HOST-PARASITE RELATIONSHIPS IN EXPERIMENTAL PNEUMONIA DUE TO PNEUMOCOCCUS TYPE III*

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PLATES 3 AND 4

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Most strains of type III pneumococcus possess a particularly high virulence for man (1) once infection has become established. Recent study of this organism in relation to phagocytosis (2) has revealed that strains of maximum virulence exhibit during logarithmic growth, a wide capsular "slime layer" which interferes with surface phagocytosis. The slime layer can be visualized by electronic microscopy and stains metachromatically with methylene blue. The metachromasia is due to the presence in the slime layer of viscous type III polysaccharide, a high molecular polymer of 4-β-glucuronosidoglucose (3). In broth cultures the capsular slime layer is lost with aging of the bacterial population, and pneumococcus III is then, like other encapsulated bacteria, susceptible to surface phagocytosis.

Recovery from acute bacterial pneumonia has been shown to depend in part upon surface phagocytosis, a defense reaction of the host that operates in the absence of specific antibodies (4–6). Since virulent strains of type III pneumococci in the logarithmic phase of growth are resistant to this form of phagocytosis, experiments have been performed to determine the influence of the slime layer upon the *in vivo* behavior of pneumococcus III. Systematic study of induced pulmonary infection has revealed certain host-parasite relationships that appear to account for the relative malignancy of type III pneumonia.

Characteristics of Pneumonic Lesion Caused by Highly Virulent Strain of Type III Pneumococcus

Fatality Rate and Survival Time.—Experimental pneumonia was produced in albino rats by the method previously described (7). A strain of type III pneumococcus (8HCC), the capsular properties of which have been determined by *in vitro* studies (2), was grown for 4 hours in serum-beef infusion broth (2), suspended in mucin, and inoculated intrabronchially. The number of organisms introduced into the left main bronchus varied from 500 to 5000. The resulting pneumonia was uniformly fatal, all of 72 untreated rats dying within 96 hours after inoculation. Although 14 of the infected animals failed to survive for 24 hours, the great majority (46) died on the 2nd day of the infection. The mean survival time was shorter than that observed in comparable experiments with type I pneumococcus (Text-fig. 1).

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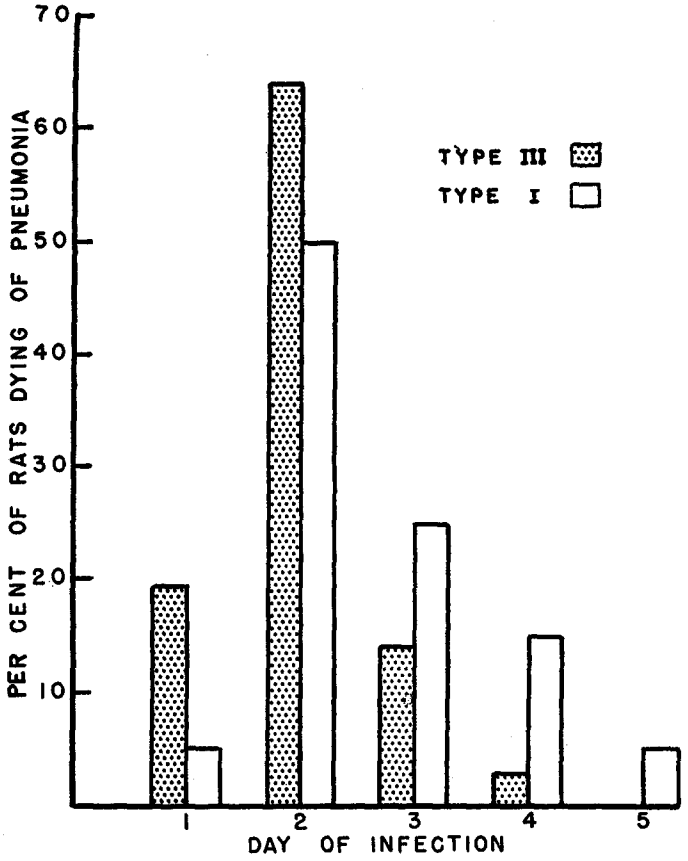
Gross Pathology.—All rats dying of untreated type III pneumonia exhibited both pleurisy and pericarditis. The pleurisy was usually fibrinous and was accompanied by a cloudy effusion which more often than not was bilateral. Similar changes were noted in the pericardial cavity. In animals surviving for more than 36 hours the exudate was thick and gelatinous.



TEXT-FIG. 1. Comparative survival times of rats dying of type I and type III pneumonias.

The progress of the pneumonic lesion in the left lobe was studied by sacrificing animals at various stages of the infection as shown in Text-fig. 2. It will be noted that the lesion continued to spread until most of the lobe was involved at the end of 36 hours.

In its gross appearance the pneumonic lesion caused by type III pneumococcus simulated that of experimental type I pneumonia (7) with one important exception. The type III lesions in animals sacrificed after 36 hours frequently

showed evidence of abscess formation similar to that seen in Friedländer's pneumonia (8).

Histopathology.—Details of the histopathology of experimental pneumonia caused by type I pneumococcus and Friedländer's bacillus have been published elsewhere (7, 8). The same techniques of fixing the lungs and of sectioning and staining the tissues (Gram-Weigert stain) were used in the present experiments. Animals were sacrificed for study at the intervals designated in Text-fig. 2. Microscopic findings were, with the exceptions mentioned below, identical with those of experimental type I pneumonia. The same edema zone was noted at the margins of the spreading lesions (Fig. 1). In the more central area of consolidation phagocytosis by polymorphonuclear leucocytes was prominent (Fig. 2).

