PNEUMONIC LESIONS MADE BY INTRABRONCHIAL INSUFFLATION OF NON-VIRULENT PNEUMOCOCCI.*

By MARTHA WOLLSTEIN, M.D., and S. J. MELTZER, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

INTRODUCTION.

In two series of investigations pneumonia has been produced experimentally in dogs by means of intrabronchial insufflation of virulent organisms. Lamar and Meltzer[1] produced lobar pneumonia in a large number of animals by insufflation of cultures of a highly virulent pneumococcus, and in a smaller number by the insufflation of *Streptococcus mucosus* and of Friedländer's pneumobacillus. We[2] produced lobular pneumonia in twenty dogs by the insufflation of cultures of streptococcus, and in eleven dogs by the insufflation of cultures of the influenza bacillus. These experimental results agree with the pathological experience with human beings; in lobar pneumonia it is the pneumococcus which is found in the exudate, and in bronchopneumonia the streptococcus is more often present. Both forms of experimental pneumonia have, of course, essential features in common; namely, there is in both an acute exudative inflammation, the exudate containing polymorphonuclear leucocytes, serum, and desquamated epithelial cells. Later large mononuclear phagocytes and cell debris also appear in both. But the pneumonias have also some features which sharply distinguish one from the other. We discussed these distinguishing features in our last paper.[3] For the purpose of our present paper it is well to enumerate them again. However, we wish to say expressly that these features have reference only to the experimental

* Received for publication, December 10, 1912.

[1] Lamar, R. V., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xv, 133.

[2] Wollstein, M., and Meltzer, S. J., *Jour. Exper. Med.*, 1912, xvi, 126.

[3] Wollstein, M., and Meltzer, S. J., *loc. cit.*

pneumonias produced by the method of intrabronchial insufflation, and only as we know them at present.

1. In experimental lobar pneumonia there was a mortality of 16 per cent.; in experimental bronchopneumonia there was no mortality. We should, however, add that in this respect the two series of experiments were not sufficienty comparable to permit of a final conclusion. Not only was the number of experiments with the streptococcus only about one half as large as the number of those of Lamar and Meltzer with the pneumococcus, but most of the animals were killed at too early a stage after the inoculation to permit drawing a definite conclusion as to the actual mortality of this infection. For the present we have to be satisfied with the statement that in none of our uncomplicated cases of experimental bronchopneumonia was there a fatal tendency of the disease. Furthermore in the experiments with the pneumococcus the course seemed to depend upon the quantity of injected culture ("most of the deaths followed the injection of a large quantity of culture, and, conversely, the injection of a large quantity was usually fatal"),[4] while in our experiments with the streptococcus large doses did not seem to affect the final outcome. For instance, in an animal which received thirty cubic centimeters of culture and which was killed on the sixth day, the animal was not sicker than other animals that received fifteen or ten cubic centimeters of the culture, nor was the resolution of the exudate retarded.

2. In the pneumococcus inoculations a pneumococcus septicemia developed in all the fatal cases; no bacteremia occurred in the streptococcus cases.

3. In the pneumococcus lesions the consolidated part of the lung was always dense and air-free, *i. e.*, lobar in character, no matter how large or small the affected area was; in the lesions produced by the streptococcus, no matter how large the area or how intense the inflammatory process was, aerated lobules were always discernible among the solid foci; *i. e.*, the streptococcus lesion was lobular.

4. In pneumococcus pneumonia there was always a definite pleurisy present even in all the non-fatal cases, while it was practically absent in all the streptococcus infections.

[4] Lamar, R. V., and Meltzer, S. J., *loc. cit.*, p. 141.

5. In the pneumococcus lesions the cut surface was rather dry and granular; in the lesion produced by the streptococcus the cut surface was moist and smooth.

6. Fibrin was an important element in the exudate of the lesions brought about by the pneumococcus, while it played practically no part in the streptococcus pneumonia.

