultures were positive at 6 hours. The blood stream was invaded in roughly half of the animals after 12 hours, and in over 90 per cent at the end of the first day. Rats with early bacteriemia showed the most extensive pulmonary lesions.

Cloudy pleural fluid was present occasionally as early as 6 hours in rats having positive blood cultures. The first signs of pleurisy usually occurred, however, at 18 or 24 hours, when most of the animals had developed bacteriemia. Pleurisy in the absence of bacteriemia was extremely uncommon.

Histopathology and Pathogenesis of Pneumonia in Untreated Rats.—The early changes occurring in the lung following the intrabronchial injection of pneumococci suspended in mucin have been carefully described by Gunn and Nungester (20). A lag of several hours occurs before the pneumococci become numerous enough to be seen in the alveoli, and the lesion does not take on the characteristic appearance of a spreading pneumonia until after approximately 12 hours. The histology of later lesions observed in the present study differed somewhat from that described by Gunn and Nungester, probably because different methods were used in producing the pneumonia and in fixing the lungs. Certain essential features of the present histological findings are therefore briefly described, since they have a direct bearing upon the interpretation of later experiments.

12 to 18 hours after inoculation three definite zones became recognizable in the pneumonic lesion. These zones were even more clearly defined after 24 and 36 hours, and although the transition between them was gradual, the histology of each was sufficiently characteristic to bear particular emphasis.

1. *Outer Edema Zone.*—The margin of the advancing pneumonic lesion was found to be characterized by the presence of edema fluid in the alveoli (Fig. 2). The edema fluid, which stained a light pink, contained a great many pneumococci apparently multiplying freely in this favorable medium (Fig. 3). The presence of the fluid also appeared to afford the pneumococci an excellent means of spreading into adjacent areas of normal lung. In the outer zone very few, if any, leucocytes were present in the edema-filled alveoli.

2. *Zone of Early Consolidation and Phagocytosis.*—Inside the outer edema zone a second zone could be distinguished in which the alveoli contained both leucocytes and organisms. In the outer portion of this area, where the leucocytes were few, pneumococci were numerous (Fig. 6); and in the inner portion the leucocytes were more prevalent and the bacteria scarce. Phagocytosis of the organisms by polymorphonuclear leucocytes was a conspicuous feature of this portion of the lesion. In connection with later experiments, it should be emphasized that the phagocytic reaction, though definitely present, was less marked than that seen in rats treated with type specific antibody, there being many fewer organisms ingested per leucocyte in the untreated animals.

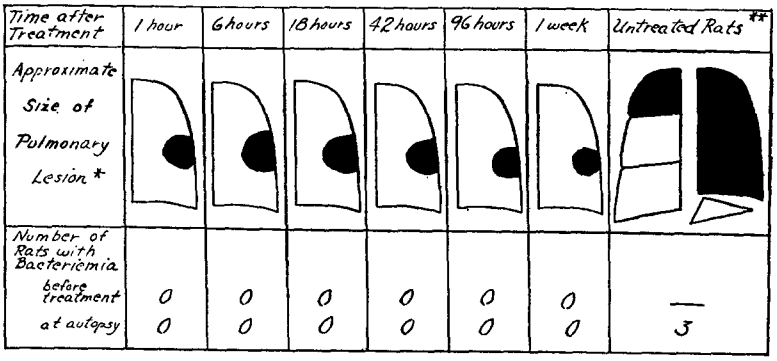
3. *Inner Zone of Advanced Consolidation.*—The thoroughness with which the polymorphonuclear leucocytes of the alveolar exudate are able to destroy pneumococci was clearly shown by the complete absence of organisms within the alveoli of the central portion of the lesion (Figs. 7 and 8). The alveoli and many of the bronchi in this inner zone were packed with leucocytes. Only occasional organisms could still be detected in the exudate, and these were easily distinguished from the large mucin granules seen in a few areas within the alveolar phagocytes. Heavy deposits of fibrin were not uncommonly observed after 18 hours (Fig. 9). In the older parts of the lesion, local areas of clearing were prominent, macrophages having appeared in the resolving alveolar exudate (Fig. 8).

Two additional histological findings deserve special emphasis, since they suggest the mode of spread of pneumococci to other lobes and to the pleural cavity. The presence in large bronchi of appreciable amounts of pneumococcus-laden edema fluid was repeatedly noted in sections from rats with rapidly advancing pneumonia (Fig. 5). Hamburger and Robertson have demonstrated (21) in dogs that this watery bronchial exudate contains myriads of organisms and is responsible for the spread of the infection from one lobe to another. The frequency with which the bronchial edema fluid has been noted in the present study indicates that the same mechanism may operate in rats. The presence of large numbers of pneumococci in edema-filled alveoli bordering on the pleural cavity (Fig. 4) suggests that pleurisy may be caused by the direct penetration of pneumococci through the visceral pleura. It is conceivable that the organisms invade the pleura by way of the lymphatics, draining outward at the periphery of the lung, but in the face of the present histological findings, the possibility of pneumococci passing directly into the pleural cavity cannot be excluded.

The histopathology of the 24 and 36 hour pneumonia was essentially the same as at 18 hours, except for the extent of the lesion. Rats dying after 2 or more days also showed the same histological changes in the lungs, except that the older lesions were characterized by a more extensive zone of advanced consolidation in which local areas of clearing were relatively numerous.

Experiments on the Action of Antiserum.—Five separate experiments were carried out in which rats were treated 2, 6, 12, 18, and 24 hours after inoculation. 21 rats were inoculated in each experiment, 3 serving as untreated controls. The 18 treated animals were sacrificed in groups of 3 at different intervals following treatment. The lungs of each rat were carefully examined in the gross before being fixed for histological study. The results of the first four experiments are summarized in Text-figs. 4 to 7; the fifth

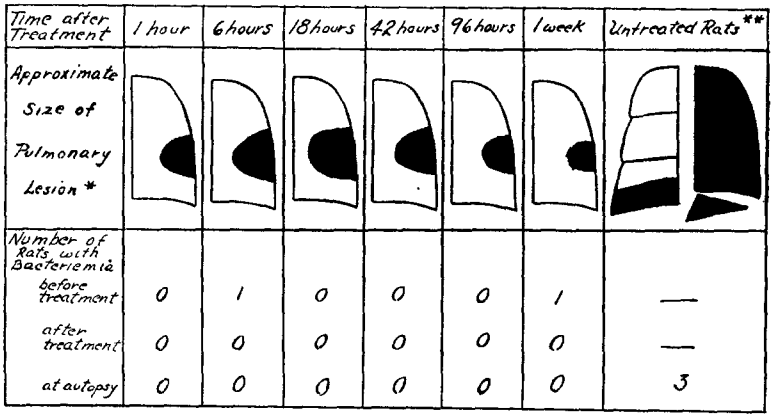
experiment, in which rats were treated after 24 hours, is described later under a separate heading.



TEXT-FIG. 4. Effect upon pulmonary lesion of type specific antiserum administered intravenously 2 hours after inoculation.

