

PATHOLOGY OF EXPERIMENTAL PNEUMOCOCCUS
PNEUMONIA IN MICE.

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PLATES 29 TO 31.

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In preceding papers (1) it has been shown that virulent pneumococci generally disappear from the lungs of normal mice within a few hours after inhalation and do not give rise to generalized infection. In alcohol-intoxicated mice, however, pneumococci persist in the lungs for a longer period and a fatal septicemia frequently follows. It has further been shown (2) that a high degree of immunity is produced in mice following repeated inhalations of live pneumococci and that a less marked immunity results from exposure to a spray of dead organisms. In the instances in which non-immune mice succumb as the result of infection following a pneumococcus spraying while alcoholized, death is due to general septicemia and there is no evidence of localized infection in the lung. However, mice which have been rendered partially immune by inhalation of live or killed pneumococci and are later alcoholized and exposed to virulent pneumococci show definite evidence of localized pulmonary infection.

In the present paper we shall report upon (1) the normal histology and the pathological lesions which have been encountered in routine examinations of the lungs of a large series of stock mice; (2) the effect of alcohol on the lungs of normal mice together with the changes brought about by the inhalation of pneumococci; and finally (3), the pathological lesions produced in the lungs of normal and immunized mice sprayed with pneumococci while under the influence of alcohol.

Technique.

At the time of autopsy sections of lung tissue were fixed in Zenker's fluid containing 5 per cent acetic acid. They were later embedded in paraffin and stained by Gram's and Mallory's eosin-methylene blue methods, respectively.

Normal Histology.—Except for occasional small or more extensive areas of congestion the lungs of normal mice appear salmon pink and crepitant. These congested areas have been noted by Branham, Maitland, Cowan, and Detweiler (4) and are ascribed to the methods whereby the animals were sacrificed. Certain points of histological difference from the human lung should be emphasized. In the mouse there are no secondary pulmonary septa and the pleura has no definite sub-pleural layer, but appears to consist of a single layer of endothelial cells covering the peripheral alveoli. Lymphoid tissue is sparsely distributed and lymphatics are not prominent, only becoming so when dilated, when they may be seen around blood vessels and bronchi.

Pathological Lesions in Stock Mice.—The lesion most frequently encountered was a chronic consolidation of the lung which occurred in about 3 per cent of apparently healthy stock mice. Both in the gross and microscopically the lesions were readily differentiated from the pneumococcus lesions to be described. These evidences of chronic pneumonic change are probably the result of some intercurrent infection, but no attempt has been made so far to isolate the etiological agent. The consolidated areas usually occur in multiple patchy fashion scattered through numerous lobes, and the right medial lobe is most often affected. They may, however, involve the whole of a single lobe. In the early stage the consolidated areas appear red, firm, dry, and liver-like. Later they appear grayish and gelatinous, almost transparent, and the surface is irregularly granular and puckered. Pleurisy occurred rarely.

On microscopic examination the most prominent and constant feature was a mononuclear reaction around the bronchi and blood vessels. These areas occurred around both large and small radicals as a mantle or collar and could be traced from the hilum outward. The accumulations of mononuclear cells probably represent a massive increase in the normally inconspicuous lymphoid elements, but large numbers of plasma cells were also present. Areas of true pneumonic consolidation also occurred, the exudate being composed predominantly of large mononuclear cells. As a rule, only a few polymorphonuclear leucocytes were seen in the alveolar exudate whereas the bronchial exudate contained many. The consolidated areas were often small and situated around the hilum, but when large extended from pleura to hilum, while occasionally the whole of a single lobe was involved. Fibrin was rarely found and the exudate was chiefly cellular. In some instances the exudate in the bronchi and alveoli was definitely necrotic, the focal changes bearing some resemblance to tuberculosis.

The Pulmonary Lesions Following Alcohol Administration.—In order to determine whether the administration of alcohol causes any demonstrable changes in the lungs, twelve animals, which had received alcohol either by spray or intraperitoneally, were killed at intervals and the lungs examined histologically. A few animals which had died of an overdose of alcohol were also examined. In no instance were any lesions found in the lungs of the alcoholized mice which did not occur in normal animals.