7. In the pneumococcus lesions the walls of the alveoli and bronchioles remained free from infiltration although their lumina may have been densely packed with exudate; on the other hand, in the lobular pneumonia produced by the streptococcus not only the walls of the alveoli and bronchioles but also the connective tissue septa and the adventitia of the blood vessels were the seat of marked infiltration.

8. The resolution of the pneumonic lesions set in and terminated earlier in the consolidations from streptococci than in those caused by pneumococci.

We may now add a further point of differentiation. In the course of further study we made many experiments with insufflation of virulent pneumococci. We found that in every case twenty-four hours after insufflation of a virulent pneumococcus this organism could be cultivated from the heart's blood, whereas three or four days later in the non-fatal cases the lungs as well as the blood were free from the pneumococcus. This is an interesting point and *probably has its analogy in clinical findings.* On the other hand, in pneumonia produced by the insufflation of streptococcus the blood remained free from the infecting organism. We may therefore state:

9. Insufflation of virulent pneumococci leads in all cases to bacteremia at the end of the first day after inoculation. The bacteremia disappears in the non-fatal cases, while after insufflation of streptococci no bacteremia develops.

From the above it seems that in general the inoculation of the streptococcus produced a milder effect than the pneumococcus. In discussing the results of both series of investigations, we said that since different organisms introduced in the same way and under conditions which are apparently the same produce distinctly different pneumonic lesions in animals of the same species, the con-

clusion presents itself that different types of pneumonia are produced by specifically different bacteria. We added, however, that further investigation may show that the differences in the nature of the lesions are due rather to the degree of virulence of the causative microörganism than to differences in the species, that even apperently virulent streptococci may nevertheless be less virulent than certain virulent pneumococci, and that such different lesions as were here obtained by different organisms may perhaps be obtained by organisms of the same species possessing different degrees of virulence.

This point has now been tested in a series of experiments on dogs with the intrabronchial insufflation of a non-virulent strain of pneumococcus. The non-virulent strain was obtained from Dr. Dochez of the Rockefeller Institute Hospital, by whom it was isolated from the blood of a patient with lobar pneumonia who recovered. The microörganism[5] had the morphological and cultural features of the pneumococcus, but failed to kill a mouse in doses less than one half cubic centimeter of a twenty-four hour broth culture when freshly isolated from the patient's blood. After cultivation in the laboratory it failed to kill in any dose.

EXPERIMENTAL DATA.

Twenty to twenty-four hour broth cultures of the avirulent pneumococcus were injected by intrabronchial insufflation into eighteen dogs, in doses varying from ten to thirty cubic centimeters. In three instances the dose of cocci was enriched three times; that is, it contained three times the number of organisms, as the result of centrifugalization of a large quantity of culture. The method of injection by intrabronchial insufflation has been described in detail by Lamar and Meltzer,[6] and consists essentially of the introduction of a tube through the mouth, larynx, and trachea deeply into a

[5] This strain of pneumococcus was soluble in rabbit bile, though it was avirulent for white mice, and, as will be shown later, it differed from the virulent pneumococcus in its effect on the lungs of dogs. This solubility of an avirulent pneumococcus is contrary to the statement of Neufeld and Haendel (Kolle, W., and Wasserman, A., Handbuch der pathogenen Mikroorganismen, Jena, 1912, iv, 527) that avirulent strains are not dissolved in bile.

[6] Lamar, R. V., and Meltzer, S. J., *loc. cit.*

bronchus, the culture being introduced through the tube. Usually the tube slips into the right bronchus.

None of the dogs died as the result of the inoculation of these doses of avirulent pneumococci. All were killed at periods varying from one to seven days after the dose had been administered. The animals did not appear to be very ill, and only three showed a rise of temperature to 41.1° C., all three having received a dose of ten cubic centimeters. The animals receiving the enriched doses, as well as one dog to whom thirty cubic centimeters were given, had even less fever and no more severe local reaction than the dogs to whom the smaller dose had been administered.