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

** Untreated rats died in less than 70 hours.



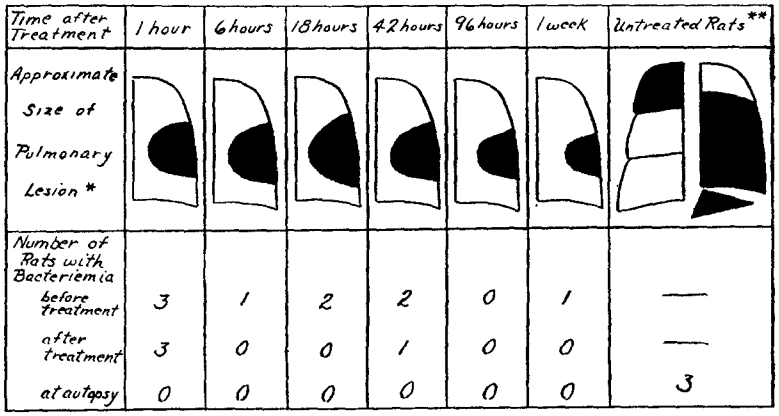
TEXT-FIG. 5. Effect upon pulmonary lesion and bacteriemia of type specific antiserum administered intravenously 6 hours after inoculation.

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

** Untreated rats died in less than 70 hours.

The Effect of Serum Treatment upon Fatality Rate.—No deaths due to pneumonia occurred among the 76 animals treated within 18 hours after inoculation, although many showed bacteriemia at the time of treatment. 12 rats were allowed to live for one week and 12 more for 4 days before being sacrificed. Histological examination of the lungs indicated in each case that the pneumococcal infection had completely subsided.

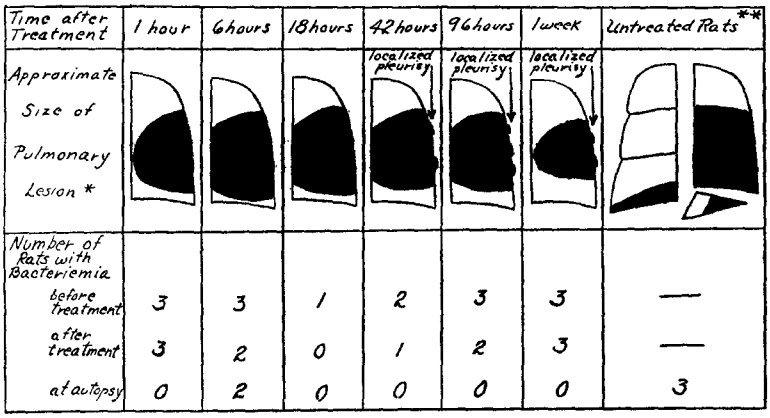
The Effect of Antiserum upon the Spread of the Pneumonic Lesion.—In each of the first four experiments the spreading lesion was promptly arrested



TEXT-FIG. 6. Effect upon pulmonary lesion and bacteriemia of type specific antiserum administered intravenously 12 hours after inoculation.

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

** Untreated rats died in less than 90 hours.



TEXT-FIG. 7. Effect upon pulmonary lesion and bacteriemia of type specific antiserum administered intravenously 18 hours after inoculation.

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

** Untreated rats died in less than 70 hours.

by treatment. When serum was administered 2 hours after inoculation, the lesion remained small; it was slightly larger 18 hours after treatment, but had regressed to its original size by the end of one week. The effect of antiserum upon the 6, 12, and 18 hour pneumonias was likewise pronounced.

Although the area of consolidation was somewhat larger at the time of treatment in each succeeding experiment, the infection was always quickly controlled by serum therapy.

The gross appearance of the spreading lesion changed noticeably following treatment. The margin of the consolidated area became sharply demarcated and lost the irregular hemorrhagic border characteristic of an advancing pneumonia. The entire lesion gradually became gray in color and in its center there appeared small patches of soft normal lung indicating that resolution had begun. These changes in the gross appearance of the lesion made it a simple matter to differentiate at autopsy between an actively spreading pneumonia and one arrested by serum therapy.

The original area of lung injected with mucin remained firm and dark red, simulating exactly the lesion observed in rats inoculated with mucin alone. The mucin area showed no signs of spreading and could be clearly distinguished from the surrounding pneumococcal lesion.

The Effect of Antiserum upon Bacteriemia and Pleurisy.—As shown in Text-figs. 5, 6, and 7 the blood stream of bacteriemic rats cleared rapidly following serum therapy. None of the animals treated at 2 hours had bacteriemia, but 2 at 6 hours, exactly half at 12 hours, and roughly 80 per cent at 18 hours had positive blood cultures just before treatment. When the bacteriemia was light, the blood became sterile a few minutes after the injection of serum. When the initial blood culture showed many colonies per cc., the second culture taken 5 minutes after treatment usually contained many fewer organisms but seldom was sterile. In all but 2 rats the blood cultures were negative at the time of autopsy. These 2 showed positive cultures 6 hours after treatment, although in both cases the number of colonies per cc. was markedly diminished. No rat developed bacteriemia following therapy.

Signs of pleurisy were noted only in animals treated 18 hours after being infected. In this group all 9 rats sacrificed 42 hours or more after treatment showed between the visceral and parietal pleura small areas of dense adhesions indicative of localized pleural infections (see Text-fig. 7). No generalized pleurisy or empyema, as seen in untreated rats, was observed in any of these animals, the serum treatment having apparently caused a localization of the pleural infection.

The Action of Type Specific Antibody upon the Pulmonary Lesion.—In order to investigate the effect of antibody upon the pneumococci in the lesion, microscopic sections were made of the lungs of all rats killed at intervals after treatment. In rats treated 2 and 6 hours after inoculation the pneumonic process had not developed sufficiently to make histological study

profitable. Under the influence of antibody the organisms were apparently destroyed by phagocytosis before they had had a chance to multiply sufficiently to produce the typical pneumococcal lesion. In rats treated after 12 and 18 hours, however, the pneumonia was well advanced. Although the 18 hour pneumonia was more extensive than that treated at 12 hours, the evolution of the lesion following treatment was essentially the same in both cases and may be described as follows:—

One hour after treatment pneumococci could be seen agglutinated in the alveoli,[7] particularly in the edema zone at the margin of the lesion (Figs. 10 and 11). This agglutination reaction became more marked at 6 hours, when practically all of the extracellular organisms had congregated in large clumps.[8] Many of the pneumococci seemed to adhere to the alveolar walls as well as to each other (Fig. 11). Even the organisms in large bronchi (Fig. 12) and those well within the area of consolidation (Fig. 13) were agglutinated. Careful examination of the clumped cocci revealed capsular swelling typical of the Neufeld *Quellung* reaction (Figs. 14 and 15). In many areas where leucocytes and pneumococci were present in the alveoli marked phagocytosis had occurred (Fig. 16); each leucocyte was loaded with bacteria, the capsules of which were often visibly swollen (Fig. 17). The phagocytic reaction was most marked in the zone of early consolidation bordering on the edema-filled alveoli at the margin of the lesion. In the central zone of advanced consolidation, as in untreated rats no pneumococci were present.

