

THE CHARACTER OF THE PNEUMONIC LESIONS PRODUCED BY INTRABRONCHIAL INSUFFLATION OF VIRULENT STREPTOCOCCI.*

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INTRODUCTION.

In our first studies¹ on the effect of intrabronchial insufflation of the streptococcus in dogs, we found that the nature of the pneumonic lesion produced by this organism was one which in human pathology is usually described as lobular pneumonia. In the insufflation experiments of Lamar and Meltzer² with the pneumococcus, the lesion produced was that of a lobar pneumonia. The conclusion which presented itself was that the different organisms cause experimentally different pneumonic lesions. In a later communication we³ enumerated in detail the differences in the two forms of pneumonic lesions resulting experimentally from the method of intrabronchial insufflation. The question arose whether the differences in the lesions were indeed specific; that is, whether the pneumococcus always produces a lobar pneumonia. The two series of experiments did not exclude the possibility that the difference in the results was merely due to a difference in the degree of virulence of the two species of organisms employed in the two investigations. The pneumococcus employed in the experiments of Lamar and Meltzer was highly virulent. It was fatal to mice in doses of 0.000,001 of a cubic centimeter. The strain of streptococcus which we employed in our first experiments and which had been isolated from a fatal case of bronchopneumonia caused the death of a mouse weighing fifteen grams only after the intraperitoneal injection of

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¹ Wollstein, M., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xvi, 126.

² Lamar, R. V., and Meltzer, S. J., *idem*, 1912, xv, 133.

³ Wollstein, M., and Meltzer, S. J., *idem*, 1913, xvii, 353.

0.5 of a cubic centimeter. We⁴ tried to answer the question in part, at least, by studying the character of the pneumonic lesions which followed insufflation of a non-virulent pneumococcus isolated from the blood of a patient with lobar pneumonia who recovered. In the present series of experiments we attempted to meet the same question by studying the character of the pneumonic lesion produced by a strain of streptococcus whose virulence is comparable with that of the strain of the pneumococcus employed in the experiments of Lamar and Meltzer, inasmuch as it is also fatal to mice in doses of 0.000,001 of a cubic centimeter.

EXPERIMENTAL DATA.

From a fatal case of purulent leptomeningitis a streptococcus was isolated from the spinal fluid and from the heart's blood at autopsy. The coccus grew in chains of six to twenty elements, did not ferment inulin, and was not dissolved in rabbit bile. It formed the streptococcus type of capsules. On blood agar plates a distinct hemolytic zone surrounded the small round colonies. From the first generation on agar broth tubes were inoculated, and after twenty-four hours' growth 0.01 of a cubic centimeter was injected intraperitoneally into a white mouse weighing fifteen grams. The following morning the mouse was dead, and the streptococcus was recovered from the heart's blood in pure culture. Smaller doses were inoculated into successive mice, until 0.000,001 of a cubic centimeter killed the animals in from twenty-four to thirty-six hours. Broth cultures from this virulent streptococcus were used in the following series of experiments on dogs. From time to time the coccus was reinoculated into mice in order to test its virulence, which was thus kept undiminished throughout the period of our experiments.

Seventeen dogs were inoculated by intrabronchial insufflation of broth cultures eighteen to twenty-four hours old, in doses of fifteen to twenty-five cubic centimeters. No one of the animals died, nor were they very ill. The temperature generally rose within six or seven hours after the injection, but in almost every case it had fallen quite or almost to normal by the following morning. The highest rise recorded was 40.8° C.

⁴ Wollstein, M., and Meltzer, S. J., *loc. cit.*, 1913, xvii, 353.

Four dogs to which fifteen cubic centimeters had been given were killed in twenty-four hours. In all the lesion was confined to the right lower lobe, the greater part of which was somewhat swollen, heavy, mottled with dark and paler areas, while on section the moist cut surface was distinctly that of a lobular pneumonia in which aerated, pink areas of small extent were present among the more solid peribronchial, dark red masses of smaller and larger size. There was no pleural exudate present in any of the cases. Smears from the lung showed many cocci in very short chains, almost all lying outside of the leucocytes. There was practically no phagocytosis. Cultures from the lung and from the heart's blood gave pure growths of streptococci. The bronchial lymph nodes were swollen, moist, and soft.