Histological Examination of Lungs of Mice Following Spraying with Pneumococci.—With a view of determining the relative number of organisms inspired by the inhalation method, twenty-five mice were killed at intervals of from 5 minutes to 24 hours after exposure to a pneumococcus spray. In the microscopic sections of the lungs of those mice killed shortly after exposure, large numbers of pneumococci were to be seen in the upper respiratory tract, especially in the pharynx, and also in the esophagus. They were found only occasionally, however, in the trachea and never in the bronchi or alveoli. That they were present, however, in the lower respiratory tract was proved by the fact that when small pieces of the periphery of the lobes were placed in broth, cultures of pneumococci were obtained. From this it is evident that the inspired bacteria reach the deeper parts of the lung following inhalation, but in such small numbers as to be demonstrable only by cultural methods.

Pathology of Pneumococcus Pulmonary Infections in Mice.—This report is based on the examination of the lungs of 133 animals which developed pulmonary lesions. The total number of animals exposed was over 900. In each instance in which death occurred following exposure to pneumococcus spray the organism was recovered from the heart's blood. The lesions may be conveniently discussed under (1) pleurisy and congestion, and (2) exudative pneumonitis.

1. Pleurisy and Congestion.—In 55 instances congestion and pleurisy were found in the absence of any exudative inflammation in the lobes of the lung examined. These lesions occurred about as frequently in the previously normal mice as in the animals immunized in various ways. Usually there was a considerable amount of serofibrinous fluid in the pleural cavities. One or both pleural cavities were involved while in some cases pericarditis was also present. Less often the exudate was fibrinous and formed a thin coating over the pleura of one or more lobes. The

lung itself was dark red and congested, and in the gross gave the impression of being consolidated. On microscopic examination the prominent feature was a widening and engorgement of the alveolar capillaries and an exudative pleurisy. Red blood cells and a granular serous fluid were sometimes found in the alveolar spaces. In no instance was any exudate of white cells encountered in the lung tissue proper, even in places contiguous to the pleura. Mononuclear cells were prominent in the earliest stage, whereas, later polymorphonuclear leucocytes predominated. The exudate varied greatly in its fibrin content, and pneumococci were found in large numbers especially in the superficial layer. The bacteria were chiefly extracellular, but occasionally within leucocytes. The most interesting feature was the sharp line of demarcation between the inflamed pleura and the congested lung. Thus, in sections which included the interlobar fissures, the inflammation was found to have spread along the pleural invaginations almost to the hilum of the lung, without any involvement of the adjacent lung tissue (Fig. 3). In a few instances the inflammation had also implicated the loose connective tissue around the hilum, peribronchial glands, and esophagus. We do not as yet understand the mode of primary invasion of the pleura by the pneumococcus. A study of serial sections of a whole lung is essential to this, and is being undertaken. In most instances no lymphatic involvement was evident in the lobe examined.

2. *Exudative Pneumonitis*.—An exudative inflammation was noted in the lungs of 78 mice. In eighteen pleurisy was also present. Twenty-seven of these instances were not considered in the paper preceding this one since they occurred in preliminary experiments in which accurate record of the method of immunization was not obtainable. The pulmonary lesions consisted of (1) interstitial inflammation, (2) red, and (3) gray hepatization, presumably the results of attempts on the part of the body to localize the infection.

In the pneumonias in which pleurisy occurred it was not infrequent to find the pleural exudate covering not only the surface of the consolidated lobe but also other lobes which merely showed congestion. In the early instances of interstitial inflammation the lungs often appeared merely congested or even normal on gross examination. In the stage of red hepatization one or more lobes were involved, sometimes on opposite sides. The lower lobes were most frequently affected. The affected lobes were moderately enlarged, dark red, firm and smooth (Fig. 1). On section they were moist and exuded serosanguineous material. In the gray stage the lobe was larger and of a dull grayish color (Fig. 2).

Microscopical examination showed that in the early interstitial inflammations congestion and edema were much more frequent when pleurisy was also present. The congestion was greater in the tissue surrounding the area of exudation than in the infiltrated area itself. Although the exudate in the interstitial tissue was rich in polymorphonuclear leucocytes, pneumococci could rarely be demonstrated histologically. The initial lesion was in the region of bronchioli, atria, and alveoli and these structures were involved even when the initial focus was near the hilum (Figs. 4 and 5). From this point in the lower respiratory tract the cellulitis

spread rapidly in irregular fashion either in the alveolar septa or in the interstitial tissue around the vessels and bronchi. These perivascular lesions were often extensive and were to be seen following the large blood vessels to their termination at the periphery of the lung. The perivascular lymphatics were greatly distended and filled with cell detritus, and there was often comparatively little actual interalveolar spread (Fig. 6); in other cases, however, the interalveolar spread was more marked than the perivascular, and the lymphatics were less involved. The lining of the bronchi was intact and their walls were rarely infiltrated. The lumina of the small bronchioli early contained an exudate rich in polymorphonuclear leucocytes. These cells apparently had emigrated from the initial interstitial foci. Vascular thrombi in capillaries and smaller vessels were occasionally seen but none were found in the larger vessels.