HOURS AFTER INOCULATION	2	6	12	18	24	36
APPROXIMATE SIZE OF PULMONARY LESION*						
BLOOD CULTURE POSITIVE	0	0	11	21	17	10
BLOOD CULTURE NEGATIVE	4	28	17	2	1	10
INCIDENCE OF BACTERIEMIA	0%	0%	39%	87%	94%	100%

TEXT-FIG. 2. Extent of pulmonary lesion and incidence of bacteriemia at various stages of experimental type III pneumonia.

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

Clearing was seen in some of the older parts of the lesions and was characterized by the presence of macrophages (Fig. 3).

Three histologic features, not present in type I pneumonia, were prominent in the type III lesions. First, as already stated, areas of frank abscess formation were frequently noted after 36 hours (Fig. 4). The pneumococci seen in the necrotic exudate of these abscesses were often pleomorphic and poorly stained, indicating local bacteriostasis. Secondly, the number of pneumococci per alveolus in certain parts of the lesions was far greater than that ever observed in type I infections (Fig. 5). In these most heavily infected areas there was often little or no phagocytosis, and necrosis of leucocytes and cells of the alveolar walls was noted, suggesting the early stages of an abscess. Thirdly, a coarse reticular precipitate was visible in certain of the infected edema-filled alveoli at the periphery of the lesion (Fig. 6). Evidence is presented in the next section that this

reticular precipitate contained excess capsular polysaccharide synthesized by the growing pneumococci.

Relation of Capsular Polysaccharide to Histologic Features of Lesion

Method of Study.—The capsular polysaccharide, when present either in the wide slime layer of type III pneumococcus or in the surrounding medium, stains metachromatically with methylene blue. Treatment with chemical fixatives, such as Zenker's solution, formalin, or alcohol so alters the high molecular polysaccharide as to prevent the metachromasia. In order to study the capsular carbohydrate in lung sections, therefore, it is necessary to avoid the use of chemical fixatives in preparing the tissue for staining with methylene blue. The following method was found to be satisfactory for this purpose.

At necropsy the aorta was clamped while the heart was still beating; the pulmonary vessels were tied off with a single ligature (7), and the lungs were sufficiently reinflated with air to fill the thoracic cage. When the trachea had been ligated, the lungs were removed from the thorax, wrapped in filter paper, and frozen immediately in the dry ice box. The frozen tissues were cut on a rotary microtome in the cold room (0–5°C.), the knife blade being "frozen" with CO_2 gas just prior to the sectioning of each block. The sections cut at a thickness of 5 micra were transferred immediately to clean glass slides where they were allowed to dry. They were then stained for 1 minute with alkaline methylene blue (2), washed with tap water, dried, and mounted in balsam.

To supplement this frozen section technique impression smears were made of representative fresh lesions and were likewise air-dried and stained with methylene blue. Such smears contained leucocytes, bacteria, and tissue fluid from the alveolar exudate, but in contrast to the frozen sections they did not depict the general architecture of the pneumonic process and contained extraneous red blood cells derived from pulmonary vessels transected in the cutting of the fresh tissue.

The Capsular Slime Layer and Phagocytosis.—The capsular slime layer of type III pneumococcus renders the organism relatively resistant to surface phagocytosis *in vitro* (2). The effect of this outer capsular structure *in vivo* was determined by study of frozen sections and impression smears of lesions from animals sacrificed 6, 18, 30, and 48 hours after inoculation. For the most part only organisms that had lost their metachromatic slime layers, presumably through aging of the local bacterial population, were phagocyted by the polymorphonuclear leucocytes. The partially denuded organisms were most numerous in the older, more heavily consolidated parts of the lesion. Most of the pneumococci that had not been phagocyted and were located in the more recently developed peripheral portions of the lesion still exhibited the wide outer capsular layer (Fig. 8). Intermediate areas often contained pneumococci both with and without slime layers, and in these alveoli the preferential phagocytosis of the denuded organisms was particularly striking (Fig. 9). As in the *in vitro* experiments some intracellular slime-covered pneumococci were occasionally

found, particularly in macrophages, but this finding was the exception rather than the rule.[1]

Polysaccharide in the Alveolar Exudate.—Free capsular substance, which stained metachromatically, was visible in the alveolar exudate of many parts of the more advanced pneumonic lesions. The metachromatically stained material in the exudate was shown to contain capsular polysaccharide by the fact that its characteristic staining properties were destroyed by pretreatment of the impression smears with homologous type-specific antiserum (2). Addition of heterologous antiserum to the smears prior to application of the methylene blue stain had no effect upon the metachromasia. Although the free capsular substance usually appeared as a pink-staining, amorphous precipitate in the exudate (Fig. 10), it sometimes formed a reticular matrix (Fig. 11). Some of the organisms visible in the precipitate had prominent capsular slime layers, whereas others, having lost this outer capsular structure, exhibited no stainable capsule at all. Intermediate forms that appeared only partially denuded were also present.

The metachromatic capsular substance in the exudate was most concentrated in those areas in which bacterial growth was maximum. Since early necrosis of leucocytes and other tissue cells was also evident in these same regions of maximum infection, the question arose as to whether the free capsular substance might not be cytotoxic and thus contribute to abscess formation.