Within 6 hours after treatment the edema zone at the edge of the lesion became narrower than that seen in untreated animals and after 18 hours it had disappeared entirely (Fig. 18). Its disappearance was apparently due to the agglutination of the pneumococci which immobilized them in edema-filled alveoli and allowed them to be overtaken by leucocytes. The extension of the leucocytic exudate into alveoli previously containing only edema fluid and organisms accounted for the slight progression of the gross lesion which occurred during the first 18 hours after treatment. At the same time more organisms were phagocytized, until at the end of 18 hours few could be found extracellularly. Within 42 hours most of the pneumococci had been completely destroyed by phagocytic cells (Fig. 19). 4 days after treatment the alveolar walls were

[7] In experiments on untreated rats it was found that pneumococci growing in the presence of free mucin are often clumped together. No conclusions, therefore, can be drawn regarding agglutination of organisms in alveoli containing free mucin. By 18 hours relatively little mucin remains in the lesion, and only organisms contained in mucin-free alveoli are considered in describing the effect of the antiserum.

[8] The increase in the size of the clumps seen at 6 hours was apparently due not only to a more complete agglutination reaction but also to the multiplication of organisms already agglutinated. It is well known that pneumococci will continue to multiply in clumps when grown in the presence of homologous antibody. It seems unlikely that such masses of organisms can be destroyed by phagocytosis alone, and it is suggested that autolysis may be an important factor in the process of destruction. This view is supported by the fact that many of the clumped cocci are Gram-negative after 6 to 18 hours in sections stained by the method of Brown and Brenn (22).

noticeably thickened, and the macrophage reaction (23) became prominent. At the end of one week only large pale macrophages remained in the alveoli and the lesion showed clearing.

Lesion Produced by Mucin Alone.—In rats inoculated with mucin alone two histological findings of importance were noted after the 4th day. One was the presence of occasional localized sterile abscesses usually arising from small bronchi near the periphery of the lung; the other was metaplasia of the walls of a few peribronchial alveoli which became lined with columnar epithelium. Both of these changes were found in the lungs of rats recovering from pneumonia following serum therapy. They were evidently due to the action of mucin and were easily distinguished from the pneumococcal portion of the lesion.

The Effect of Antiserum Administered after 24 Hours.—18 rats were treated 24 hours after inoculation and the surviving animals were sacrificed at the same intervals as in the previous experiments. All but 2 had bacteriemia at the time of treatment. 11 of the animals survived and at autopsy showed gross lesions similar to those seen in rats treated 18 hours after inoculation. The lesions were slightly more extensive but otherwise similar to those already described. Localized pleurisy was noted in the rats sacrificed after 4 days, and in one animal killed at the end of a week there was a massive pericardial effusion containing viable Type I pneumococci.

The 7 remaining rats all died within 18 hours of treatment; 2 succumbed during the injection of serum. Each animal showed evidence of marked pulmonary edema at the time of death with foamy hemorrhagic fluid exuding from the nostrils. Autopsy findings were essentially similar in each case. The lung was extremely hemorrhagic and edematous in the gross; histological examination revealed large clumps of agglutinated pneumococci and extensive hemorrhages into the alveoli. In many areas there was necrosis of cells in the alveolar exudate and in the alveolar walls. In all but one rat surviving more than 6 hours after treatment the blood stream had become sterilized as the result of therapy.

No definite explanation can be given for the fatal reaction observed in this group of animals. Because all of the rats died within 18 hours after receiving serum, and more than half had negative blood cultures at the time of death, it would appear that they died of a violent reaction to the antiserum rather than of the pneumococcal infection itself. All of the animals were acutely ill at the time of treatment; several were moribund. The reaction closely resembled that occasionally observed when a large dose of antiserum is given to a patient already in a state of collapse from severe pneumococcal pneumonia. Under these conditions death not uncommonly

results from a reaction characterized by pronounced pulmonary edema (24). The mechanism of the reaction is not understood, and an attempt is being made to study it further.

Because of the failure of the majority of infected rats to live more than 48 hours, it was impossible to investigate systematically the effect of antiserum upon later lesions. Since there had invariably developed by the end of the 2nd day an advanced pleural or pericardial infection, which was unaffected by antiserum, accurate evaluation of late treatment was not possible. As will be pointed out below, however, there is little reason to believe that the action of antibody upon the intrapulmonary infection is significantly changed by the age of the lesion.

Promptness of Agglutination Reaction.—Agglutination of pneumococci was observed in lungs fixed as soon as 10 minutes after the start of serum therapy. The reaction began quickly but was not complete until sometime between 1 and 6 hours after treatment.

Necessary Dosage of Antiserum.—Although a dose of 6,000 units of antibody was used in all of the experiments described above, as little as 120 units was found to be effective in treating rats with 12 hour pneumonia. The rats not only survived the infection when given this small dose but the lungs of animals sacrificed 6 hours after treatment contained agglutinated and opsonized pneumococci in the alveoli. When only 12 units of antibody were administered, the rats failed to survive and no agglutination or opsonic effect was observed in the lungs.

The Action of Horse Serum upon the Pulmonary Lesion.—The antibody contained in antipneumococcal horse serum is known to be of larger molecular size than the corresponding rabbit antibody (25, 26). It has been suggested that the antibody of rabbit serum may be able to penetrate tissues more readily (27) and may, therefore, be more effective as a therapeutic agent. In the present study antipneumococcal horse serum was found to exert the same effect upon the pulmonary lesion as did rabbit serum. The specific antibodies contained in horse serum penetrated the lung, as evidenced by the presence of agglutinated and opsonized pneumococci within the alveoli.

The Type Specificity of the Antibody Effect.—Histological examination of the lungs of rats treated with Type II antiserum showed that heterologous antibody had no effect upon the pneumonic lesion.

COMMENT

The pathology of experimental pneumonia in rats was found to simulate closely that of spontaneous lobar pneumonia in man. The histology of the

pulmonary lesion was essentially the same as that described by Loeschcke (28) in human patients. The significance of bacteriemia and the occurrence of extrapulmonary complications such as pleurisy, empyema, and pericarditis also seemed to be the same in the two diseases. The study of the pathology of the experimental disease in rats was greatly facilitated by the fact that a sagittal section of the unilobar left lung could be mounted on a single microscopic slide. This made it possible to examine the lesion as a whole, to note the transition between histological changes in different areas, and particularly to determine the relation of the margin of the pneumonia to the surrounding normal lung.

The mode of spread of pneumococci through the lung in lobar pneumonia has long been a subject of controversy. Two general theories have been evolved from the observations of many investigators: The first favors an extension of the infection through the interstitial tissues and particularly the lymphatics of the lung; the second emphasizes the passive spread of organisms in the alveolar edema fluid in response to the action of gravity and variations in intrapulmonary pressure. The importance of lymphangitis in the extension of the lesion was stressed particularly in early studies made on mice (29), rabbits (30), and monkeys (31). Lauche (32) in 1927 considered that the reversed flow of lymph resulting from blocked lymphatics was responsible for the spread of pneumococci through the human lung. More recent investigations in man, however, as well as in dogs and rats, indicate that pneumococcal pneumonia extends principally by way of the edema-filled alveoli at the margin of the lesion. Loeschcke (28) made a detailed examination of serial sagittal sections of the lungs of 50 patients dead of pneumonia and concluded that the organisms were carried into adjacent alveoli through the pores of Cohn by infected edema fluid. He suggested that the process was aided by the changes in the intra-alveolar pressure associated with breathing and coughing. Robertson and his coworkers (33) and Gunn and Nungester (20) expressed similar views after studying the pathogenesis of experimental pneumonia in dogs and rats, respectively.

