SURFACE PHAGOCYTOSIS

Further Evidence of Its Destructive Action upon Fully Encapsulated Pneumococci in the Absence of Type-Specific Antibody*

By MARY RUTH SMITH and W. BARRY WOOD, Jr., M.D.

(From the Department of Microbiology, The Johns Hopkins University School of Medicine and School of Hygiene and Public Health, Baltimore)

Plate 1

(Received for publication, June 29, 1957)

The role of surface phagocytosis in antibacterial defense has been described in previous publications (1–13). Its destructive action upon certain common species of encapsulated bacteria in the absence of type-specific antibody has been confirmed by a number of investigators (14–17). Recently, however, Lerner (18) has reported findings which fail to substantiate these earlier studies. He has criticized them as not having been sufficiently quantitative. Francis (19) also has questioned the significance of surface phagocytosis in pneumococcal infections on the grounds that it may be of only "limited efficiency."

The publication of Lerner's paper has prompted us to repeat his experiments as exactly as possible. By so doing, we have determined the factors which account for the conflicting observations. In addition, we have gained further knowledge as to the quantitative aspects of surface phagocytosis, by repeating previously reported *in vitro* tests, using a more precise method for recording the end results of the phagocytic reaction. The data thus obtained confirm the findings originally reported and further support the conclusion that surface phagocytosis provides an important mechanism of natural defense in infections due to pneumococcus (2, 9).

Methods

Bacterial Strains.—Two strains of organisms were used in the phagocytic tests. The first was the A-5 strain of pneumococcus type I employed in earlier studies (20). Stored in rabbit blood under vaseline as previously described (20), it was subjected to mouse passage at frequent intervals to maintain maximum capsule formation. The second was a rough pneumococcus (strain R36NC[1]) possessing no demonstrable capsule but originally derived from a type II pneumococcus. It was stored in the same manner but was not passed serially in mice.

* This study was supported by a grant from the United States Public Health Service (E-662).

[1] Kindly supplied by Dr. Colin MacLeod of the University of Pennsylvania School of Medicine.

Cultural Methods.—All cultures were made in beef infusion broth containing 10 per cent sheep serum and 0.2 per cent dextrose. Transfers[2] were made from 16 hour broth cultures and were incubated for 4 hours at 37°C. before being harvested.

Bacterial Suspension.—The organisms centrifuged from 5 ml. of each 4 hour culture were washed twice in iced gelatin-Locke's solution (21) and the final centrifugate was diluted so that each ml. contained 2×10^{10} pneumococci.[3] All procedures were performed at approximately 4°C. in order to minimize loss of capsular material from the pneumococcal cells. Centrifugations were carried out in an International refrigerated centrifuge (PR 2) equipped with an angle head (No. 813a), and driven at a rate of 3000 R.P.M. (1500 *g*) for 30 minutes. The Krebs-gelatin solution used by Lerner (18) was substituted for the gelatin-Locke solution in several experiments. It was found, however, to be unsatisfactory because its gelatin content was so high that it solidified when cooled to 4°C.

Leucocytes.—Leucocytes were obtained from 21-hour-old peritoneal exudates produced in albino rats by intraperitoneal injections of a sterile mixture of starch-aleuronat and tryptose phosphate broth. A detailed description of the method used has been published elsewhere (2).[4] The cells were washed from the peritoneal cavity of each of four rats with 15 ml. of iced gelatin-Locke's solution containing heparin in a concentration of 1 mg. per cent. After being washed once more in this solution all four of the cell centrifugates were pooled and brought to a volume of 5 ml. A cell count was performed on this last suspension, and an aliquot containing 5×10^8 cells was again centrifuged. When the supernatant was removed, the final volume of packed cells was 0.3 ml. Centrifugation of the leucocytes (unless otherwise stated) was performed in the refrigerated centrifuge with a horizontal head spun for 5 minutes at 800 R.P.M. (150*g*). Throughout all of the procedures the cells were kept in the cold to preserve their functional activity.

Cell-Pneumococcus Suspension.—A volume of 0.05 ml. of the pneumococcal suspension containing 2×10^{10} organisms per ml. (or a total of 1×10^9 pneumococci) was added to the 0.3 ml. of cell centrifugate which contained 5×10^8 leucocytes. The combined suspension was then thoroughly mixed, and a stained smear was made for study of: (*a*) the condition of the leucocytes, and (*b*) the differential leucocyte count. The average ratio thus obtained was 2 pneumococci per cell. The cell population contained approximately 70 per cent polymorphonuclear leucocytes.