Possible Leucocidal Action of Type III Polysaccharide.—Accordingly *in vitro* phagocytic tests on filter paper using pneumococcus type I (4) were carried out in the presence of an excess of capsular material derived both from serum-broth cultures and from infected pleural fluid.[2] The presence of the capsular carbohydrate in the test mixtures was easily identified by: (*a*) its metachromatic staining properties and (*b*) its precipitation by type III antiserum. No evidence could be obtained that the polysaccharide affected the leucocytes sufficiently to interfere with surface phagocytosis. Likewise phagocytosis of encapsulated

[1] In unpublished *in vitro* experiments high concentrations (5 to 50 per cent) of normal rat serum, containing no demonstrable type-specific opsonins, have been found to increase slightly the susceptibility of slime-covered type III pneumococci to surface phagocytosis. This observation may account in part for the phagocytosis of slime-covered organisms occasionally noted *in vivo* in the apparent absence of specific antibody.

[2] When serum-broth cultures were used for the source of excess polysaccharide, the cultures were incubated for 24 to 48 hours and the bacteria were removed by centrifugation. Pleural fluid of high polysaccharide content was obtained from rats inoculated intrapleurally with approximately 20 type III pneumococci and treated 18 hours after inoculation with 3000 units of procaine penicillin (duracillin). Treatment was administered intramuscularly every 12 hours, and the animals were sacrificed at the end of 3 days. When the thorax was opened, 0.2 ml. of liquaemin (Roche Organon, Inc.) was added to the pleural fluid to prevent clotting, and the fluid, which was shown by culture to contain no viable pneumococci, was centrifuged for 30 minutes at 3000 R.P.M. The final supernatant contained large amounts of polysaccharide.

test organisms (type I pneumococcus) took place as usual when leucocyte-bacterial mixtures were incubated on the cut surfaces of type III lesions containing large amounts of polysaccharide. In addition, fluid from cultures and pleural exudates of high polysaccharide concentration were injected both intrabronchially and subcutaneously to determine the effect of the capsular carbohydrate upon normal tissues. No signs of necrotoxicity were noted in the histologic sections from any of the test animals. It is concluded, therefore, that type III polysaccharide, in the form in which it exists in the animal body, does not injure significantly either leucocytes or other tissue cells.

Growth and Survival of Type III Pneumococcus in Pneumonic Lesion

As already stated, histologic study of type III pneumonia revealed an excessive number of organisms in some parts of the lesion. Also, in these areas of maximum bacterial growth there was relatively little phagocytosis, most of the extracellular organisms having prominent slime layers. Because of the previously demonstrated resistance of the slime-covered type III organisms to surface phagocytosis (2), it seemed likely that pneumococcus III might survive more readily in the alveolar exudate than pneumococcus I and thus might produce a more heavily infected lesion. The number of viable organisms per gram of consolidated lung was therefore determined at various stages of both type I and type III pneumonia. The method used to obtain these "*in vivo* growth curves" was as follows:—

Three rats with each of the two types of pneumonia were killed 6, 18, 30, and 48 hours after inoculation. The thorax was opened aseptically and the infected left lung was removed and placed in a sterile Petri dish. From a representative portion of the consolidated lesion a wedge-shaped sample was cut, weighed, and ground with sterile sand in a mortar. The ground tissue was diluted 1 to 10 with tryptose phosphate broth and bacterial counts were then made in the usual manner.

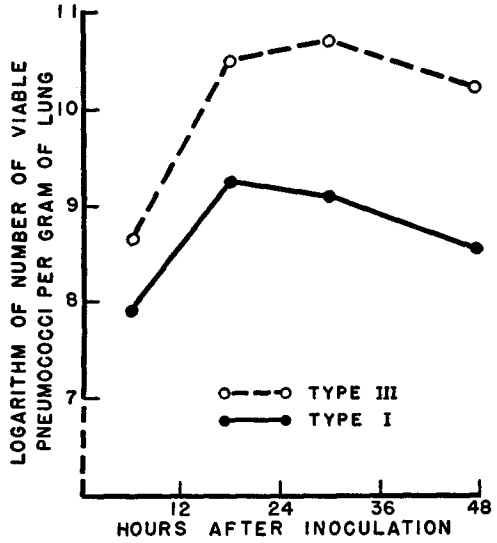
As shown in Text-fig. 3, there were, in all but the earliest lesions, more than ten times as many viable pneumococci per gram of tissue in the type III infections as in those caused by pneumococcus I. Although the method of quantitation used was admittedly crude, the mean differences noted in the two infections were found to be statistically significant.[3]

Response to Chemotherapy

Rats with both type III and type I pneumonia were treated with sulfadiazine according to the method previously described (9). 300 mg. of sulfadiazine was given by stomach tube 6 hours after inoculation, 12 hours later, and every 24

[3] The statistical analyses were kindly performed by Dr. Robert Shank of the Department of Preventive Medicine. The ratios of the $\frac{\text{Difference in means}}{\text{Standard error of difference}}$ for the four points on each of the curves were found to be 3.06, 6.6, 8.8, and 1.02 respectively.

hours thereafter throughout the period of observation. The effect of the drug upon the type III infection[4] was similar to that previously reported in type I pneumonia (10). Spread of the lesion stopped within 18 hours after the start of treatment. Serial histologic studies revealed the same prominent phagocytic reaction noted in the type I infection. The one important difference observed in the type III animals was the frequent occurrence of abscesses in lesions that otherwise appeared to be responding favorably to the chemotherapy. Nearly all the lungs examined after the first day of infection had definite abscesses, and multiple areas of suppuration were common particularly in the older lesions.



TEXT-FIG. 3. Bacterial counts in the pulmonary lesions of experimental type I and type III pneumonias.