No sharp line can be drawn between the stage of interstitial inflammation and that of red hepatization. Frequently a typical area of red hepatization would be seen in one area while the adjoining showed a spreading interstitial inflammation. The stage of red hepatization was found as early as the 1st day but generally was encountered in mice dying on the 2nd day. At this stage the alveoli were filled with an exudate of red blood cells and polymorphonuclear leucocytes. Occasionally many mononuclear cells were noted. Fibrin was prominent in only a few instances. The alveolar walls now contained fewer inflammatory cells and the capillaries appeared engorged. In some instances fibrin could be seen in the capillaries. The numbers of pneumococci varied, sometimes only a few being present and in other instances large numbers.

As the transition from the stage of early interstitial inflammation to that of red hepatization was gradual, so was there a gradual transition from red to gray hepatization. In a single lobe areas resembling both red and gray hepatization frequently occurred. The stage of gray hepatization was reached at about the 3rd day. The alveoli were then completely filled with the polymorphonuclear and mononuclear exudate and the alveolar walls were anemic (Fig. 7). In some places the latter could be made out only with difficulty, the section showing what appeared to be a mass of clumped white blood cells. Vascular thrombi were not prominent although present in a few instances. Only occasionally were dilated lymphatics visible in the pleura. For these reasons observations on the lymphatics and the rôle they play were limited. The lymphatic system is apparently much less developed in the lungs of mice than of animals with secondary septa. The lymphatics, when distinguishable in the inflamed areas, were seen only in the perivascular and peribronchial tissue, and in some cases were filled with the cellular exudate.

Although the lesions described above are typical of the kind most frequently observed, other lesions were found which, in the hemorrhagic nature of the exudate, resembled more closely those described by Wadsworth (5) as occurring in the least immunized rabbits of his

series, and also by Hirsch and McKinney (6) in an epidemic of bronchopneumonia due to Pneumococcus Type I and Group IV. We are under the impressions that lesions of this type occurred, just as was the case with Wadsworth's rabbits, oftenest in mice with a relatively slight immunity as judged by the number of previous pneumococcus inhalations.

DISCUSSION.

There seem to be three factors necessary to produce pneumococcus pneumonia in mice, namely, the presence in the respiratory tract of a sufficient number of virulent organisms, penetration of the lung tissue, and some degree of local resistance or reactivity in the lung. This is, in some instances, apparently an expression of a general immunity rather than an allergic phenomenon. Mackenzie¹ has recently made the interesting observation that following reinjections of pneumococcus antigen intradermally, guinea pigs exhibit a marked local "hyper-reactivity" (allergy) without developing a general immunity. As we were able to produce pneumonia in mice immunized by spraying dead organisms, which animals exhibit little general immunity to intraperitoneal inoculation, it may possibly be that the increased reactivity in the lung is in other instances an expression of a purely local immunity.

The initial lesion, when the reaction is focal, appears to be in the neighborhood of the alveolar ducts, atria, and alveoli. The infection spreads just as readily from the hilum outward as from the periphery inward. The earliest lesion is an interstitial inflammation or cellulitis which spreads in the interstitial tissues around the vessels and bronchi and in the alveolar walls. The lymphatics become filled with exudate but probably play a minor part in the spread of the infection, with the exception perhaps of the pleurisy. The extravasation of the exudate into the alveoli in the later stages of red and gray hepatization is a secondary phenomenon. The exact point of invasion of the organisms has not been determined beyond question, that is to say whether the pneumococci penetrate into the lower respiratory tract, in which case the infection would be a direct one, or lodge primarily in the upper respiratory tract. In the latter instance they would then reach the

¹ Mackenzie, G., personal communication.

lung by the lymphatics through reversal of the stream or pass through the lymphatic glands into the blood or enter the blood stream direct. In any of these events, lobar pneumonia would have to be considered, like acute splenitis, as a local reaction of an organ to a general septicemia.