Three dogs received twenty cubic centimeters of the culture and showed a more extensive lesion in twenty-four hours. In one of these animals the subcardiac lobe was involved as well as the right lower, and in the others the entire lobe was solid, the picture being that of a pseudo-lobar bronchopneumonia. Only one case showed a narrow strip of fibrinous exudate on the pleura, along the posterior border of the subcardiac lobe. In two of these cases the consolidated lung contained areas that were as firmly solidified as were the control specimens of pneumococcus pneumonia, but these solid areas were less extensive and were separated, or interrupted, by softer, lighter areas of aerated, congested, or edematous lung substance.

In two dogs a blood culture from the jugular vein was taken every half hour for two hours after the insufflation of twenty cubic centimeters of streptococcus culture. In one of the animals a growth appeared in the culture made after one hour, while in the other no bacteriemia was apparent two hours after the cocci had been injected into the bronchus. Both dogs, however, showed streptococci in the blood just before death twenty-four hours after inoculation.

Three dogs were killed two days after a dose of fifteen cubic centimeters of streptococci had been given them. In two the right lower lobe showed a bronchopneumonia in its posterior third, while in the other dog the subcardiac lobe was also involved. The lesions were not more intense than were those studied at the end of twenty-

four hours. There was no pleurisy present. Smears from the lungs showed few cocci; the cultures from the heart's blood and lungs were less profuse than they had been in dogs killed on the first day after inoculation.

In one case a dog to which fifteen cubic centimeters had been given was allowed to live until the sixth day, when a large area of resolving bronchopneumonia was found in the right lower lobe, and a growth of streptococci was obtained from it. The heart's blood gave no growth.

Four dogs received twenty-five cubic centimeters and were allowed to live seven days. In two cases a resolving area of bronchopneumonia was found in one of the lower lobes. In the other two dogs the right lower lobe was merely darker red in color than the rest of the lungs. No growth took place from the lungs or heart's blood.

Two dogs were kept alive thirteen days after intrabronchial insufflation of fifteen cubic centimeters of a streptococcus broth culture. Their lungs were well aerated, elastic, and pink in color, showing no evidence of any pneumonic lesion.

Microscopic Examination.—Microscopic examination of the pulmonary lesion found twenty-four hours after the intrabronchial inoculation of fifteen cubic centimeters of a broth culture of streptococcus into a dog showed that the alveoli were filled with an exudate composed very largely of polymorphonuclear leucocytes, many swollen epithelial cells and few red blood cells. Fibrin was present in very small amounts in the alveoli, but about the larger blood vessels there was often an appreciable amount of it. Toward the periphery of the lesion it sometimes happened that an area of intense congestion with intra-alveolar hemorrhage and edema intervened between the consolidated lung and the aerated portions. In such areas fibrin was not uncommon, due to the coagulation of the exuded blood. Within the alveoli of the pneumonic lesion proper, however, the amount of fibrin was never as large as is the case in lesions caused by virulent pneumococci in the dog's lung. The capillaries as well as the larger blood vessels were very much congested. The alveolar walls as well as all the connective tissue septa were densely infiltrated with polymorphonuclear leucocytes. The

small bronchi showed much swelling and peeling of their epithelial lining cells and infiltration of their walls, some of them being filled with masses of pus cells.

Streptococci in large numbers were present in the alveoli and in the bronchi, lying in heaps between the cells. Only an occasional leucocyte could be seen with cocci in its cytoplasm.

At the end of forty-eight hours the exudate was more distinctly purulent in character, and the diffuse leucocytic infiltration more marked. There was less, not more fibrin, and less edema. In some sections the alveoli were so distended with leucocytes that the capillaries in the walls were compressed, instead of distended with blood, as was the case in the earlier lesions. Streptococci were present in the alveoli, and there was some phagocytosis.

In the resolving lesions of the sixth and seventh days there was the usual evidence of macrophage activity.

DISCUSSION AND SUMMARY.

Comparing the pneumonic lesions produced by this very virulent streptococcus with those caused by a mildly virulent strain of the same organism, we find that with one exception the differences between them are only of a quantitative nature and not striking. There are the same intense leucocytic exudation into the alveoli and the leucocytic infiltration of the framework of the lungs. Macroscopically the pneumonia produced by the virulent organism was, as a rule, lobular in character. Occasionally, however, especially when large doses of the culture had been given, the gross appearance of the lesion gave the impression of a lobar pneumonia. But even in these cases the cut surface showed that the solid areas were separated by softer and lighter areas of aerated, congested, or edematous lung substance. Pleurisy was practically absent from the lungs in this series of experiments; only one case showed a narrow strip of fibrinous exudate on the pleura. Although in some instances large doses of the cultures were given and some dogs were permitted to live six or seven days, there was no mortality among the dogs in these experiments, just as there had been none among the animals experimented upon with the streptococcus of very low virulence. The course of the pneumonia caused by the virulent organism

seemed to be somewhat longer than that produced by the less virulent one; areas of resolving pneumonia were found to persist on the sixth and seventh days after the injection, and in one such instance the solid focus contained viable organisms. There was some difference in the amount of fibrin present in the exudate caused by the two strains of streptococci. Although even in these experiments the amount of fibrin present was less than that found in the lesion produced by the virulent pneumococcus, it was perceptibly larger than the very small amount found in the lesions caused by the less virulent strain of streptococcus.