By the 5th day the abscesses were beginning to be walled off by fibroblastic proliferation in the alveoli adjacent to the dense leucocytic barrier which surrounded the necrotic core of the abscess (Fig. 7). The central zone was characterized by ischemia, the presence of fibrin, and relatively few phagocytic cells, most of which were in varying stages of disintegration. Pleomorphic bacteria were visible in small numbers within the necrotic exudate. When cultured at

[4] When pneumococcus III is incubated in beef infusion broth (pH 7.8) containing 1 per cent rabbit serum and 10 per cent sulfadiazine, a ropy sediment of relatively small volume containing chains of pneumococci entangled in a fibrous precipitate of polysaccharide forms at the bottom of the tube. The sediment is in marked contrast to the smooth viscous centrifugate of large volume obtained from cultures grown in the plain broth. This finding suggests that sulfadiazine disturbs the synthesis of type III polysaccharide, either through bacteriostasis or through some other more direct mechanism.

the end of 2 weeks abscesses were found still to contain viable type III pneumococci despite a full week's treatment with sulfadiazine.

Similar experiments were also done with penicillin, and a detailed account of the results will be published elsewhere (11). Penicillin acted more promptly than sulfadiazine on both the type III and the type I lesions, but again phagocytosis was prominent in the alveolar exudate and appeared to be responsible for the destruction of many of the organisms. In contrast to the findings in type I pneumonia, most of the type III lesions contained abscesses in spite of the chemotherapy. Prolonged treatment with penicillin (2 weeks) failed to rid the abscesses of pneumococci. Estimation of the number of viable organisms in the infected lungs of animals treated with penicillin showed that the destruction of bacteria in the type III lesions was not only less complete (*i.e.* lung abscesses) but also significantly less prompt than in the type I pneumonia, in spite of the fact that the two organisms were equally sensitive to penicillin *in vitro*.

Process of Resolution

Resolution of the pneumonic exudate in the non-suppurating portions of the lesion involved not only the destruction of bacteria and clearing of the exudate but also the removal of the large amounts of capsular polysaccharide which had accumulated in the alveoli. The latter process was found to be relatively slow, there being specific carbohydrate demonstrable in the lesion more than 2 weeks after inoculation. Serial histologic studies revealed that some of the polysaccharide was carried away by lymphatic drainage (Fig.12) to regional lymph nodes. The rest was phagocyted in the alveoli, particularly by macrophages (Fig. 13), which likewise appeared to be removed from the lesion *via* the peritruncal lymphatics. The fact that the polysaccharide antigen could be identified by its metachromatic staining within the cytoplasm of phagocytic cells suggests that it had not combined with specific antibody before being phagocyted (2).

Pathogenesis of Abscess Formation

Since type III pneumococcus, in contrast to type I, was found to produce lung abscesses with surprising regularity, experiments were designed to ascertain, if possible, the factors responsible for the pulmonary suppuration. Type I pneumococcus, when inoculated intrabronchially with mucin in doses of 1000 to 5000, uniformly produces fatal lobar pneumonia, but at no stage of the infection do excessive numbers of extracellular bacteria accumulate in the lesion, and suppuration rarely occurs either in untreated animals (7) or in those receiving penicillin after 18 hours (11).[5] It was found, however, that if the intra-

[5] Suppuration, apparently caused by the irritative action of the mucin, may occur at the original site of inoculation. In the present study only those abscesses clearly located outside the "mucin area" are recorded as significant.

bronchial inoculum is increased to 500,000 or more, the bacterial multiplication is so rapid during the early stages of the infection that the cellular defenses of the lung are overwhelmed, and a dense population of extracellular organisms accumulates in the infected alveoli. By intensive penicillin therapy[6] begun after 18 hours, such malignant infections can be controlled, but examination of the lungs after 5 days invariably reveals extensive abscess formation.

Thus, under the proper conditions, type I pneumococcus will produce the same form of pulmonary suppuration as that caused by maximally virulent strains of pneumococcus III. The type I organisms must be injected intrabronchially in large numbers in order to attain a sufficient population density in the lesion to cause necrosis. A relatively small inoculum (100 organisms) of maximally virulent type III pneumococci, on the other hand, will ultimately cause the same result. The greater tendency of type III pneumococci of maximum virulence to produce lung abscesses appears, therefore, to be due, not to the presence of excessive amounts of free polysaccharide, but to the fact that the capsular slime layer interferes with surface phagocytosis (2) and thereby permits the accumulation of a sufficiently large extracellular population of bacteria to cause tissue necrosis.

Selective Destruction in Vivo of Intermediate Mutant

Evidence has already been presented that phagocytic cells in the lung preferentially phagocyte those type III pneumococci which possess no slime layer. The efficiency with which such organisms are destroyed in the lung as compared to those with intact slime layers is conclusively demonstrated by the following experiment.

Each of 45 rats was inoculated intrabronchially with 4000 to 6000 intermediate type III pneumococci suspended in 0.1 ml. of 6 per cent mucin (7). The intermediate mutant employed was that derived from the 8HCC strain of pneumococcus III in this laboratory (2). Although considerably less virulent than the parent strain, the intermediate mutant caused 31 of the 45 rats to die within 1 week of inoculation. At postmortem examination the lesions were extensive, and all but 3 animals (which died during the first 2 days) showed multiple areas of suppuration. Impression smears of the late lesions, when stained with methylene blue, revealed many slime-covered pneumococci and much excess polysaccharide in the alveolar exudate. Blood cultures taken at the time of death were positive, but the organism present was invariably the mucoid parent strain rather than the intermediate. Only in animals dying within the first 48 hours was the intermediate strain isolated from the blood. Two rats that died at 24 and 25 hours respectively had both intermediate and mucoid organisms in the blood at the time of death. A third animal had intermediates in the blood on day 1, negative blood cultures on days 2 and 3, and a terminal bacteriemia due to the mucoid parent on day 4.