Lobar pneumonia in mice is not of strict anatomical distribution, the infection spreading in the interstitial tissue like any other cellulitis until it is controlled. The area of demarcation is usually sharp, and although the disease tends to spread within the lobe in which it arises, it may readily extend into another lobe without involving the whole of the lobe first invaded. This is well known to be true in man also, in whom the margin of the most affected lobe may be perfectly healthy although part of another lobe, the middle lobe especially, also shows involvement by direct spread of the pneumonic consolidation.

It is interesting to note that the pleura often forms a sharp line delimiting the pulmonary lesion. One may see not infrequently a normal pleura overlying an inflamed lung. This state of affairs would seem to be indirect argument in favor of the pleural involvement being due to lymphatic rather than to direct spread of the infection.

MacCallum's (7) conception that the difference between lobular and interstitial pneumonias is largely a matter of degree of resistance in the tissue seems well founded. One can readily understand how it is that the pneumococcus is responsible for all of the lesions here described.

The comparative rarity in lobar pneumonia of true reinfection of the other lung, or parts of the same lung, may be explained by the assumption that even if the infectious agent reaches other parts of the lower respiratory tract, in the absence of some preceding injury, as for instance that produced by alcohol in the case of the experimental animals, invasion of the tissues fails to take place and the organisms are ultimately removed from the respiratory tract without causing harm. As an explanation of the "lobar" distribution of pneumococcus pneumonia the fact may be offered that penetration and infection only occur at some point where the normal defensive barriers have been broken down by a preliminary injury even when virulent organisms are present deep within the respiratory tract. This is a somewhat different conception from that offered by Blake and Cecil (8) or by

Permar (9). The authors first mentioned conceive of the organisms as entering the lung at the hilum and being carried along the lymphatics of a lobe to the alveoli where the interstitial lesion starts, while Permar interprets pneumonia as an exudative alveolitis, with the interstitial lesion as secondary and the spread mainly lymphatic. The lesions here produced in mice seem to be identical with those obtained by Blake and Cecil in monkeys, when one allows for the differences in anatomical structure of the two animals as further with those occurring in lobar pneumonia in man.

Alcohol may be taken as the prototype of many possible agencies which break down the effective protective barriers of the body and allow a penetration of organisms to occur. The exact nature of its action has not been determined. So far we have not succeeded in substituting other agencies.

The literature shows that previous attempts to produce pneumococcus pneumonia in mice have been unsuccessful because the necessary factors of penetration by the organism and partial immunity of the host were not present. In the small number of cases in which penetration was accomplished, the animals died of a general septicemia without localizing signs in the lung.

CONCLUSIONS.

1. The administration of alcohol produces no evident histological changes in the lungs of mice.
2. Following inhalations of pneumococci the organisms are not visible histologically in the lungs of mice either in the bronchi or alveoli.
3. In partially immunized mice which have been exposed to a pneumococcus spray while alcoholized, true lobar pneumonia not infrequently develops.
4. The primary lesion of such lobar pneumonia in mice is an interstitial inflammation of the alveolar wall and the infection spreads in the interstitial tissue like a cellulitis.
5. A tentative explanation of the "lobar" distribution of pneumococcus pneumonia is offered.

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EXPLANATION OF PLATES.

PLATE 29.

FIG. 1. Mouse A.B., 11-8. Red hepatization in the lung of an immune mouse. The animal had recovered from first treatment of alcohol intraperitoneally and inhalation of virulent Type I pneumococci. It died on the 3rd day following a repetition of the treatment. Natural size.

FIG. 2. Mouse A.B., 88-4. Gray hepatization in the lung of an immune mouse. The animal had been sprayed four times with virulent Type I pneumococci. After the usual interval it was given alcohol intraperitoneally and sprayed again with Type I pneumococci. It recovered. Again after the usual interval it was given alcohol intraperitoneally and sprayed with a virulent Type II culture. It died 2 days later. Natural size.

FIG. 3. Mouse A.B., 57-3. Pleurisy and congestion without pneumonia in an immune mouse. The animal had been sprayed ten times with a virulent Type I culture. After the usual interval it was given alcohol intraperitoneally and sprayed with virulent Type I pneumococci. It died on the 2nd day. $\times 200$.