The Lesions in the Lungs.—The gross appearance of the lesions in the lungs of the dogs varied according to the length of time elapsing between the injection and the killing of the animal; but in parellel stages the differences were very slight. The size of the dose influenced the course of the disease and the severity of the lesion but little, a point in marked contrast to the experience of Lamar and Meltzer with virulent pneumococci. There was no exudate on the surface of the pleura in any one of the eighteen cases, but a lack of luster was noted in three instances, one and two days after inoculation. In twenty-four hours after insufflation of a dose of ten or fifteen cubic centimeters of the avirulent organisms, the whole or part of one lobe was the seat of hepatization. In two cases the right posterior lobe, in two cases the left posterior lobe, and in one case the right anterior lobe was involved. In two cases the entire lobe was solid, in two others only two thirds of the lung was involved, and in one all but the superior anterior angle of the lobe was solid. The lobe was heavy, firm, but not much larger than its uninvolved neighbors. On section the hepatized lung was red, moist, and evenly consolidated; only blood could be expressed. From the bronchi frothy fluid ran, thin, not viscid. Aerated lobules were not seen within the solid areas, but at their periphery, the line of demarcation being irregular. Pneumococci were found in small numbers in smears made from the lung, and they grew sparingly in cultures; the heart blood was sterile. The anatomical diagnosis was that of an early, not severe lobar pneumonia in the stage of red hepatization, the condition of engorgement being still present in portions of the lobe.

In forty-eight hours the lesion had become more marked; hepatization was more advanced, though no larger area of lung was involved. Thus the left posterior lobe except its inferior border was solid in one case, three quarters of the right posterior in another, two thirds of the left anterior and the left posterior in another, and two thirds of the right posterior with a small area in the subcardiac lobe in still another animal. These lungs were more solid and heavy than those seen on the earlier day; on section they were mottled red and gray, smoothly consolidated, friable, and less moist. It was difficult to find pneumococci in smears, and none grew in cultures.

Microscopic examination after twenty-four hours showed that the alveoli were filled with polymorphonuclear leucocytes in large numbers, very few epithelial cells, and little fibrin. The capillaries in the alveolar walls were distended with blood, but the walls of the alveoli were not infiltrated with cells nor with fibrin. The congestion was most marked just beneath the pleura, and some of the most solidly packed areas were also there. The alveoli were not equally filled with exudate, some containing only fibrin and a few desquamated epithelial cells, others a few red cells in addition to these, and in others the leucocytes were most abundant, nuclear fragmentation being well under way. The smallest bronchi contained plugs of fibrin and leucocytes, while their walls showed leucocytic infiltration; the larger bronchi, on the other hand, were quite normal, showing neither desquamation of their epithelia nor infiltration of their walls; they were empty. Some medium sized bronchi contained masses of fibrin, leucocytes, and red cells quite detached from their lining cells, evidently sputum in the course of expectoration. Pneumococci were few in number in the alveoli and in the bronchi. Areas of leucocytic exudation about small veins were numerous.

In forty-eight hours the exudate was found to have become more leucocytic in character, the epithelial cells being few in number and the red blood cells only occasional. The amount of fibrin had not increased. The most solid areas were again found beneath the pleura. The bronchi showed the same lesions as in the earlier stage, the infiltration of their walls not being more marked or more general.

Five days after insufflation of a dose of ten cubic centimeters resolution was going on in a large portion of the right posterior lobe, though a small area of pneumonia still remained. In six days there were no solidly packed alveoli remaining, and resolution was almost complete.

A larger dose of twenty cubic centimeters had caused a lobar pneumonia still at its height on the third day, with an exudate consisting mostly of polynuclear leucocytes and with little fibrin. That the disease was still progressing on the third day was evidenced in the animal by the fact that while the right posterior lobe showed the lesion of red hepatization, the upper half of the left posterior lobe showed an earlier stage of engorgement and edema, with an exudation of serum, epithelial cells, red blood cells in small numbers, and less fibrin than was present in the later stage. This left posterior lobe was not swollen, but dark, firm, heavier than its aerated neighbor, and its section was moist, not crepitant, but not entirely airless.