In those experiments in which Lerner's method (18) was followed, equal volumes of a pneumococcal suspension containing 1×10^{10} organisms per ml. and a leucocyte suspension containing 1×10^8 cells per ml. were mixed in a similar manner. The resulting cell-pneumococcus ratio was 1 to 100. The concentration of cells in these mixtures, however, was only between 5×10^6 and 5×10^7 per ml., whereas in the standard method, outlined above, the concentration of cells was approximately 1.5×10^9 per ml. In other words, the cellular concentration in the standard method was 30 to 300 times greater than in Lerner's. The critical effect of this difference is indicated by the comparative experiments described below.

Phagocytic Tests.—Three different techniques were used to test for phagocytosis.

(*a*) *On Filter Paper.*—To test for surface phagocytosis the originally described filter paper method (2) was modified as follows to increase the precision of the test. Each sample of filter paper used (standard American No. 42700, A. S. Aloe Co., St. Louis,)[5] was cut to a size of 1 by 2 cm., and was placed on a coverslip contained within a Petri dish lined with filter

[2] In each transfer 0.5 ml. of culture was added to 4.5 ml. of fresh broth.

[3] The bacterial counts were made in a Petroff-Hausser chamber.

[4] Treatment of the injected rats with sulfapyridine to prevent infection of the exudate was found to be unnecessary and therefore was not carried out in the present experiments.

[5] The brand of filter paper used by Lerner (Whatman No. 42) was also tested and was found to yield identical results.

paper saturated with gelatin-Locke's solution to prevent drying. After the test sample of filter paper had been moistened with 0.01 ml. of gelatin-Locke's solution, 0.03 ml. of the cold cell-pneumococcus suspension was evenly spread over its surface.[6] The Petri dish was then promptly sealed with adhesive tape and incubated at 37°C. for 30 minutes. At the end of the period of incubation the test sample of filter paper was transferred to an iced Petri dish and was washed thoroughly with 0.5 ml. of cold gelatin-Locke's solution containing heparin. The washings were centrifuged for 3 minutes (150 *g*), the supernatant was discarded, and the centrifugate was thoroughly mixed with a drop of 3 per cent gelatin-0.9 per cent saline solution. The final exudate was washed from the filter paper and centrifuged in order to separate the leucocytes from the extracellular bacteria.[7] The gelatin was added to sharpen the outline of the leucocytes in the stained smears, which were prepared by the standard coverslip method and were stained with Loeffler's alkaline methylene blue.

(*b*) *On Glass.*—To test for phagocytosis on glass the same procedure was followed except that a glass slide was substituted for the coverslip-mounted filter paper. Care was taken to spread the same volume of cell-pneumococcus suspension (0.03 ml.) over the same surface area (1 by 2 cm.) as in the filter paper method.

(*c*) *In Roller Tube.*—To determine the quantitative effect of motion upon the phagocytosis of pneumococci on glass, tests were also carried out by a roller tube method (22). A volume of 0.1 ml. of the pneumococcus-cell mixture was placed in a 7 by 44 mm. test tube which was stoppered and rotated parallel to the shaft of the motor at a rate of 9 R.P.M. for 30 minutes at 37°C. Smears were prepared directly from the rotated suspension and were stained as in the other phagocytic tests.

Method of Recording Phagocytosis.—In each stained smear 200 polymorphonuclear leucocytes were examined, and the number of cells containing one or more pneumococci was counted. The results were expressed as the percentage of polymorphonuclear leucocytes exhibiting phagocytosis (23).

Test for Viability of Leucocytes.—A gross measure of the viability of the polymorphonuclear leucocytes at the end of each phagocytic test was determined by their uptake of trypan blue (24).

Nomenclature.—The term "standard method," as it refers to phagocytic tests, is used throughout this report to indicate the method generally followed in earlier studies (2, 4) and further refined as described above. The method was originally designed to approximate as closely as possible the conditions which commonly obtain in naturally occurring pneumococcal exudates.

RESULTS

A. Quantitative Measurement of Surface Phagocytosis.—

Since earlier experiments relating to surface phagocytosis have been criticized by Lerner (18) because of the fact that they did not include a quantitative measurement of the resulting phagocytosis, they were repeated as described in the section on methods. The quantitative relationships revealed by the per cent phagocytosis recorded in each of the tests (Table I) fully confirm the

[6] The coverslip was placed beneath the test strip of filter paper to prevent absorption of additional fluid from the saturated filter paper in the bottom of the Petri dish (see question raised by Lerner in Addendum (18)).

[7] This important step was not carried out in any of Lerner's experiments. Its omission, as is emphasized below in the discussion, significantly increased the counting error in those phagocytic tests in which the ratio of pneumococci to leucocytes was high, *i.e.*, 100 to 1.

earlier observation that the presence of a suitable surface upon which the leucocytes may operate is a critical factor in the phagocytosis of encapsulated pneumococci in the absence of opsonins (1–3, 5–13, 25). The results also indicate that in the case of non-encapsulated (rough) pneumococci surface phagocytosis is of little significance (9, 25).

