

PATHOLOGY OF THE EXPERIMENTAL PNEUMONIAS IN
MICE FOLLOWING INHALATION OF STREPTOCOCCUS
HÆMOLYTICUS, OF FRIEDLÄNDER'S BACILLUS, AND
OF PNEUMOCOCCUS.

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PLATE 21.

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In previous papers (1) it has been shown that lesions comparable with those occurring in lobar pneumonia in man may be produced in partially immunized mice by the inhalation while intoxicated of virulent pneumococci. It has likewise been shown (2) that pneumonia may develop in non-immune mice after exposure to a spray of virulent *Streptococcus hæmolyticus* or of Friedländer's bacillus.

It is the purpose of this paper to describe the lesions produced in mice by inhalation of *Streptococcus hæmolyticus* and of Friedländer's bacillus, to contrast them with those found in the lungs of mice following inhalation of pneumococcus; and to compare them with the lesions present in pneumonia caused by these organisms in man.

Technique.

Sections of the lungs were fixed in Zenker's fluid with 5 per cent acetic acid; paraffin embedding was employed; and sections were stained with eosin and methylene blue and a modified Gram's stain.

A. The Pneumonia of Streptococcus hæmolyticus.

The association of this organism with pneumonia in human beings has been recognized for years and its importance has recently been emphasized in the numerous reports on the epidemics of pneumonia occurring in Army camps during the late war. Attention will be called to but one of these, that of MacCallum (3) who has classified the lesions in a manner applicable in the present relation.

MacCallum divided the pneumonias into interstitial and lobular, combinations of both forms occurring. The lobular lesions were sometimes confluent, involving practically a whole lobe. In the interstitial form the initial lesion was around the bronchioles, the consolidated areas were peribronchial, and the peribronchial tissue and septa were densely infiltrated with leucocytes. There was a tendency to cicatrization. In the lobular form there was more exudate in the alveoli, streptococci were very numerous, and necrosis occurred. In both forms, especially the latter, the lymphatics were extensively involved. Blake and Cecil (4) confirmed these observations experimentally in monkeys by injecting streptococci into the trachea. Even as early as 1899 Silfvast (5) claimed to have produced pneumonia in rabbits by the inhalation of streptococci.

The present report is based on the examination of the lungs of 80 mice, in 28 of which pneumonic lesions occurred following inhalation of *Streptococcus hemolyticus*. In every case the organism was cultured from the lungs and the heart's blood.

Gross Pathology.—One or more lobes were involved, the lesion in some instances affecting the whole lobe in typical lobar distribution. The earliest lesion noted in the gross was a small area of pinkish grey consolidation surrounded by an irregular, narrow, dark red, hemorrhagic zone. This sometimes was around the hilum, but in other instances peripheral. The tip of the apex was sometimes involved, the angry red limiting zone forming a ring around this portion of the lung. Extension of the consolidation occurred irregularly from this point so that the greater part of the whole of a single lobe was solid. A noticeable early feature was the relatively great size that the affected lobe attained. At times it seemed inconceivable that the lung in question could be that of a mouse. In the oldest lesions the lung was very large and the consolidated areas were a pale, greyish white color streaked with red. Pleurisy was not frequent, occurring with pneumonia in only five instances, and three times alone. The pneumonic lesion was readily distinguishable from that caused by pneumococcus and death practically always occurred later than in the case of this organism, the 5th to the 10th day being the period of greatest mortality.

Microscopic Pathology.—The lesions were more protean in character than those associated with the pneumococcus. In no instance was there the typical "interstitial" lesion observed by MacCallum in man, all the lesions falling into the group of lobular and confluent lobular or lobar consolidations. There was, however, early, evidence of exudation in the interstitial tissue of the alveolar septa. In view of the fact that MacCallum conceives of the "interstitial" lesion as evidence of a marked resistance our failure to note this type of lesion is interesting