The pneumonia caused by a dose of twenty cubic centimeters was quite resolved on the sixth and seventh days, leaving only a dark, congested, inelastic area to indicate grossly where the lesion had been. On microscopic examination there was evidence of phagocytosis of cellular fragments and the presence of granular, poorly staining debris in some alveoli.

In a dog that received thirty cubic centimeters the lungs presented on the third day a well marked but not intense lobar pneumonia (red hepatization) in the upper half of the right posterior lobe. The amount of fibrin present in the alveolar exudate was only moderate, leucocytes being very numerous.

An enriched dose, containing in fifteen cubic centimeters of broth the cocci grown in forty-five cubic centimeters, produced a lesion in twenty-four hours which occupied the right posterior lobe except its anterior border; the lobe was red, solid, not friable, evenly consolidated, moist, and without pleurisy. Its exudate consisted of polymorphonuclear leucocytes, few epithelial cells, and very little fibrin contained in alveoli whose walls were quite free from infiltration of any kind. The lesion was not more severe than one produced by a dose of fifteen cubic centimeters. Another

dog, after an enriched dose similar to the above, showed a resolving lobar pneumonia on the third day, about two thirds of the left posterior lobe having been involved. On microscopic examination the extent of the resolution was surprisingly far advanced, considering the size of the dose of cocci administered.

DISCUSSION.

The experiments established, in the first place, the fact that the insufflation of a culture of avirulent pneumococcus invariably caused the development of a pulmonary lesion which macroscopically had the appearance of a lobar pneumonia. The consolidated part was firm, solid, heavy, and contained no aerated lobules in its midst. The inflammation, however, was less intense than it usually is in the lesions of lobar pneumonia caused by virulent pneumococci, the progress was generally slower, and the resolution set in earlier. Furthermore, in none of the animals had the disease a fatal termination, and the increase of the quantity of the insufflated culture had not the same effect upon the course of the inflammatory process that it undoubtedly had in the experiments with the virulent pneumococcus. Still more important perhaps are the following differences. The exudate produced by the avirulent pneumococcus contained strikingly less fibrin than the exudates of the virulent pneumococci, the quantities of fibrin present being only slightly larger than in the streptococcus exudates. The pneumonia produced by the avirulent pneumococcus differed further from the pneumonia of the virulent pneumococcus by the complete absence of the organism from the blood at any time after the inoculation. Even the pulmonary lesion itself contained no more culturable organisms on the second day after the inoculation.

By the non-fatal character of the disease, by the absence of bacteremia, by the scantiness of fibrin in the exudate, and by the tendency to earlier resolution, the pneumonia of the non-virulent pneumococcus manifests similarities with the experimental pneumonia produced by insufflation of the streptococcus. However, it differs from the latter by leaving the framework of the lung tissue and the bronchi practically free from injury. While in the advanced lesions caused by the streptococcus there was often a

marked purulent infiltration of the walls of the alveoli and of the bronchioles, of the connective tissue septa, and even of the adventitia of the blood vessels, in the pneumonic lesions brought on by the non-virulent pneumococcus there was at the most only a slight leucocytic infiltration of the walls of the small bronchi.

SUMMARY.

The intrabronchial insufflation of a non-virulent pneumococcus causes, like the insufflation of a virulent pneumococcus, the development of an exudate in the lungs which, in general, leaves the framework unaffected, and the lesion presents the gross appearance of a lobar pneumonia. It differs, however, materially from the pneumonia produced by virulent pneumococci in the important points that the consolidation tends to a more rapid resolution, the disease is non-fatal, the blood is not invaded by the organism, and the exudate is strikingly poor in fibrin.

As to the question which was the starting point for the foregoing investigation, namely, whether the pneumonic lesion produced by the streptococcus is merely a form of pneumonia caused by a less virulent organism, it may be answered for the dog, it seems, in the negative. The leucocytic infiltration of the framework of the lungs which occurs invariably in streptococcus pneumonia, and which is practically absent in the lesions caused by the virulent as well as by the non-virulent pneumococcus, is a strong enough feature to form a dividing line between the two forms of experimental pneumonia previously described.