TABLE I

Quantitative Measurements of Phagocytosis of Pneumococci on Glass and Filter Paper

Organism	Method of recording phagocytosis	Degree of phagocytosis		
		Glass	Roller tube	Filter paper
Pneumococcus type I (capsule intact)	− to ++++	−	−	++++
Pneumococcus type I (capsule intact)	Per cent leucocytes containing pneumococci	0.5	0	62
Rough pneumococcus (no capsule)	− to ++++	++++	++++	++++
Rough pneumococcus (no capsule)	Per cent leucocytes containing pneumococci	50	64	64

TABLE II

Comparative Results of Phagocytic Tests Made with Concentrated and Relatively Dilute Leucocytic Exudates

Organism	Exudate	Per cent leucocytes containing pneumococci		
		Glass	Roller tube	Filter paper
Pneumococcus type I (capsule intact)	Concentrated (standard)	0.5	0.5	66
Pneumococcus type I (capsule intact)	Dilute (Lerner)	0	0	0

B. The Failure of Surface Phagocytosis to Occur in Relatively Dilute Suspensions of Leucocytes.—

In systematic histologic studies of the pre-antibody phase of experimental pneumococcal pneumonia (9, 20, 26, 27), appreciable phagocytosis of pneumococci has been shown to occur only in those portions of the lesion where the leucocytic exudate has become relatively concentrated (see Figs. 1 and 2). Furthermore, it has been demonstrated that surface phagocytosis *in vitro* may be markedly inhibited by the addition of fluid to the test mixture of leucocytes and pneumococci (10).

As already mentioned under methods, the exudate used in Lerner's phagocytic tests was between 30 and 300 times more dilute than that employed in the standard method (see Figs. 3 and 4). The importance of this difference is shown by the experiments summarized in Table II.

It will be noted that in the concentrated exudate, the fully encapsulated A-5 strain is resistant to phagocytosis on glass (in both stationary and moving preparations) but is relatively susceptible on filter paper (Fig. 5). In the dilute exudate, employed by Lerner (18), on the other hand, the encapsulated pneumococci are not phagocyted on either glass or filter paper (Fig. 6).

C. The Inhibition of Phagocytosis Resulting from the Presence of an Excessive Number of Pneumococci.—

Lerner (18) has also criticized the earlier studies of surface phagocytosis on the grounds that the ratio of pneumococci to cells was too low to achieve optimal phagocytic activity. He has contended that this ratio should be at

TABLE III

The Inhibitory Effect of a High Bacterium-Phagocyte Ratio upon the Phagocytosis of Fully Encapsulated Pneumococci

Ratio of pneumococci to leucocytes	Per cent leucocytes containing pneumococci		
	Glass	Roller tube	Filter paper
2 to 1 (standard)	0.5	0.5	66
100 to 1 (Lerner)	0.5	0	0

least 100 to 1. As shown in Table III, such a high concentration of virulent pneumococci markedly inhibits phagocytosis when the exudate is sufficiently concentrated to allow surface phagocytosis to take place at lower pneumococcus-leucocyte ratios ("standard" exudate).

This inhibitory effect has been previously described (8). At the conclusion of phagocytic tests with concentrated exudates in which such a high bacterium-leucocyte ratio is employed, between 60 and 85 per cent of the leucocytes are found to be non-viable as indicated by the trypan blue test (24). In contrast, the nuclei of less than 5 per cent of the leucocytes are stainable with trypan blue when either (*a*) the ratio of pneumococci to cells is maintained at approximately the 2 to 1 level adhered to in the standard procedure, or (*b*) the mixture is diluted to the concentration used by Lerner (18). In the latter mixtures, as stated above, surface phagocytosis does not occur because of the dilution factor. It may be concluded therefore, that the inhibitory effect of the high pneumococcus-cell ratio in the concentrated mixtures is due to a toxic action of the virulent organisms upon the leucocytes.

D. The Effect of Parahydroxybenzoic Acid upon the Phagocytosis of Fully Encapsulated Pneumococci.—

Lerner and Victor (28) have suggested that the phagocytosis of pneumococci on filter paper is due to the presence of traces of parahydroxybenzoic acid in the paper rather than to the surface provided for the phagocytic action of the

leucocytes. To test their hypothesis, we have exposed fully encapsulated pneumococci (type I—strain A-5) to parahydroxybenzoic acid and have subjected them to the standard phagocytic tests on glass and filter paper. The concentration of parahydroxybenzoic acid (1 mg. per ml.) and the conditions of exposure (30 minutes at room temperature) were those advocated by Lerner and Victor (28). The data recorded in Table IV indicate that such treatment only slightly increases the susceptibility of virulent pneumococci to phagocytosis on glass. The resulting phagocytic reaction does not approach in intensity that which occurs on filter paper.