The results of the present study support the views of Loeschcke. Pneumococci were seldom found in lymphatics in any of the sections studied. On the other hand, the edema zone at its margin was a striking feature of the spreading lesion. Pneumococci, which were most numerous in this outer area, could be seen in great numbers floating freely in the edema fluid. The fact that inert mucin particles were washed outward to the margin of the lesion by the advancing edema fluid suggested strongly that organisms were likewise transported to new alveoli by the liquid medium.

In addition, evidence has been mentioned that infected fluid probably causes the extension of pneumonia to other lobes and may even be responsible for invasion of the pleural cavity. In connection with the rôle of edema fluid in causing the spread of pneumococcal pneumonia, it is of great theoretical interest that Sutliff and Friedemann (34) have isolated from growing pneumococci an "edema-producing substance" which is thought to be responsible for the local outpouring of fluid at the site of pneumococcic infections. The substance has been separated from the capsular polysaccharide of the pneumococcus, is species specific rather than type specific, and seems to be non-antigenic.

No adequate explanation can at present be given for the phagocytic reaction consistently observed in the lungs of rats dying of pneumonia. Phagocytosis of pneumococci by alveolar leucocytes was observed in all lesions more than 12 hours old, even though many of the animals had bacteriemia when sacrificed. In both man (35) and experimental animals (36) circulating opsonins are rarely found in the presence of positive blood cultures, and although serological tests were not done in these experiments, it is only reasonable to suppose that specific antibodies were absent from the blood in many instances. In spite of this, not only was unmistakable phagocytosis observed in the lungs, but the fact that pneumococci were absent from the older, central portion of the lesion indicated that organisms were being efficiently destroyed by the phagocytic cells. As repeatedly emphasized, fully encapsulated living pneumococci cannot be phagocytized unless they are first opsonized; therefore, the presence of active phagocytosis in the lungs of these animals is not easily explained. Robertson (33) has observed phagocytosis in the pneumonic lungs of dogs under the same circumstances. He has suggested that immune bodies may be produced locally in the lungs and thus cause phagocytosis and clearing of the lesion without overflowing into the blood stream in detectable amounts. No data are as yet available supporting Robertson's hypothesis, but an attempt is being made to investigate further this important phenomenon, since it obviously has a direct bearing upon the mechanism of recovery. Regardless of the explanation of the phagocytic reaction, its presence, and particularly its efficiency, indicate that the lung possesses a strong natural defence against pneumococcal infections. In an actively spreading lesion, however, leucocytes have not had time to reach the alveoli in the advancing edema zone, and here in the absence of phagocytes, pneumococci can multiply freely and invade the surrounding normal lung.

Although the effectiveness of antipneumococcal serum in the treatment of lobar pneumonia is well established, the action of antibody upon the pul-

monary lesion has not been understood. Considerable experimental evidence has accumulated indicating that antibody cannot penetrate areas of consolidation within the lung. It has been shown that the circulation in the pneumonic lung is impaired, and this fact has been advanced as evidence that antibody cannot enter the lesion (7, 37, 38). Fox (8) investigated the permeability of the lungs of rabbits to antibodies and reported that the lung-blood barrier was only slightly permeable even in the presence of an inflammatory process. More recently, Kempf and Nungester (38) have studied the penetration of antipneumococcal immune bodies into pneumonic lesions produced in rats and have been unable to demonstrate the presence of either horse or rabbit antibody in the lungs following intravenous treatment. Nye and Harris (9) measured the amount of capsular polysaccharide in human lungs examined at autopsy, and calculated that in certain cases as much as 60 liters of potent antiserum would be required to precipitate the pneumococcal carbohydrate contained in the consolidated lobes. They concluded that, even if antibody were able to enter the lesion, it could not neutralize all of the antiphagocytic polysaccharide and, therefore, could not be expected to opsonize the offending pneumococci.

In the present paper, however, experiments are reported which demonstrate that antipneumococcal immune bodies not only enter the pulmonary lesion of experimental pneumonia, but do so in amounts sufficient to control its spread. None of the 76 animals treated with antiserum within 18 hours after inoculation died of pneumonia. Study of the lungs at various intervals after treatment revealed that the type specific antibody had caused agglutination and thus fixation of the pneumococci in the edema zone and even in large bronchi. Pneumococci in the "zone of early consolidation," though fewer in number, were also agglutinated. The type specific antibody at times appeared to cause the agglutinated organisms to stick to the surrounding tissue as well as to each other. This phenomenon has been emphasized by Rich (39) as an important factor in bringing about localization of pneumococcal infections in immunized animals. Besides being clumped, many of the organisms showed swollen capsules. The *Quellung* reaction is highly type specific and its presence in the microscopic sections proves conclusively that the changes observed were due to type specific antibody.

Although phagocytosis of pneumococci occurs in the lungs of untreated animals the process is relatively slow, and only a few organisms are ingested by any one leucocyte. In serum-treated rats, on the other hand, the phagocytic reaction appeared to be greatly accelerated by the opsonic action of antibody. Many of the leucocytes became engorged with pneumococci

and within 42 hours after treatment practically all of the organisms in the lesion had been destroyed by phagocytic cells. It is of interest that most of the pneumococci were phagocytized and digested by polymorphonuclear leucocytes and that the process was completed before any appreciable number of macrophages had appeared in the alveolar exudate. Robertson (33) has emphasized the importance of the macrophage reaction in bringing about the final destruction of pneumococci in the lungs of untreated human patients and also of untreated dogs with experimental pneumonia. The fact that the polymorphonuclear leucocytes appear to play a more important rôle in the present experiments may well be explained by the accelerated phagocytic reaction caused by the specific opsonins. Evidence will be presented in a subsequent report that macrophages take a more active part in the destruction of pneumococci when infected rats are treated with sulfapyridine.

The blood of animals naturally resistant to pneumococcal infections is capable of destroying large numbers of pneumococci (40). Bacteriemia results only when the number of organisms entering the circulation from the pulmonary lesion exceeds that which can be handled by the pneumococcidal properties of the blood. Bacteriemia may be controlled by depressing the source of pneumococci in the lung, or by increasing the killing power of the blood. Both mechanisms are involved in the action of antiserum. The intravenous injection of specific antibody has been shown to cause immediate agglutination of circulating pneumococci; the clumped organisms become lodged in the capillaries of the liver, spleen, lungs, and possibly other organs where they are destroyed by phagocytic cells (41). In the course of the present study antibody has also been found to cause agglutination and immobilization of pneumococci within the alveoli of the pneumonic lesion. Thus it becomes evident that antiserum exerts its dramatic effect upon bacteriemia not only by hastening the destruction of organisms already in the blood stream, but also by preventing pneumococci still multiplying in the lung from invading the circulation. The resultant effect is rapid sterilization of the blood.

No definite explanation can as yet be offered for the effect of antiserum upon early pleurisy. Since organisms could not be found in any of the areas of localized pleurisy observed in these experiments, no conclusions can be drawn regarding the action of antibody upon the pleural infection. The mere fact, however, that serum treatment should lead to the localization of an infection already involving the pleural cavity would seem of itself to be of considerable importance.

