PHAGOCYTIC IMMUNITY IN PNEUMOCOCCUS INFECTIONS, AND IN PNEUMONIA WITH RELATION TO THE CRISIS.*

By S. STROUSE, M.D.

(From the Laboratories of the Michael Reese Hospital, Chicago.)

This work was originally planned to determine the presence of immune opsonins in the circulating blood of pneumonia patients after crisis. However, while the work was under way, Neufeld and his associates (1) published their results on the same subject, in which they claim to have found immune opsonins or bacteriotropins in practically every case. They also found that post-critical sera protected mice from many times the minimum lethal dose of virulent pneumococci. On the basis of this work, Neufeld has undertaken to make a serum for the treatment of pneumonia. Subsequently, Seligmann and Klopstock (2), and Boettcher (3) have been unable to confirm Neufeld's results.

Since Mennes' (4) first work on the subject, phagocytosis has been considered an important means of defense in pneumonia; and yet so far no successful practical application has been made of this biological reaction in treatment. Lamar (5) has recently given a conservative review of the subject of phagocytosis and pneumococcus immunity, which he concludes as follows:

"With our present knowledge, we must view the power of an antipneumococcus serum to promote phagocytosis as the index of its therapeutic activity, and we must also view the property of spontaneous phagocytosis of the pneumococci, which certain animals exhibit, as the determining cause of their refractoriness to infection. The only known exception to this is the pigeon. . . . These considerations do not exclude the probability that there may

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be still other and undetermined factors concerned in the prevention of infection by the pneumococcus."

Seligmann (6) questions whether modern biological methods can explain the crisis in pneumonia, and Lamar has turned his attention to the chemical side of the problem.

However, in view of the importance which Neufeld attached to the presence of immune opsonins, and the inability of later workers to substantiate his findings, it seemed worth while to continue these studies. Neufeld showed in his first communications the presence of what were apparently general pneumococcus immune opsonins, but later work proved that certain strains of pneumococci were not acted upon by serum possessing opsonic powers for other pneumococci. Such strains were called "serum-fast," but they were opsonized by the post-critical sera of pneumonic patients from whom they were derived. In other words, a certain marked degree of specificity was obtained in the immune opsonins of post-critical pneumonic sera.

**EXPERIMENTAL PART.**

The question of the specificity of opsonic immunity in animals injected with pneumococci and the relation of such immunity to the virulence of the strain used were studied in a series of experiments. It is unnecessary to give this work in detail, as the results all coincided. With avirulent organisms it was possible to produce in the serum of injected rabbits immune opsonins for the homologous organisms, but none for heterologous strains. In all test-tube reactions, Neufeld's technique, followed precisely, was employed, as he has frequently criticized other workers on the ground that they varied the technique. In addition, however, to the test-tube experiments, the reaction was studied in the peritoneal cavity of rabbits and guinea pigs immunized to avirulent pneumococci by introducing virulent organisms and removing the peritoneal exudate by glass pipettes at different intervals. As this method of testing for immune bodies may interfere with the demonstration of a protective immunity, these experiments were always performed in duplicate. With each attempt to demonstrate phagocytic immunity in the peritoneal cavity, an exactly identical control experiment
was made without removing peritoneal fluid. In spite of the fact that the animals withstood large doses of the avirulent culture, no phagocytosis of the virulent pneumococci could be detected in the peritoneal cavity, nor were the animals rendered resistant to virulent cultures. One of these experiments is given in the following protocol:

January 23, 1911.—Guinea pigs 1-10 and 2-10. Have been immunized since December 29, 1910, with increasing doses of avirulent pneumococci. Last injection January 18, 1911.

January 23, 1911.—Guinea pig 1-10. Received 1 c.c. of virulent pneumococci intraperitoneally. Peritoneal fluid removed every 2 hours showed no phagocytosis. Pneumococci increased in number. Death in 36 hours.

Guinea pig 2-10. Same dose. No withdrawal of fluid from peritoneal cavity. Death in 20 hours.

Guinea pig X (normal control). Received intraperitoneal injection of 0.5 c.c. of the same culture. Death in 60 hours.

Perhaps the most interesting results were obtained with organisms of a moderate degree of virulence. Here the technical difficulties of estimating immune opsonins on the basis of spontaneous phagocytosis in salt solution were more easily overcome, and the attempts at immunizing were much simpler than with extremely virulent pneumococci. In this series, carried out in a parallel manner, a high degree of opsonic immunity for the homologous organism was produced. Likewise, to a certain degree, phagocytic immunity could be shown to exist for virulent strains. With some strains of pneumococci which showed slight spontaneous phagocytosis and which were only moderately virulent for rabbits, the ability to produce opsonins against virulent strains and to protect from injections of such strains was in marked contrast to the inability of avirulent organisms to produce immunity toward heterologous strains.

With very virulent organisms, there was some difficulty in raising the animals to a sufficiently high grade of immunity to withstand large doses, and in none of them could we demonstrate immune opsonins either toward the homologous virulent strain or toward heterologous avirulent or moderately virulent ones. Boettcher was likewise unable to produce phagocytic immunity for very virulent pneumococci; but he succeeded in producing in rabbits immune
Phagocytic Immunity in Pneumococcus Infections.

opsonins for moderately virulent organisms. He also claims to have protected mice against moderately virulent organisms by previous injection of avirulent pneumococci.