since mice are susceptible animals and no attempt had been made to increase their resistance. The earliest lesion recognized as such occurred in animals which died on the 2nd day. Here there was some involvement of the interalveolar tissue, but extensive exudation had already occurred into the alveolar lumina and this dominated the picture. The exudate contained large numbers of streptococci in long chains. The lining of the bronchi appeared intact and only occasionally was there inflammatory infiltration in the walls. An exudate was noted early, however, in the lumen of the bronchioli but this was probably derived from the exudate in the alveoli. The perivascular and peribronchial lymphatics were early filled with polymorphonuclear leucocytes and seemed thrombosed. The interstitial process did not long persist as such and the picture soon became one of so called confluent bronchopneumonia. The pneumonic exudate was much more serous than in pneumococcus pneumonia and often contained relatively few cellular elements. Fibrin was not present in any considerable amount. Red blood cells did not form a large part of the cellular content of the exudate, except at the periphery of the lesion. In fact, in some instances the alveolar exudate was almost entirely composed of granular serous material, and organisms, the few cellular elements present almost making one doubt the presence of a true pneumonitis. The spread of the lesion was probably of the nature of a cellulitis and it took place in the alveolar, perivascular, and peribronchial tissue in irregular fashion, the lesion involving in the majority of instances the greater part or the whole of a single lobe in typical confluent lobular or lobar form. We were unable to form an opinion as to what rôle was played by the lymphatics in the spread of the infection but we are inclined to consider it of secondary importance. Nor can one state how frequently reinfection and new foci developed from the original focus. Necrosis of the alveolar walls occurred in some instances but in no case did the bronchial wall appear necrotic, nor was there any marked desquamation of the lining membrane. Capillary thrombi were noted only occasionally. Pleurisy occurred infrequently and when present was localized over the involved lobe. The pleural exudate was composed of polymorphonuclears, granular and serous fluid, and organisms in very large numbers. Fibrin was not present in any marked amount. We failed to ascertain whether extension to the pleura occurred by the lymphatics or by other paths.

In general, then, it would seem that the lesions resemble quite closely MacCallum's confluent lobar pneumonias in man if one allows for the differing anatomic structure of the mouse lung.

B. The Pneumonia of Bacillus friedländeri.

As early as 1883 during the discussion regarding the significance of the pneumococcus and *Bacillus mucosus capsulatus*, respectively, as the etiological agent of lobar pneumonia, Friedländer (6) caused mice

to inhale a spray containing the latter organism and he claimed to have produced a typical pneumonia. On the whole, sufficient clinical and experimental evidence has accumulated since to show that Friedländer's bacillus may cause lobar pneumonia in a certain percentage of cases (Sisson and Walker (7), Sisson and Thompson (8), etc.). The local changes in this form of pneumonia in man differ in some respects from pneumococcus lesions. They are, briefly, as follows:

The involvement is often lobar, though intervening healthy lung gives the clue that the lesion is really of the confluent bronchopneumonic type. Pleurisy is common. The lungs are often a reddish color, voluminous, and the exudate on section is abundant, sanguinous, tenacious, and slimy. One or more lobes may be involved. Abscesses and necrosis may occur. Microscopically, the alveoli appear dilated and the exudate is seen to be composed of leucocytes, mucoid material, and numerous bacteria. Interspersed between the affected areas are normal alveoli. The bronchial walls are uninvolved.

Our observations are based on a histological examination of the lungs of 60 mice, in 21 of which pneumonic lesions were present. In every case the organism was recovered from the heart's blood at autopsy. Death occurred later than in the case of the pneumococcus pneumonias.

Gross Pathology.—As in the case of the streptococcus infection the earliest lesion occurs either at the periphery or at the hilum, and is grossly indistinguishable from the streptococcus consolidation. Later, however, the *B. friedländeri*, and streptococcus pneumonias have certain characteristic differences besides features in common. In both instances the lobes involved are massive and voluminous, but in the *B. friedländeri* pneumonias pleurisy occurs more frequently. The lungs remain of a dull, greyish color, streaked with hemorrhagic areas, and are not pale as in the streptococcus lesions. Pleurisy in the absence of an exudative inflammation in the lung occurred four times.