It should be mentioned that there was no difference in the degree of phagocytosis observed in these two series of experiments. It was not marked in either case.

One point of difference in the two series was noted: during the first forty-eight hours after insufflation of the virulent strain of streptococcus the blood of the animals obtained from the jugular vein during life and from the heart after death contained living organisms, while in the experiments with the slightly virulent streptococcus no living cocci were recovered from the blood.

The virulence of the strain of streptococcus employed in the present investigation was, as has been pointed out, similar to that of the pneumococcus employed in the investigation of Lamar and Meltzer and in many of our own experiments, as far as mice were concerned, since both killed mice weighing fifteen grams in doses of 0.000,001 of a cubic centimeter in twenty-four to thirty-six hours. The findings in the lesions caused by these two organisms are comparable and therefore some deductions may be drawn regarding the similarity or dissimilarity of their actions. One of the first things we wish to bring out is the correction of our former statement regarding the presence of the cocci in the blood. As our new investigation shows, there is evidently no difference in this respect between the streptococcus and the pneumococcus. When both organisms are highly virulent they can be found in the circulating blood of the living dog or in the heart's blood of the dead dog during the first twenty-four or forty-eight hours after an intrabronchial insufflation.

On the other hand, the present investigation rather confirms in a

general way our first contention that the pneumonic lesions produced by the two different organisms differ distinctly in many ways. There is the difference in the mortality; even with fairly large quantities of the culture of the virulent streptococcus the mortality was nil in this series, although some of the dogs were kept alive for six and seven days. The mortality of the pneumococcus infection depends upon the quantity insufflated and may amount, as Lamar and Meltzer pointed out, to 16 per cent.

The gross appearance of the lesion produced by the pneumococcus is, as frequently stated, that of a lobar pneumonia, frequently accompanied by a fibrinous exudate on the pleura. The gross appearance of the lesion produced even by a virulent streptococcus is, as a rule, that of a lobular pneumonia and is practically never accompanied by pleurisy. In the few instances in which large quantities of the streptococcus were given and the outward appearance of the lungs approached that of a lobar pneumonia, it was found that on section of the consolidated lung the solid foci proved to be separated by lighter areas of aerated, congested lung.

In the lesions produced by the virulent streptococci the walls of the finer bronchi and the framework of the lung were markedly infiltrated with leucocytes, while in the lobar pneumonia produced by the pneumococcus the framework remained free from such infiltration.

Finally there is the unmistakable difference in the amount of fibrin present in the alveolar exudate. While in the exudate of the lesion produced by the virulent streptococcus the amount was perceptibly larger than the insignificant amount present in the lesion caused by a slightly virulent strain, it is not to be compared with the large amount of fibrin which exists in the exudate of pneumococcus pneumonia.

The several investigations which we have carried out seem to show conclusively that in general the streptococcus causes a lobular pneumonia which, besides the leucocytic intra-alveolar exudation, is characterized by a leucocytic infiltration of the lung framework, and that the pneumococcus causes a lobar pneumonia, which is practically free from leucocytic infiltration of the interstitial tissue of the lung. Furthermore, a virulent pneumococcus causes a lesion in which

fibrin is a prominent element in the exudate and that element distinguishes the exudate sharply from the exudate of the lesion caused by a virulent streptococcus in which fibrin is present only in moderate amount. It distinguishes it in a still more striking manner from the exudates of the lesions caused by non-virulent pneumococci or streptococci, in which fibrin is present only in very small amounts.

It seems that the formation of fibrin is connected in some specific way with the pneumococcus on the one hand, and with the virulence of the organism on the other. For even with the relatively small amounts of fibrin present in the exudates of lesions caused by the streptococcus there is a perceptible difference in the quantity according to the virulence of the organism.

Whether fibrin is a means which enhances virulence, or whether it is a reaction product against it, our experiments so far do not entitle us to discuss.