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[6] 6000 units of procaine penicillin in oil (duracillin) injected intramuscularly 6 hours after inoculation and every 12 hours thereafter.

These observations suggest that the intermediate strain underwent a backmutation *in vivo*, the reversion rate having been sufficient to produce in the end a pneumonia due to the maximally virulent parent. Since backmutation was observed neither on repeated plating of intermediate cultures nor during peritoneal infections in mice, it was concluded that reversion to the parent form was brought about primarily by selective destruction of the easily phagocyted intermediate organisms by the highly efficient phagocytic mechanism operating in the lung (4, 5). This conclusion was substantiated by histologic study of serial lesions by the methylene blue technique. Whereas only intermediate organisms were found during the earliest stages of the infection, the pneumococci demonstrable in the older lesions were predominantly of the mucoid type. The transition from intermediate to slime-covered pneumococci could be seen to result from the preferential phagocytosis of organisms lacking the slime layer. The failure of reversion to take place during peritoneal infections in mice was thought to be due to the relative inefficiency of the phagocytic mechanism operating in the peritoneal cavity (4).

Similar experiments were carried out with a transformed intermediate strain originally obtained by growing a rough variant of type II pneumococcus in the presence of transforming substance extracted from an intermediate mutant of type III (12). The stock cultures used for intrabronchial inoculation were made from single cell preparations of the transformed intermediate strain.[7] Although somewhat more stable than naturally occurring intermediate mutants (13), the transformed strain likewise changed to the mucoid form in the pneumonic exudate and ultimately caused a fatal infection. The mucoid organisms were shown to be derived from the parent intermediate strain by the fact that they possessed the specific protein antigen (14) of the original type II pneumococcus.[8] This reversion, occurring in a bacterial population originally derived from a single intermediate cell, clearly indicates the occurrence of a backmutation.

DISCUSSION

The relative malignancy of experimental pneumonia produced with a maximally virulent type III pneumococcus is indicated by the comparatively short survival time of rats infected with the 8HCC strain of pneumococcus III and by the high incidence of multiple lung abscesses complicating the infection. Both of these findings are in keeping with clinical observations relating to type III pneumonia. Case fatality rates in untreated lobar pneumonia of pneumococcal etiology are usually higher in type III cases than in those of any other

[7] Kindly supplied by Dr. Colin MacLeod and Dr. Robert Austrian, Department of Microbiology, New York University College of Medicine.

[8] The tests for type-specific protein antigen were done by Dr. Austrian.

type (1),[9] and only in type III pneumococcal infections does pulmonary suppuration often occur (16, 17).

Maximally virulent strains of pneumococcus III, during the logarithmic phase of growth, produce excessive amounts of capsular polysaccharide and possess on their capsular surfaces a wide slime layer containing loosely packed macromolecules of type-specific carbohydrate (2). During the course of experimental pneumonia, the excess polysaccharide resulting from growth of the organism accumulates in large quantities within the infected alveoli, bronchi, and pleural fluid. Similar deposits of capsular carbohydrate are demonstrable in the lungs of patients dying of type III pneumonia (17, 18) (Fig. 14). Since the long-chained glucose-glucuronic acid polymers of type III carbohydrate are highly asymmetrical molecules, and since they cause liquid media in which they are dispersed to be of high viscosity (19), it is assumed that the characteristic viscous consistency of type III exudates is due to the presence of free polysaccharide. Likewise, the sputum from patients with type III pneumonia is often of a jelly-like consistency and contains large quantities of capsular carbohydrate (20) which forms a characteristic reticulum (21).

It might be assumed that the presence of such large amounts of type-specific carbohydrate in the lesion would interfere with recovery of the host, either by damaging phagocytic or other cells in the lung or by neutralizing type-specific antibody. No evidence was obtained in the present study that type III polysaccharide is either leucocidal or of sufficient toxicity to tissue cells to cause necrosis.[10] Its possible neutralization of antibody would appear to be of significance only in relatively long standing infections, since evidence has been presented elsewhere that type-specific antibody does not play an important role in recovery from experimental pneumonia until after the 3rd day of the disease (22). The excess polysaccharide which forms the capsular slime layer on the surface of the type III pneumococcal cell does, on the other hand, have a definite effect upon pathogenicity. Its presence interferes with surface phagocytosis in the lung and thus allows the organism to produce a more heavily infected pneumonic lesion than is caused by other types of pneumococci.

Fortunately for the host the slime layer of pneumococcus III is present only during the logarithmic phase of growth (2) and therefore fails to protect the organism against surface phagocytosis except during the earliest stages of the bacterial invasion. When spread of the pneumonic process is checked by effective chemotherapy, those pneumococci not killed outright by the antimicrobial

[9] Case fatality rates in pneumococcal pneumonia vary considerably in different series, many of which include too few cases to be statistically significant. In the largest single series of type III cases reported (15) the fatality rate was 60.6 per cent—a figure considerably higher than any encountered in pneumococcal pneumonia of other types.

[10] Its effect on chemotaxis was not studied.

drug ultimately lose their slime layers and are destroyed by alveolar phagocytes. Only in foci of necrosis, where the majority of phagocytic cells are non-viable, can organisms survive indefinitely.