The results of the present experiments leave no doubt as to the ability of type specific antibody to penetrate the pulmonary lesion of experimental pneumonia. The presence of agglutinated pneumococci in the alveoli, the swelling of their capsules, and the marked increase in the phagocytosis of organisms by the cells of the alveolar exudate all serve as ample evidence that antibody actually entered the alveoli. A study of the histological changes occurring during recovery also indicates that antipneumococcal serum exerts its curative action in experimental pneumonia by halting the pneumococci in the advancing edema zone through agglutination thus causing them to be overtaken and phagocytized by leucocytes. Experiments to be reported in a subsequent paper have shown that sulfapyridine likewise stops the spread of the organisms at the margin of the lesion but does so through a different mechanism.

It has been stated that serum therapy is relatively ineffective in lobar pneumonia if instituted after the 4th day of the disease. The explanations offered for this commonly accepted dictum are that antibody cannot penetrate the densely consolidated lesion of advanced pneumonia (42) and it cannot neutralize all of the antiphagocytic polysaccharide which has accumulated in the pneumonic exudate (9). Neither of these explanations appears to be valid in the light of the present results. It has been shown that type specific antibody acts principally upon the advancing margin of the lesion in experimental pneumonia. Since there is good evidence that most of the pneumococci in both human and experimental pneumonia are at the border of the lesion, there is no reason to believe that intravenously administered antiserum must penetrate areas of advanced consolidation in order to control the infection. On the contrary, it has been repeatedly observed in both man and laboratory animals (33) that the cells of the alveolar exudate in densely consolidated areas are themselves capable of destroying pneumococci without the aid of serotherapy. Were this not the case, lung abscesses would be frequent rather than extremely rare sequelae of untreated pneumonia. Neither is there any reason to believe that intravenously injected antibody must neutralize all of the polysaccharide in the pneumonic lesion. Much of the polysaccharide is contained within the consolidated portion of the lesion where it cannot possibly offset the action of antibody at the margin. The apparent ineffectiveness of late serum therapy, therefore, cannot be explained either by the advanced stage of consolidation or by the amount of polysaccharide in the lung. Admitting that the exact cause of death, as in any severe infection, is often obscure, it would seem probable that many failures have been due in the past to inadequate doses of antiserum (43) rather than to the inability of antibody to affect the lesion.

SUMMARY

A uniformly fatal lobar pneumonia was produced in white rats by inoculation of the left main bronchus with virulent Type I pneumococci suspended in mucin. All of the animals succumbed in less than 5 days, half of them dying within 48 hours. In only 5 of 40 rats was the lesion confined to the left lung, and all but one developed pleurisy, pericarditis, or both. All had bacteriemia at the time of death.

The pathogenesis of the pulmonary lesion was studied by examining the lungs of 35 rats killed at various intervals following inoculation. The pneumonic process spread rapidly until most of the left lung was involved in 36 hours. Frequent blood cultures showed invasion of the blood stream in a few rats at 6 hours and in over 90 per cent at the end of the first day. The first signs of pleurisy usually appeared at 18 hours.

Microscopic examination of the actively spreading lesion revealed three characteristic zones: (1) an outer "edema zone" in which the alveoli contained many pneumococci floating freely in edema fluid, (2) a middle zone where both leucocytes and organisms were present, many of the latter being phagocytized, and (3) an inner zone of advanced consolidation in which the alveoli contained many leucocytes but no organisms and where there were already local areas of early resolution. Study of numerous lesions, at intervals of from 12 to 36 hours after inoculation, indicated that the pneumococci spread into normal alveoli principally by way of the infected edema fluid in the outer zone. Pneumococcus-laden edema fluid in large bronchi and in alveoli beneath the pleura suggested the mode of spread of the infection to other lobes and possibly to the pleural cavity. No adequate explanation could be found for the presence of active phagocytosis in the lungs of animals with bacteriemia and presumably without circulating antibodies, but this conspicuous phagocytic reaction was obviously responsible for the clearing of the central part of the spreading lesion.

The action of type specific antibody upon the pulmonary lesion of experimental lobar pneumonia was studied in rats similarly infected but treated with antipneumococcal serum. When injected intravenously in a single dose within 18 hours after inoculation the antiserum was found to protect all of the rats against the otherwise fatal pneumonia. It stopped the spread of the pneumonic lesion, cleared the blood stream of organisms, and prevented the extension of early pleurisy. The antibody caused agglutination and capsular swelling of the pneumococci in the lung, particularly in the edema zone at the margin of the lesion where they were most numerous. Apparently immobilized by agglutination the organisms were

overtaken by leucocytes and destroyed by phagocytosis. The phagocytic reaction was greatly accelerated by the specific opsonins of the antiserum, and the pneumococci were destroyed by polymorphonuclear leucocytes before many macrophages appeared in the alveolar exudate. Within a week after treatment resolution of the pulmonary lesion was well in progress. Both horse and rabbit antibody were shown to penetrate the lung, and immune bodies were demonstrated in the alveoli within 10 minutes after the start of treatment.

The relation of the observed phenomena to the curative action of antipneumococcal serum has been briefly discussed, and it is pointed out that the principal effect of antiserum is to cause immobilization of the pneumococci in the advancing edema zone. Experiments to be reported in a later publication have shown that sulfapyridine exerts a similar effect through a different mechanism.

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EXPLANATION OF PLATES

Sections stained by the Gram-Weigert technique. Figs. 1, 2, 4, 7–13, 16–20 photographed by Dr. G. O. Favorite; Figs. 3, 5, 6, 14, 15 photographed by Mr. L. Talbert.

PLATE 9

FIG. 1. Gross lesion in rat dying of pneumonia 90 hours after inoculation. Unilobar left lung is completely consolidated. Interlobar fissure between upper, middle, and lower lobes can be seen in the right lung. Small postcardiac lobe is not visible in posterior view. × 2.