PLATE 30.

FIG. 4. Mouse A.B., 2-4. Interstitial lung inflammation in a previously normal mouse. The animal was given alcohol intraperitoneally and sprayed with virulent Type I pneumococci. It died on the 2nd day. This illustrates one of the few cases in which pulmonary localization occurred in a previously normal mouse. $\times 315$.

FIG. 5. Mouse A.B., 7-5. Early interstitial lesion around the atria in an immune mouse. It had recovered from a treatment of alcohol intraperitoneally and spray of viable atypical Type II pneumococci. After the usual interval it was given alcohol intraperitoneally and sprayed with virulent Type II pneumococci. It died 1 day later. $\times 450$.

PLATE 31.

FIG. 6. Mouse A.B., 79-4. Interstitial inflammation showing perivascular lymphatics distended with degenerating polymorphonuclears. Immune mouse sprayed six times with a culture of atypical Type II organisms. After the usual interval it was given alcohol intraperitoneally and sprayed with virulent Type II pneumococci. It died on the 2nd day. $\times 600$.

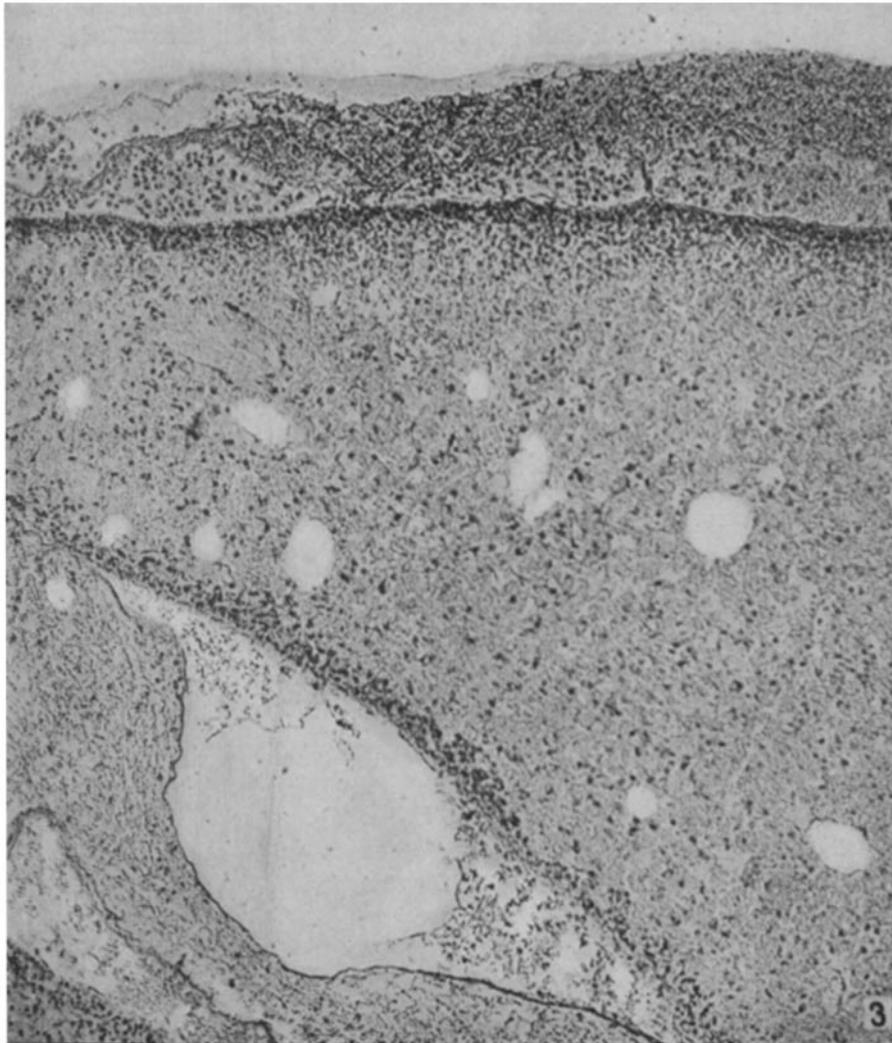
FIG. 7. Mouse A.B., 99-18. Gray hepatization in an immune mouse. The animal had recovered from two previous treatments with alcohol intraperitoneally and inhalation of virulent Type I pneumococci. On a repetition of the treatment after the usual interval it died on the 2nd day. $\times 600$.