In contrast to the rapid destruction of bacteria in the resolving lesion, the excess polysaccharide deposited in the alveoli can be demonstrated to persist *in situ* for many days. This finding is in keeping with Graeser's studies on experimental canine pneumonia (23) and with the clinical observation that specific carbohydrate may be excreted in the urine for several weeks after recovery (24). Phagocytosis of the specific polysaccharide by macrophages in the lung, and also in regional lymph nodes, raises interesting questions relating to antibody formation. Since type III polysaccharide will not stain metachromatically with methylene blue when combined with antibody, it appears that the metachromatic polysaccharide demonstrated within the cytoplasm of phagocytic cells was phagocyted in the absence of antibody. What this process of phagocytosis has to do with the mechanism of antibody formation remains to be determined. The unique staining properties of type III polysaccharide, which are nullified by neutralization with antibody and therefore permit identification of the free antigen both inside and outside of cells, should make the type III carbohydrate particularly useful in the study of antibody synthesis.

The tendency of type III pneumococcus to produce pulmonary necrosis is best demonstrated in rats treated with penicillin or sulfonamide. Whereas the pneumonia promptly resolves under treatment, the lung abscesses fail to respond and remain as obvious residua of the pulmonary infection. Their pathogenesis appears to be related to the accumulation of large numbers of viable organisms in certain parts of the pneumonic lesion. The antiphagocytic effect of the capsular slime layer enables pneumococcus III to resist phagocytosis and thus to continue to multiply in the alveolar exudate. Cultural methods have shown more viable pneumococci to be present in type III lesions than in type I, and histologic studies reveal foci of denser bacterial population in experimental type III pneumonia than are ever observed in comparable type I infections. Only when very large inocula are used to produce type I pneumonia, do sufficient organisms survive in the exudate to cause pulmonary suppuration. In the areas of excessive bacterial concentration within the experimental lesions, phagocytosis is depressed, alveolar capillaries are thrombosed, and early signs of necrosis are evident. The findings relative to phagocytosis are in keeping with the observation that leucocytes, when exposed to large enough numbers of viable pneumococci *in vitro*, not only fail to phagocyte the bacteria but also become non-motile and undergo lysis (25). Products of the viable organisms appear also to cause capillary thrombosis which leads to necrosis (26) and abscess formation.

The pathogenesis of lung abscess in Friedländer's pneumonia appears to

differ from that caused by pneumococcus III, since Friedländer's bacillus does not produce a wide slime layer either *in vitro* or *in vivo* (25) and therefore is relatively susceptible to surface phagocytosis (5). As reported previously, however, bacilli accumulate in very large numbers in certain parts of the Friedländer's lesion where they appear to cause vascular and cellular damage which eventuates in abscess formation (8). The accumulation of such large numbers of bacteria seems to be due to an ability of Friedländer's bacillus to grow rapidly in the pneumonic exudate rather than to any tendency of its capsule to protect it against surface phagocytosis. Whether the free polysaccharide which accumulates in the lesion exerts an antiphagocytic effect remains to be determined.

It should be emphasized that the incidence of lung abscesses in experimental type III pneumonia in rats is much higher than that encountered in the natural disease in man. Likewise, lung abscesses can be produced experimentally with large inocula of type I pneumococcus, whereas pulmonary suppuration is rarely, if ever, demonstrable in human patients as a result of type I pneumonia. These differences are probably accounted for by the fact that the experimental infections, which were originally designed to be uniformly fatal (7), are considerably more severe than those ordinarily encountered in clinical practice (15). Although analogies between the experimental and naturally occurring infection must be made with caution, it is considered significant that the same qualitative difference observed clinically between type I and type III pneumonia regarding abscess formation is demonstrable in experimental pneumonia when relatively small inocula are used.

The clinical significance of intermediate mutants of type III pneumococcus is not known. It is conceivable that intermediate strains lacking the protective slime layer may occasionally cause relatively mild type III pneumonia. The present studies indicate, however, that intermediate mutants will, during the course of pneumonia, backmutate and ultimately predominate in the pneumonic lesion due to selective destruction of the intermediate forms by phagocytic cells. This finding emphasizes the importance of the capsular slime layer of pneumococcus III and suggests that, once established in the human lung, initially mild intermediate infections may revert to serious pneumonia caused by type III organisms of maximum pathogenicity.

SUMMARY

Experimental pneumonia was produced with a highly virulent strain of type III pneumococcus which synthesizes, during rapid growth, large amounts of capsular polysaccharide. The type III pneumonia differed from that caused by pneumococcus I in that (*a*) death occurred more promptly in the type III infection, (*b*) the local pulmonary lesion became more heavily infected, and (*c*) frank suppuration was common even after otherwise effective chemotherapy. The

greater pathogenicity of the type III organism was shown by special histologic techniques to be due primarily to its capsular slime layer which interferes with surface phagocytosis. Capsular polysaccharide shed from the organism during growth was also demonstrated in high concentration in certain parts of the pneumonic lesion. Removal of the excess polysaccharide from the alveoli resulted from (*a*) lymphatic drainage to regional lymph nodes and (*b*) phagocytosis, particularly by macrophages. The possible relationship of the free carbohydrate to the malignancy and the characteristically viscous exudate of type III pneumonia was discussed.

The lung abscesses which resulted from type III infection were observed to occur in those areas in which the maximum number of organisms had accumulated. Evidence was obtained that suppuration was due, not to necrotoxic products peculiar to the type III pneumococcus, but rather to the survival of large numbers of bacteria in the tissues, brought about primarily by the antiphagocytic effect of the slime layer.