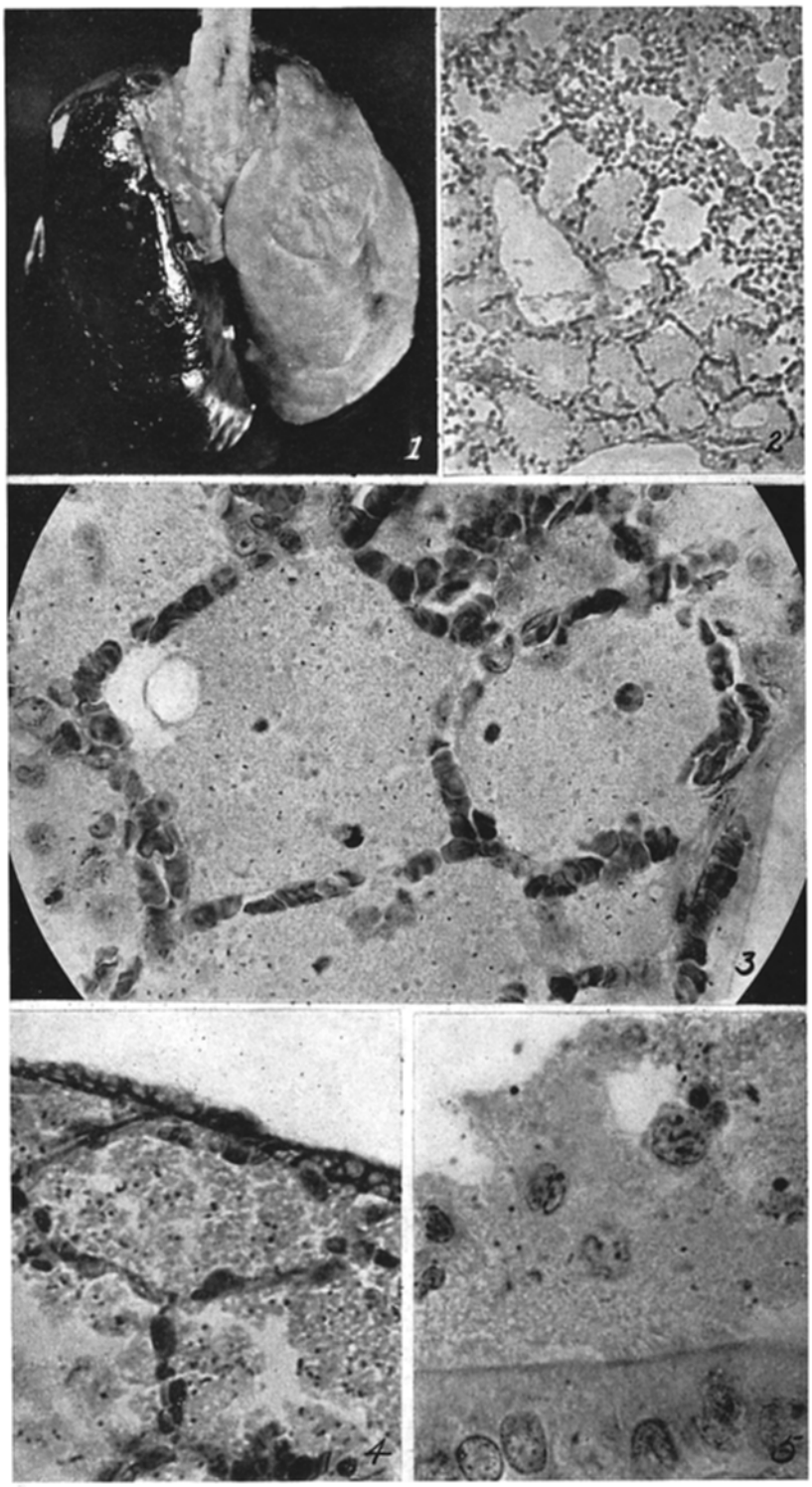
FIG. 2. Edema zone at margin of lesion. No leucocytes in edema-filled alveoli. Leucocytic exudate can be seen in alveoli bordering on inner margin of edema zone (upper right). Lesion is spreading downward toward left. × 160.

(Figs. 2 to 9 are all photomicrographs of 24 hour lesions.)

FIG. 3. Pneumococci in edema-filled alveoli at margin of lesion. Leucocytes have not yet entered these alveoli. Organisms apparently float about in edema fluid while multiplying freely. × 800.

FIG. 4. Pneumococcus-laden edema fluid in alveoli bordering on visceral pleura. Suggests possibility of direct invasion of pleural cavity. × 800.

FIG. 5. Pneumococci in edema fluid contained within the lumen of a large bronchus. Infected bronchial fluid probably causes spread of pneumonia to other lobes. × 1250.



(Wood: Mechanism of recovery in pneumococcal pneumonia. I)

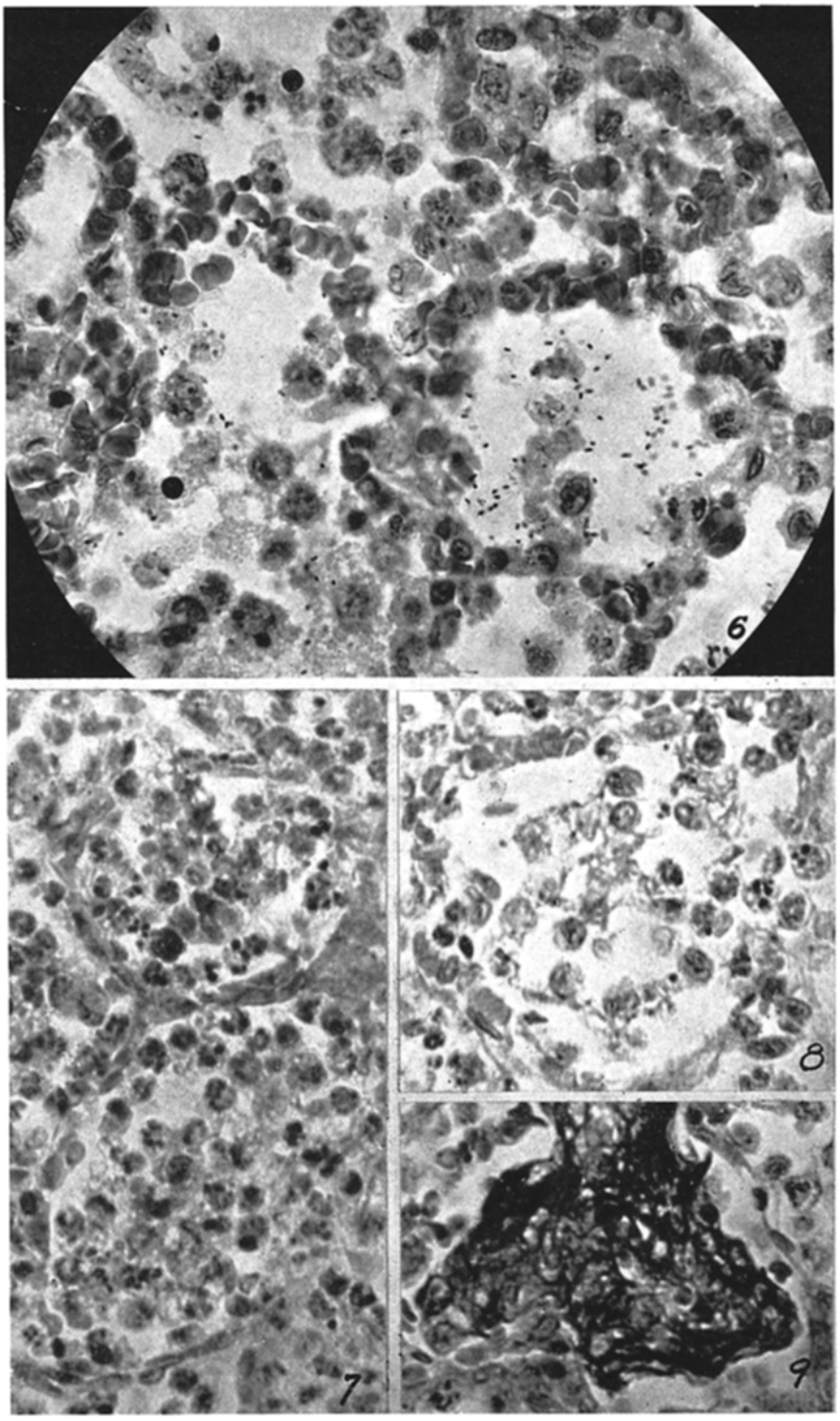
PLATE 10

FIG. 6. Both pneumococci and leucocytes in alveoli within zone of early consolidation. This area borders on outer edema zone (see Fig. 2). Organisms are plentiful, but a few are already being phagocytized by leucocytes, although rat had bacteriemia when sacrificed. × 800.

FIG. 7. Leucocytic exudate in inner zone of advanced consolidation. Pneumococci have been phagocytized and are no longer visible. Cells of exudate are still predominantly polymorphonuclear. × 800.