1

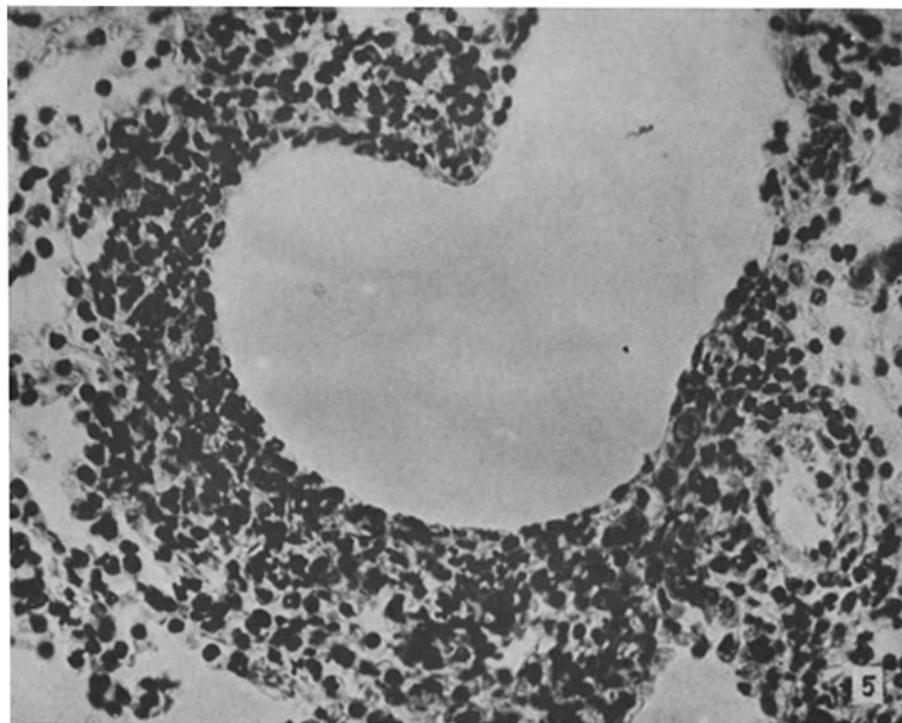
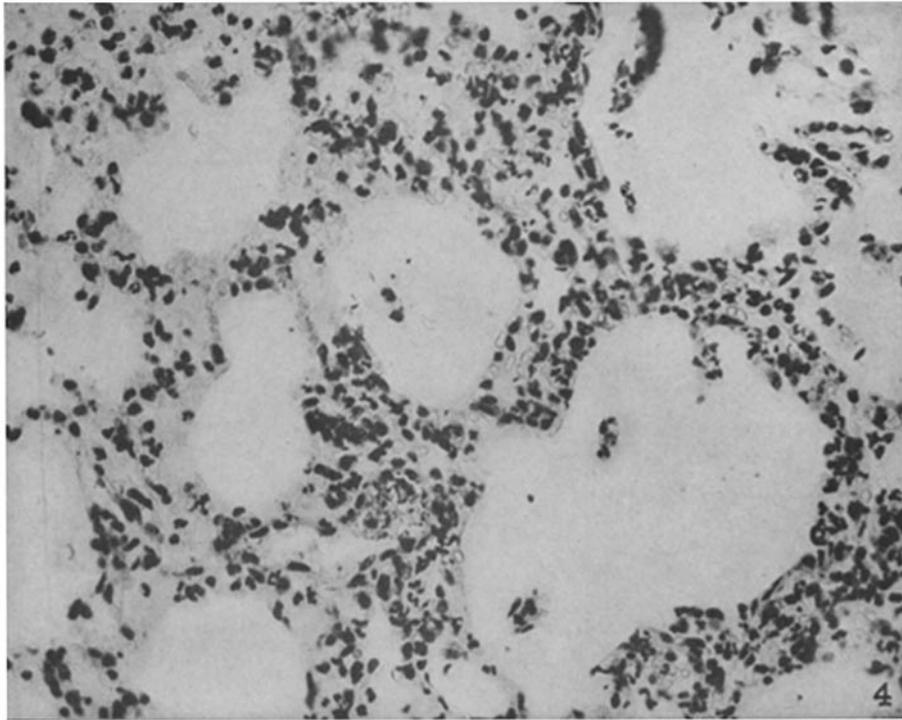