When pneumonia was produced with an intermediate type III mutant lacking the protective slime layer, back mutation to the mucoid parent occurred during the course of the infection, and the mucoid form eventually predominated in the lung as a result of selective phagocytosis of the intermediate organisms. Similar mutation to the maximally virulent type III form was noted with a transformed intermediate type III strain grown from single cell preparations.

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EXPLANATION OF PLATES

Photomicrographs made by Cramer Lewis, Department of Illustration, Washington University School of Medicine.

PLATE 3

Sections stained by Gram-Weigert technique.

FIG. 1. Infected edema fluid in alveoli at periphery of spreading lesion. Rat sacrificed 30 hours after inoculation with pneumococcus type III. × 900.

FIG. 2. Phagocytosis of type III pneumococci in consolidated portion of same lesion. × 1500

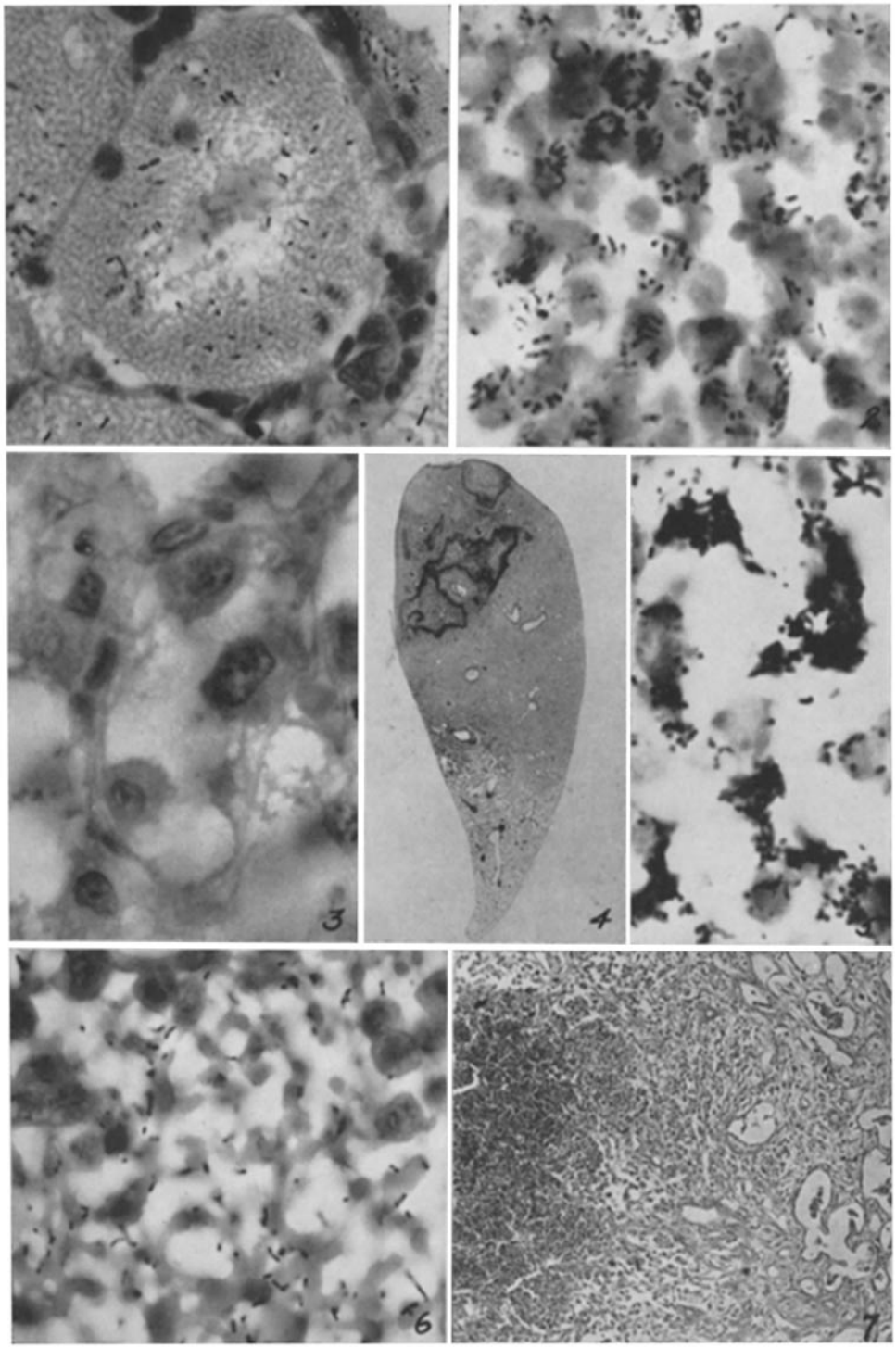
FIG. 3. Macrophage reaction in central portion of lesion where resolution has already begun. × 1500.

FIG. 4. Abscess formation in resolving type III lesion of rat treated for 78 hours with penicillin. Animal was infected 18 hours before start of therapy. × 2.5.

FIG. 5. Accumulation of a great number of pneumococci within a localized area of the lesion depicted in Figs. 1, 2, and 3. × 1500.

FIG. 6. Reticular precipitate contained within some of the alveoli in the edema zone of the same lesion. × 1000.

FIG. 7. Fibroblastic proliferation in alveoli surrounding necrotic core of abscess caused by type III pneumococcus. Rat treated with penicillin and sacrificed on day 5. × 100.