FIG. 8. Early macrophage reaction and clearing of exudate in central area of advanced consolidation. Rat had heavy bacteriemia when sacrificed. × 800.

FIG. 9. Fibrin deposits in alveoli in zone of consolidation near center of lesion. × 800.



(Wood: Mechanism of recovery in pneumococcal pneumonia. I)

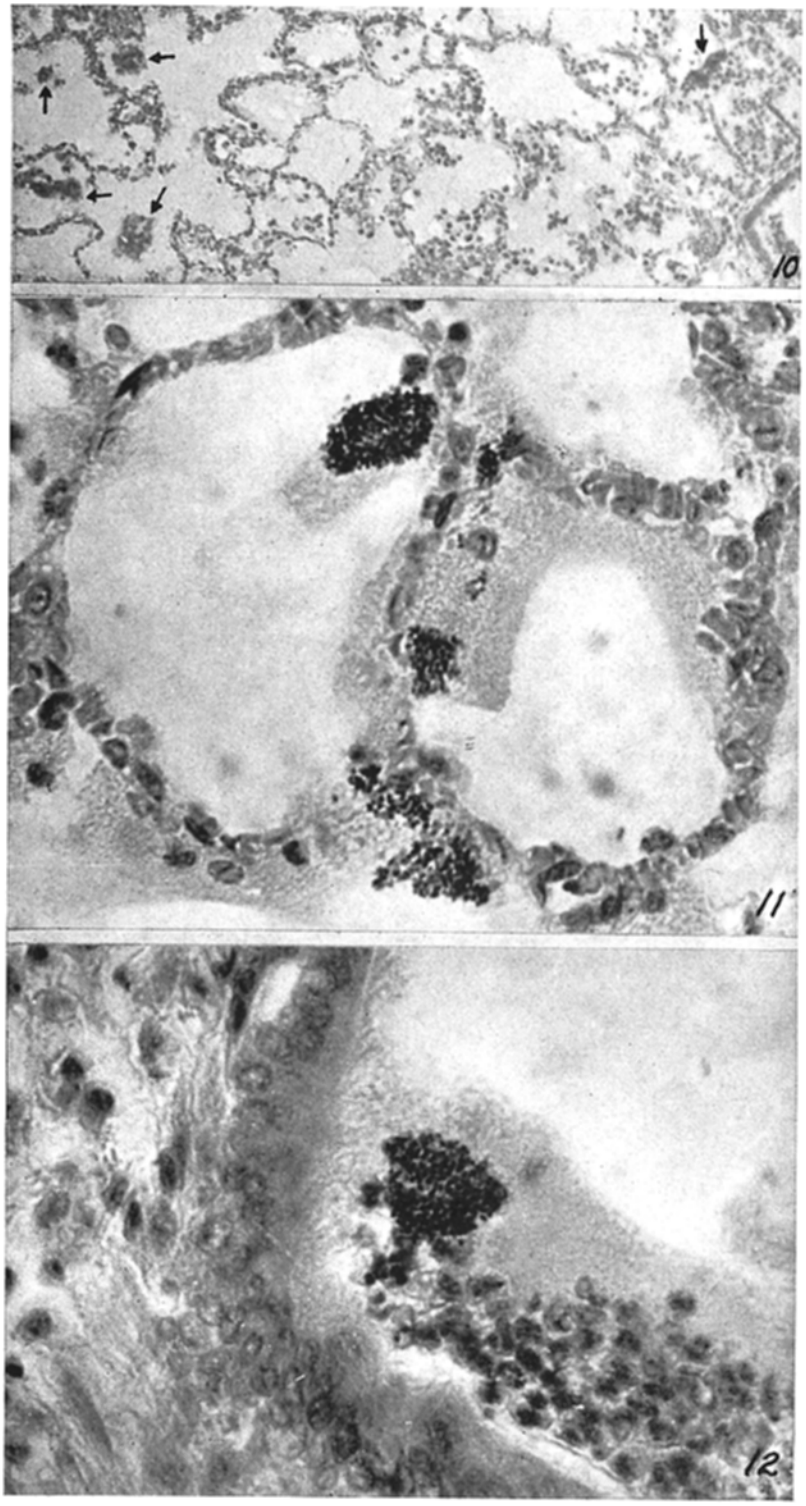
PLATE 11

FIG. 10. Clumps of agglutinated pneumococci (see arrows) in edema zone at margin of lesion. Note small vacuoles in edema fluid. The rat was sacrificed 6 hours after treatment. × 160.

(All figures except 16, 17, and 20 are photomicrographs of lungs taken from rats treated 18 hours after inoculation. Fig. 16, 17, and 20 show lesions in animals treated at 12 hours.)

FIG. 11. Agglutination of pneumococci within the alveoli 6 hours after treatment. No leucocytes have as yet reached these alveoli in the outer edema zone. Many of the organisms appear to cling to the alveolar walls as well as to each other. × 800.

FIG. 12. Agglutinated pneumococci within the lumen of a large bronchus. Although many leucocytes are present in the bronchial exudate few contain organisms. × 800.



(Wood: Mechanism of recovery in pneumococcal pneumonia. I)