Microscopic Pathology.—The earliest lesions examined were from the lungs of mice dying on the 2nd day. By this time exudation has already occurred into the alveoli, and the interalveolar septum is infiltrated with polymorphonuclears. The exudate is patchy and is composed of polymorphonuclear leucocytes and homogeneous mucoid material, staining characteristically a light blue. Organisms occur in large numbers in the alveoli even in the absence of many cellular elements. The resulting picture is that of an irregular, patchy consolidation, the alveoli being filled with leucocytes and mucoid material. These areas may fuse and involve the whole of a single lobe. The proportions of mucoid material, cellular elements, and organisms vary greatly, some alveoli containing many cells while

others show merely mucoid material and organisms. Between the consolidated areas the alveoli appear emphysematous and the line of demarcation between the involved and healthy alveoli is sharp. Fibrin occurs in some cases but usually is not abundant. The process appears to spread in the interstitial tissue of the alveolar septa. An interesting feature is the persistence of the interstitial inflammation, even in animals that die on the 9th day. Thus the walls of alveoli which contain only mucoid material and organisms may be infiltrated with leucocytes. In other words, the lobar consolidation is not as massive or as regularly distributed as the pneumococcus lesion and may be considered as a typical type of confluent lobular pneumonia. Fibrin is encountered rarely in the capillaries. There is a tendency to emphysema in the involved, as well as in the uninvolved areas and this would partly account for the massive size of the lung. Mononuclear cells occur in the exudate rather sparsely. In the areas of interstitial involvement the capillaries seem empty of blood, but as exudation occurs they again contain red cells, and occasionally hemorrhagic extravasation into the alveoli occurs. There is, however, always a partial anemia of certain areas due to the persistence, as above mentioned, of the interstitial process, even in the late lesions, and to some capillary thrombi. The bronchi contain a cellular exudate but there is little epithelial desquamation or infiltration of the bronchial wall. Lymphatic involvement is sometimes extensive.

Pleurisy without pneumonia occurred in four instances and was present in fourteen of the pneumonic cases. The pleural exudate is rich in polymorphonuclear cells and is composed of mucoid fluid and sometimes fibrin together with numerous organisms.

The lesions produced in mice by Friedländer's bacillus resemble the pneumonias occurring in man as result of infection with this organism and those produced experimentally in cats by Sisson and Walker. Although in some cases there is a persisting interstitial lesion, the pneumonia may be classed as of confluent lobular type, though often taking a lobar distribution. The mucoid material in the exudate is a characteristic feature.

DISCUSSION.

It is of interest to compare the lesions experimentally induced in the lungs of mice by air-borne infection with *Streptococcus hæmolyticus*, Friedländer's bacillus, and pneumococcus, respectively. It must be remembered, however, that the lesions caused by the first two mentioned were produced in normal mice, whereas the pneumococcus pneumonias that we studied were produced in partially immunized and alcoholized animals. The experimental conditions are, therefore,

not strictly comparable. Although consolidation of the whole of a single lobe may be produced by any of the organisms the gross and microscopical pathology both show certain points of difference in each instance when the lesions are well developed. Moreover, the pulmonary lesions experimentally induced differ from the pneumonias occurring spontaneously in stock mice, which have been described in a former paper (1). The earliest lesions, however, in the three types of experimental pneumonia being discussed may only be differentiated bacteriologically.

The initial lesion in all of the experimental pneumonias appears to be a polymorphonuclear infiltration of the interstitial tissue. In the pneumonias occurring spontaneously in stock mice the lesion is essentially a mononuclear response, though polymorphonuclears are found, especially in the bronchial exudate. The peribronchial lymph glands are very large in this condition and the lesion tends to follow and persist in the peribronchial and perivascular tissue. In the gross the lungs are liver-like in color and consistency, gelatinous, dry, not massive, and the surface is irregular and often nodular with what appears to be localized tissue necrosis.