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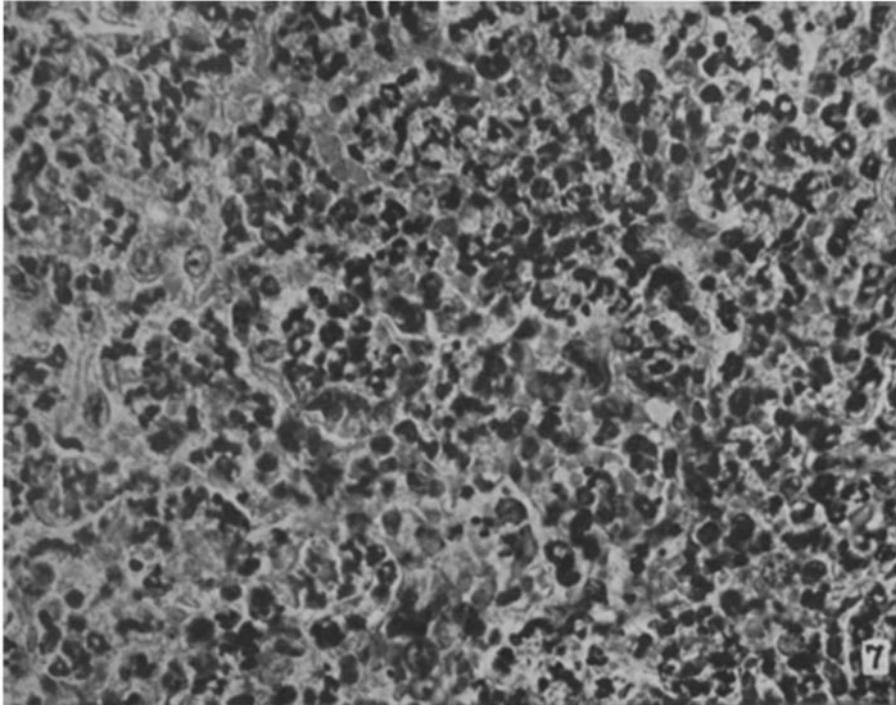
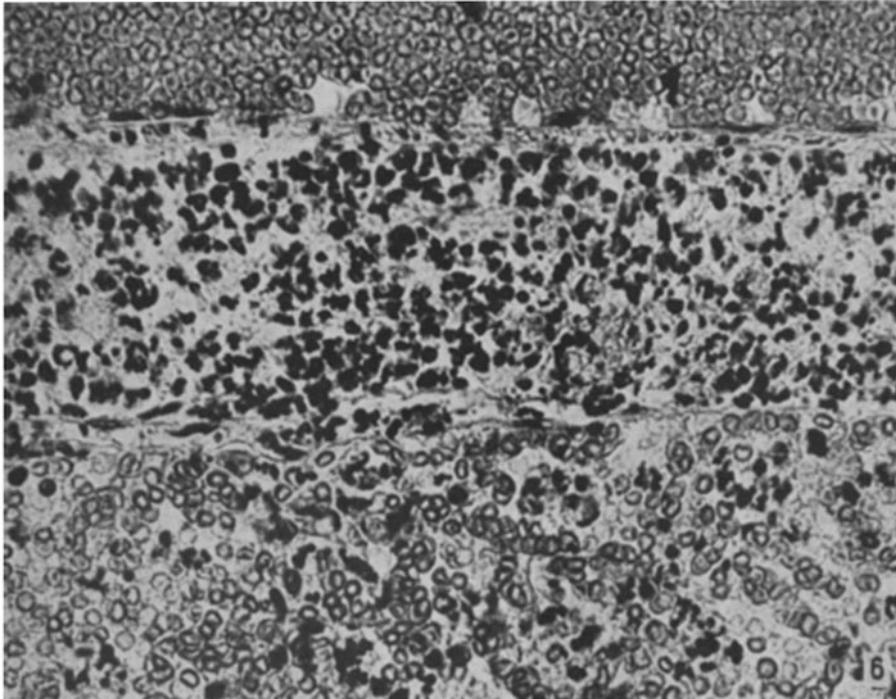


3

(Branch and Stillman: Pneumococcus pneumonia in mice.)



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