These results emphasize strongly the fact that pneumococcic immunity is, to a high degree, specific for the organism used in immunization, and further that the reaction of the injected animal depends to a great extent on the virulence of the organism. My results with certain moderately virulent organisms indicate the possibility of producing in larger animals a more general immunity. Rosenow’s (7) studies on the increased production of antibodies by autolyzed and washed virulent pneumococci, as compared to the difficulty of immunizing with the whole culture, may bear a close relation to the point under discussion, since, as Rosenow (8) has previously shown, virulent non-phagocytable pneumococci after autolysis become avirulent and phagocytable.

In the study of post-critical pneumonic sera, Neufeld’s technique was followed. The sera were taken from twenty different patients, two to four days after crisis or lysis, and heated for twenty minutes at 54° C. The pneumococci obtained either from the sputum or blood of pneumonic patients were very virulent, were fatal in doses of 0.0001 cubic centimeters to mice, and showed no spontaneous phagocytosis. They were grown in 5 per cent. beef serum bouillon. The leucocytes were obtained from the peritoneal cavity of guinea pigs following aleuronat injection. Incubation at 37° C. was usually for one and a half hours, sometimes for two and a half hours. These are the conditions laid down by Neufeld as necessary for the successful demonstration of immune opsonins. One other factor of importance was considered in each experiment; namely, the source of the pneumococcus. If “serum-fast” strains exist, opsonizable only by the homologous serum, then each strain used must be tested against its homologous serum. This was carefully controlled in each set of experiments by using several sera and at least two organisms, including one strain homologous to one serum. A protocol of a typical experiment follows.

Our results were surprisingly constant. With the virulent organisms showing no spontaneous phagocytosis, we were never able to find opsonins in heated sera taken after the crisis from pneu-
All Sera Heated to 54° C. for Twenty Minutes. Dilutions 1:10, 1:100, 1:500, 1:1000. Incubation at 37° C. for One and a Half Hours.

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<thead>
<tr>
<th>Patient</th>
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<td>A</td>
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Pneumococcus B is from the sputum of Patient B. Pneumococcus C is from the sputum of Patient C.

monic patients. This general result includes also those cases in which sera were tested against homologous organisms.

In addition to the experiments with highly virulent organisms, identical experiments were performed with pneumococci showing various degrees of spontaneous phagocytosis. In work of this sort, it is hard to overcome technical difficulties sufficiently to yield unbiased and absolute readings. Great care must be taken in striking a balance between the phagocytability of the organism and the time of incubation; there is danger of the "salt control" showing too much phagocytosis, and of the attempt to overcome this by shortening the time of incubation, in which case the serum is not permitted to opsonize the pneumococci.

The plan of Lamar (9) to sensitize the pneumococci by the sera for an hour before adding the leucocytes was followed in many experiments, and at times made the results less difficult to interpret. But even with this technique, most of the work with organisms showing spontaneous phagocytosis could not be accepted by an impartial observer, and only a few experiments gave results sufficiently sharply defined to permit of conclusions. With five sera, two of which had been tested against the more virulent pneumococci with negative results, we were able definitely to show immune opsonins at a dilution of 1:300. One case of pneumococcus endocarditis receiving autogenous vaccines showed immune opsonins in a dilution of 1:1500, but to the homologous organism only. It is interesting to compare this with my other results and with those of Meakins (10) on streptococcus endocarditis. It may also be of importance in the final correlation between opsonins and actual
pneumococcus immunity to state that this patient died although her opsonins described an upward course.

Neufeld found that the post-critical sera which showed opsonins in test-tube experiments also protected mice from many times the minimum lethal dose of virulent organisms; and in my series, sera of eight different patients were tested in an identical manner. Two hours after intraperitoneal injection of 0.2 cubic centimeters of post-critical sera, various multiples of the minimum lethal dose (0.000001 cubic centimeters) of virulent pneumococci were injected. As might be expected from the test-tube experiments, these animal inoculations were completely negative. Boettcher also failed to find any protective action of post-critical sera for mice. It is probable that with less virulent cultures the same results would be obtained in animal experiments as were shown in the test tube; but an insufficient supply of mice prevented a more extensive investigation.

SUMMARY.

These results seem definitely to show, through animal experiments, that phagocytic immunity is to a high degree specific for the organism used in immunization, and that the amount of opsonin produced in the process depends to a great extent on the virulence of the organism. The negative results obtained with post-critical sera do not mean that opsonins may not be present (our five positive cases indicate their presence), but they emphasize strongly the fact that they are not formed to any great extent. Therefore this study adds further support to the view that although opsonic immunity is produced in pneumonia, it is not the only means of defense possessed by the body, and by itself it cannot explain the crisis.

In conclusion, I wish to acknowledge my indebtedness to Dr. J. W. Jobling for many helpful criticisms and suggestions in the course of this work, and to Drs. Johnson, Goodkind, and Edwards for the privilege of studying the cases in the medical wards of the hospital.

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