In the case of the pneumococcus lesions the pneumonic lobes are quite moist and not very large. Microscopically, the involved area is uniformly consolidated and is sharply differentiated from the surrounding uninvolved tissue, and the exudate is almost entirely polymorphonuclear in character. The Friedländer and streptococcus lungs are massive and pale with a red, angry zone at the periphery of the consolidated areas. Microscopically, the exudate in the streptococcus lungs is predominantly serous and cellular, and chains of organisms occur in large numbers. Interspersed with the involved areas is an emphysematous but otherwise normal lung parenchyma. The exudate in the Friedländer pneumonias is mucoïd and cellular, the percentage of cellular elements varying in the different alveoli. Organisms occur in large numbers in both types of exudate. There are intervening areas of emphysematous healthy lung and the interstitial lesion persists. Pleurisy occurred more often in the Friedländer group. There is no difficulty in recognizing the lesions histologically when well developed.

There seems to be no evidence to warrant the term "broncho"

being applied to these any more than to the pneumococcus lesions, though the *Streptococcus hæmolyticus* and *Bacillus friedländeri* lesions are more patchy in distribution, and this despite the fact that the whole of a single lobe may be involved. We are inclined to favor MacCallum's terms "interstitial," "lobular," and "lobar," always postulating that "lobular" does not imply an anatomical unit. The character of the exudate is less cellular and more fluid than is the case in pneumococcus pneumonia. It would seem that the pneumococcus, streptococcus, and Friedländer's bacillus pneumonias are essentially a cellulitis of the lung, the pathogenesis of the lesions being the same for all three. The patchy distribution in many instances may be consequent on the circumstances that these organisms are able to invade the lungs of normal mice, unlike the pneumococcus which needs the aid of a predisposing factor (alcohol in the case of our experimental lesions); or it may merely be due to difference in the type of response of the tissues to the organisms themselves. We are unable to say whether the organisms penetrate the lungs directly, or whether the disease is hematogenous or lymphatic in origin, or is due possibly to a combination of these methods of invasion. The initial lesion is certainly in the lower respiratory tract around the bronchioli, atria, and alveoli.

It is interesting to note that the type of pulmonary reaction to the streptococcus in normal mice is a "lobular" consolidation. One is tempted to speculate as to whether the type of lesion would be "interstitial" if infection occurred in immunized animals.

SUMMARY.

1. Pulmonary consolidation may be produced in normal mice by the inhalation of Friedländer's bacillus or *Streptococcus hæmolyticus*.
2. The initial lesion is in both instances interstitial in character and the spread is by way of the interstitial tissue, though the ultimate consolidation may come to resemble in the gross a lobar pneumonia.
3. The lesions grossly and microscopically may be distinguished from the lobar consolidation associated with pneumococcus infection of the lung.
4. Friedländer's bacillus and *Streptococcus hæmolyticus* give rise to

pneumonia in mice previously normal, whereas the pneumococcus produces pulmonary lesions only under special circumstances, as when the animals are alcoholized after partial immunization.

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EXPLANATION OF PLATE 21.