(Wood and Smith: Host-parasite relationships in pneumonia)

PLATE 4

Slides stained with methylene blue.

FIG. 8. Failure of leucocytes in zone of early consolidation to phagocyte type III pneumococci possessing wide, metachromatically stained slime layer. Rat sacrificed at 18 hours. Impression smear, × 1500.

FIG. 9. Selective phagocytosis of type III pneumococci lacking wide slime layer. Photograph shows leucocytes from more central zone of lesion where consolidation is further advanced. Pneumococci with wide slime layer have resisted phagocytosis. Impression smear, × 1500.

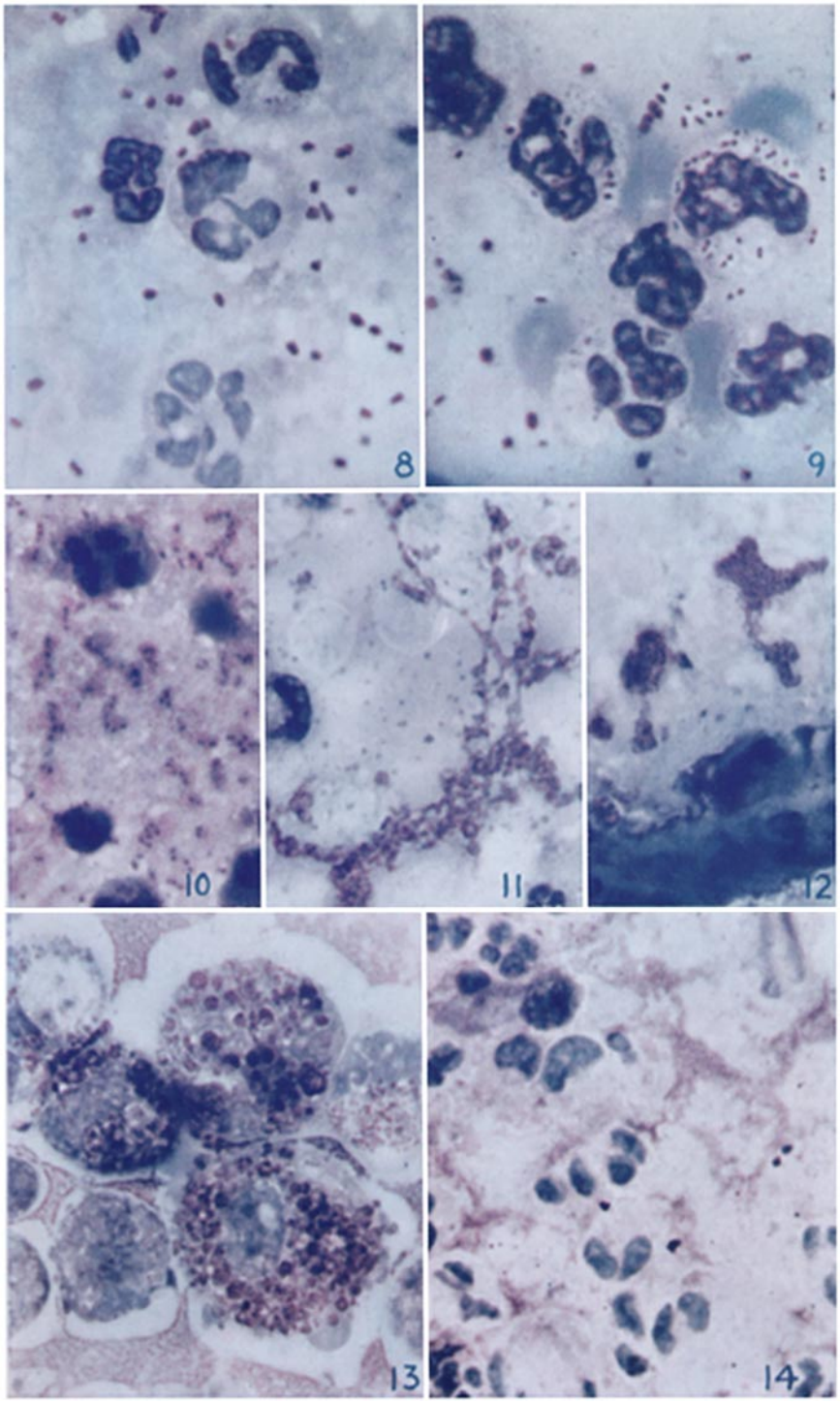
FIG. 10. Amorphous precipitate of free capsular polysaccharide in alveolar exudate of 50 hour lesion. Pneumococci both with and without visible slime layers are present in the precipitate. Impression smear, × 1500.

FIG. 11. Reticular precipitation of free polysaccharide in lesion from rat sacrificed at 48 hours. Note pneumococci with broad, metachromatically stained slime layers within meshes of reticulum. Impression smear, × 1500.

FIG. 12. Free polysaccharide within peritruncal lymphatic adjacent to bronchial wall. Rat sacrificed 120 hours after inoculation. Frozen section, × 1500.

FIG. 13. Macrophages laden with phagocyted capsular polysaccharide. Animal treated with penicillin and sacrificed after 72 hours. Note large amount of free polysaccharide still present in alveoli. Impression smear, × 1170.

FIG. 14. Capsular polysaccharide in pneumonic lesion of 58 year old man who died on the 3rd day of a type III pneumococcal pneumonia. Impression smear, × 1150.



(Wood and Smith: Host-parasite relationships in pneumonia)