PLATE 12

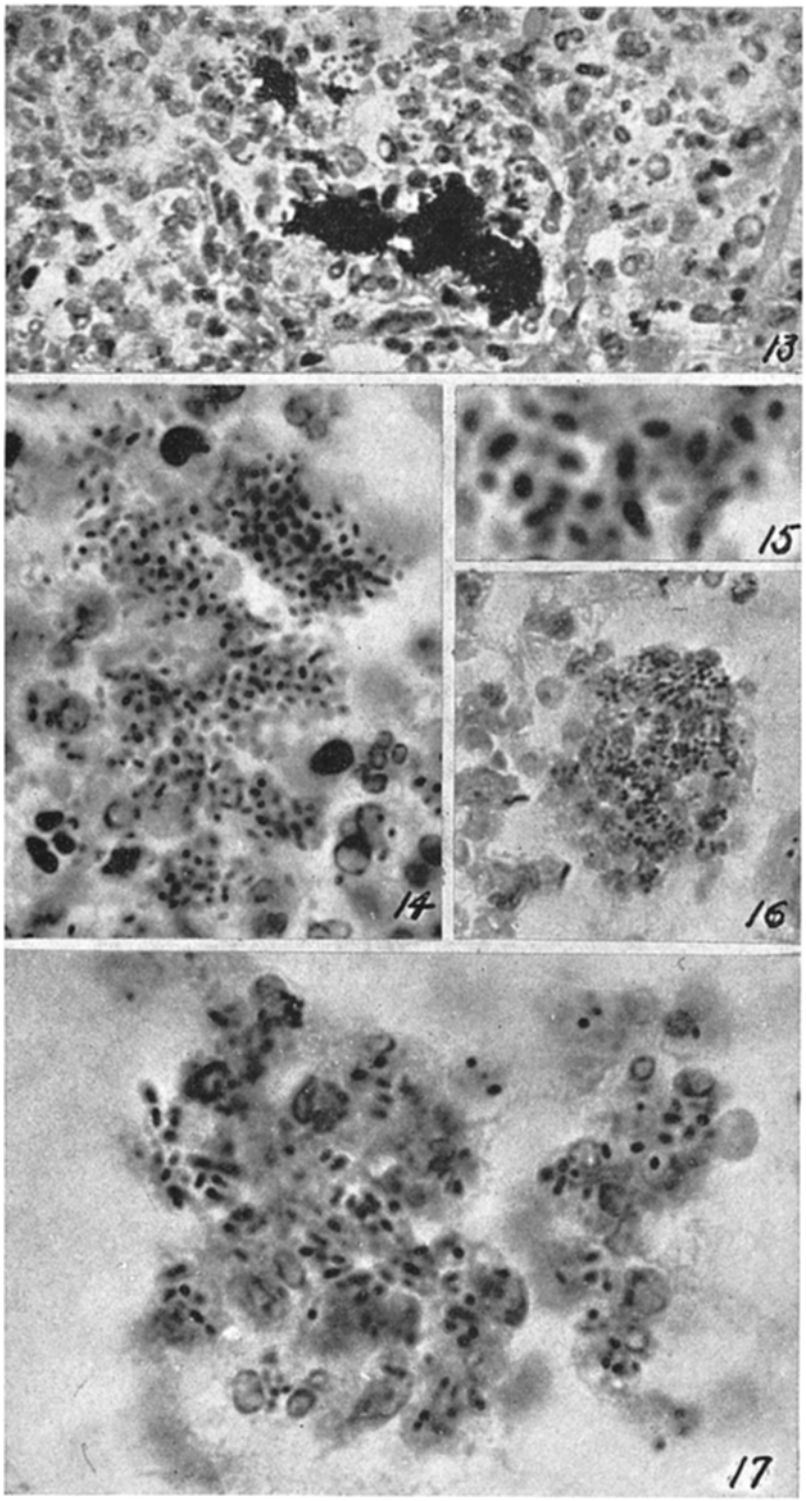
FIG. 13. Large clumps of agglutinated pneumococci in an area of early consolidation 6 hours after treatment. The large size of the clumps may be due in part to the continued multiplication of the agglutinated organisms. It seems unlikely that leucocytes can attack such large masses of bacteria directly, and it is conceivable that autolysis plays an important rôle in their final destruction. Agglutination of pneumococci in such areas indicates that antibody can penetrate the zone of early consolidation as well as the edema zone at the margin of the lesion. × 800.

FIG. 14. Agglutinated pneumococci with swollen capsules characteristic of Neufeld *Quellung* reaction. Rat sacrificed 6 hours after treatment. × 1500.

FIG. 15. Higher magnification of *Quellung* reaction. × 3750.

FIG. 16. Phagocytosis of pneumococci by polymorphonuclear leucocytes in alveolar exudate 6 hours after treatment. × 800.

FIG. 17. Higher magnification of leucocytes containing pneumococci with swollen capsules. × 1800.



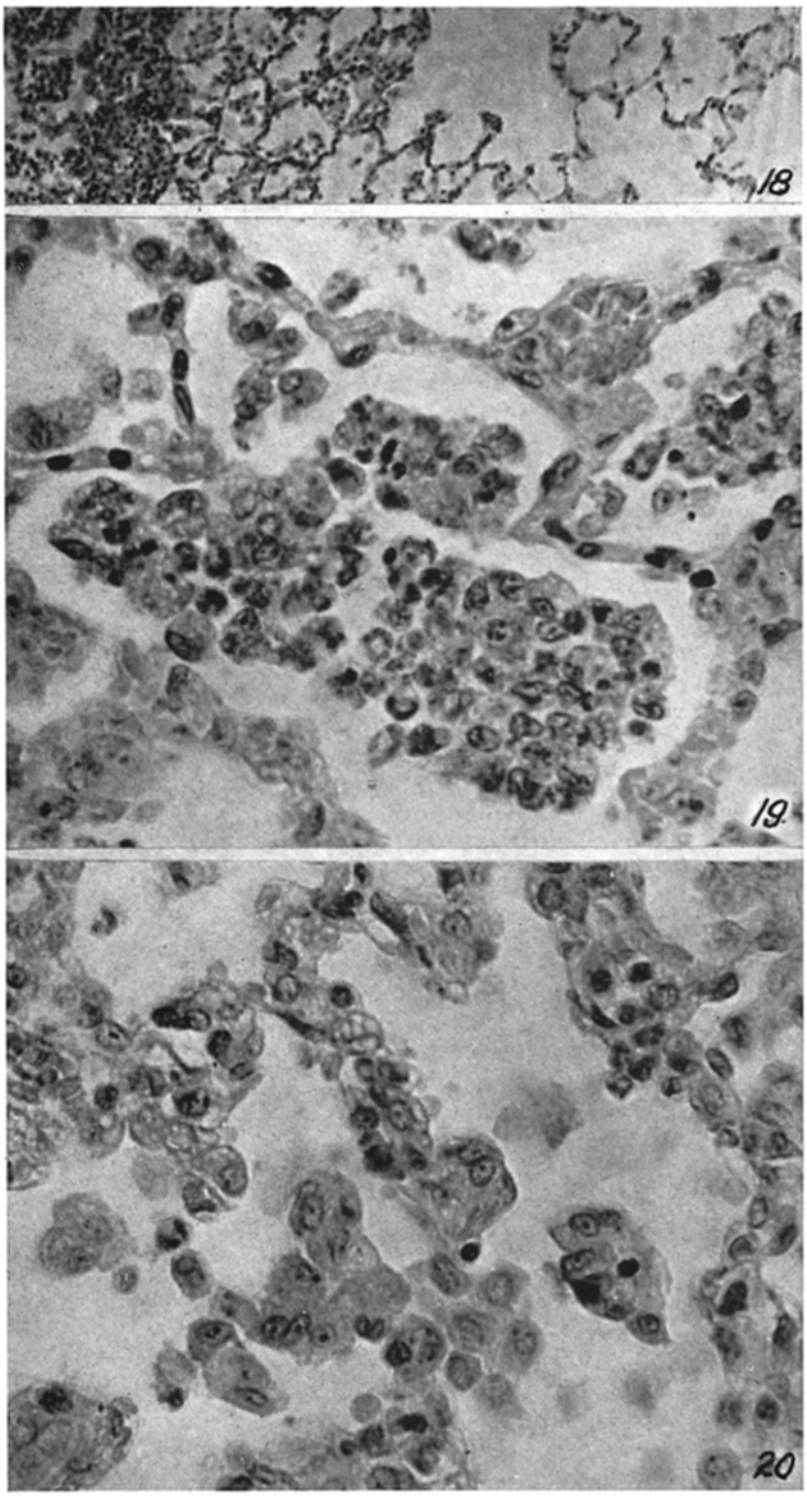
(Wood: Mechanism of recovery in pneumococcal pneumonia. I)

PLATE 13

FIG. 18. Margin of lesion 18 hours after treatment showing complete disappearance of edema zone. × 160.

FIG. 19. Alveolar exudate near margin of lesion 42 hours after treatment. The pneumococci have been destroyed by the phagocytic cells. Although macrophages are present the exudate still contains many polymorphonuclear leucocytes. × 800.

FIG. 20. Advanced macrophage reaction with marked clearing of the lesion 1 week after treatment. The alveolar walls are noticeably thickened and a few large pale macrophages can be seen free in the alveoli. × 800.



(Wood: Mechanism of recovery in pneumococcal pneumonia. I)