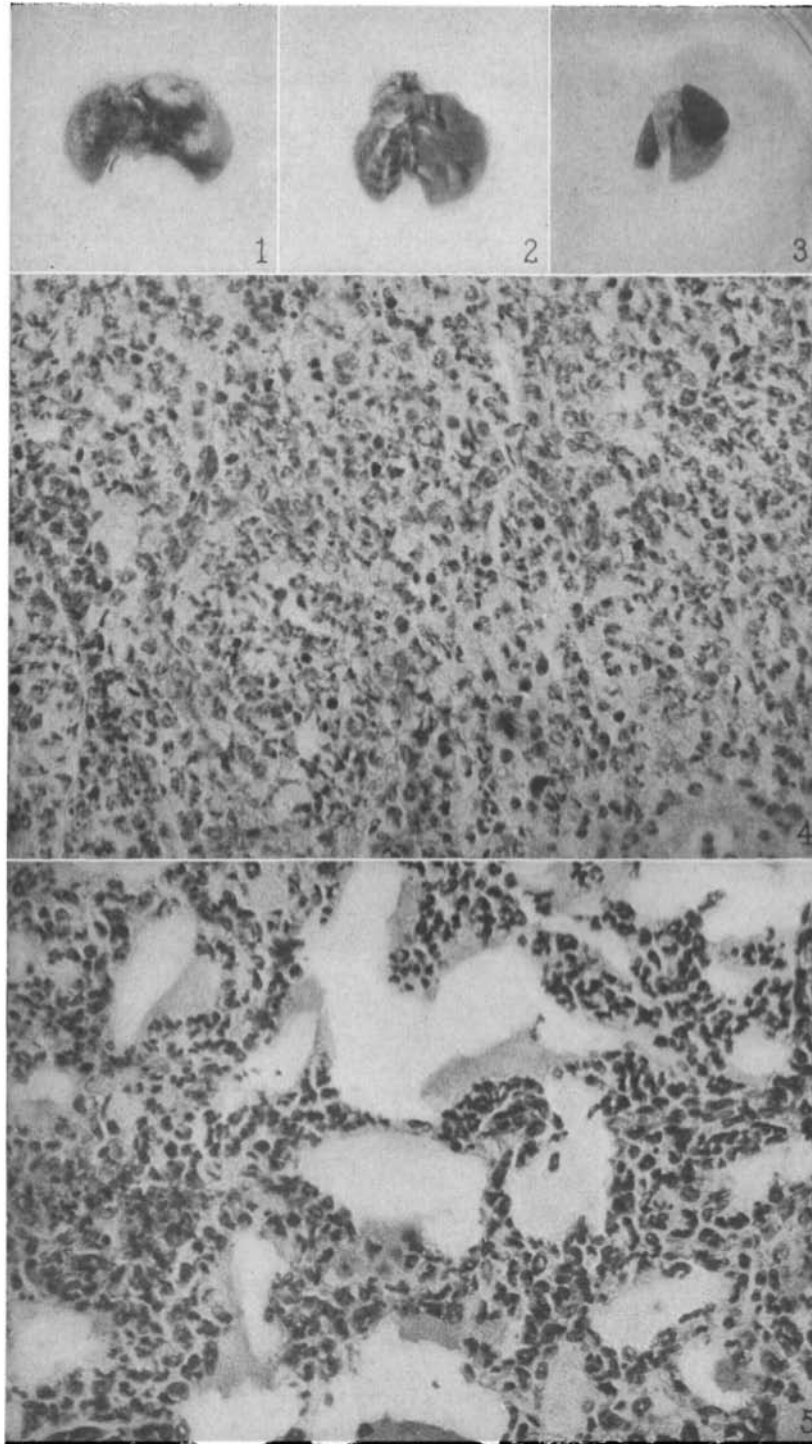
FIG. 1. Mouse 85-29. *Streptococcus hemolyticus* pneumonia. Lung from a previously normal mouse sprayed with hemolytic streptococci, which died on the 9th day. Note the great increase in the size of the affected lobe and the mottled red and white appearance. Natural size.

FIG. 2. Mouse 29-50. *B. friedländeri* pneumonia. Lung from a previously normal mouse sprayed with *B. friedländeri*. Note the large size of the consolidated lobe. Died on 17th day. Natural size.

FIG. 3. Mouse 84-1. Pneumococcus pneumonia. Lung from a partially immunized mouse, alcoholized and sprayed with pneumococcus, which died on the 2nd day. There is a consolidation of the right upper and left lower lobes. For comparison with the streptococcus and *B. friedländeri* consolidation, to show uniform red color and small size of involved lobes. Natural size.

FIG. 4. Mouse 85-10. *Streptococcus hemolyticus* pneumonia. Section of lung from a previously normal mouse sprayed with hemolytic streptococci, which died on the 4th day. $\times 420$.

FIG. 5. Mouse 115-10. *B. friedländeri* pneumonia. Section of lung from a previously normal mouse sprayed with *B. friedländeri*, which died on 13th day. $\times 420$.



(Branch and Stillman: Pathology of pneumonia in mice.)