TABLE IV

Effect of Parahydroxybenzoic Acid upon the Phagocytosis of Virulent Pneumococci

Organism	Per cent leucocytes containing pneumococci		
	Glass	Roller tube	Filter paper
Untreated pneumococcus type I.....	0.5	0	62
Pneumococcus type I exposed to parahydroxybenzoic acid.....	4	2	57

DISCUSSION

The susceptibility of pneumococcus to phagocytosis is determined primarily by the state of its capsule (29). Furthermore, in its virulent form it is known to be fully encapsulated when multiplying in infected tissues (20). Accordingly, *in vitro* studies of the ingestion of pneumococci by phagocytic cells are relatively meaningless unless the organism is handled in such a way as to preserve the integrity of its capsular gel.

To assure the maximum encapsulation of pneumococci it is necessary (*a*) to pass them through mice at frequent intervals, (*b*) to grow them under optimal cultural conditions, (*c*) to avoid subculturing them repeatedly on artificial media, (*d*) to preserve them in the cold (to minimize the diffusion of specific carbohydrate from their capsular surfaces (8)), and (*e*) to refrain from exposing them to chemical environments which may cause capsular injury (30). Most of these precautions were not taken in Lerner's experiments (18).

Equally important is the state of the phagocytic cells. They, too, must be handled with care.

Not only must the cells be obtained in such a way that they are not subjected either to physical or to chemical injury, but they should also be kept in the cold until the phagocytic tests are performed. Their *concentration* in the test mixtures is particularly critical. Only when crowded relatively close together, as in the inflammatory exudate of infected tissues (see Fig. 2), do they perform their phago-

cytic function with maximum efficiency. This cell concentration factor has been shown previously (10), as well as in the present experiments, to be crucial in surface phagocytosis. The fact that mere dilution of the exudate will inhibit or completely prevent surface phagocytosis appears to account for the relative inefficiency of antipneumococcal defenses in fluid-containing cavities such as those of the pleura, the pericardium, the peritoneum, and the subarachnoid space as compared to those of tissues with relatively dense architectures in which intercellular fluid is sparse (9, 25).

Lastly, the end result of a given phagocytic reaction is often determined by the ratio of bacteria to cells (23, 31).

Whenever the number of bacteria is great enough to cause injury to the phagocytes, the phagocytic process is inhibited. In the case of phagocytic tests with pneumococci, bacterium-cell ratios much greater than 2 to 1 will depress phagocytosis when the exudate is concentrated. This depressive effect *in vivo* may so upset the host-parasite balance as to alter drastically the outcome of the infection (8).

For an *in vitro* phagocytic test to have meaning in relation to disease, the conditions of the test must simulate as closely as possible those which obtain *in vivo*. The present experiments have shown that the conditions of Lerner's phagocytic tests differed in several important respects from those of ordinary pneumococcal infections. Accordingly, interpretation of his experiments is difficult.

The fact that Lerner consistently recorded phagocytic indices of 16 per cent or above when the tests were performed on glass indicates either that the pneumococcus had lost its capsule as a result of being repeatedly subcultured on blood agar (18), or that organisms lying on or beneath leucocytes were counted as having been ingested. The chances of introducing the latter error were, of course, greatly increased in all of the experiments in which there was an excessively high ratio of bacteria to leucocytes and in which the extracellular organisms were not removed by centrifugation (see Methods). Furthermore, comparison of phagocytic counts made by us and by Lerner on identical smears exchanged between the two laboratories have consistently shown marked discrepancies. In all smears which we have examined, for which he has recorded high phagocytic indices on glass, we have uniformly obtained counts of less than 1 per cent. It must be pointed out also that the high phagocytic indices which Lerner reports for phagocytic tests performed on glass surfaces in the absence of antibody have never been observed by other investigators (29–32).

The contention that leucocytes from whole blood should be used in phagocytic tests is also open to criticism. If the objective of an *in vitro* phagocytic experiment is to determine the mechanism by which a given species of microorganism is destroyed in infected tissues (2, 4, 9), it is preferable to use leucocytes obtained from exudates since they are more closely akin to those present in the tissues. Buffy coat leucocytes, on the other hand, might well be used in *in vitro* experiments dealing with the intravascular cellular defenses which operate during bacteriemia (11).

Lerner has suggested that the surface phagocytosis which occurs on filter paper

may be due to the opsonizing action of traces of parahydroxybenzoic acid in the paper rather than to the surface provided the leucocytes (18, 28). Although, as shown in the present experiments, previous exposure of encapsulated pneumococci to parahydroxybenzoic acid does increase slightly their susceptibility to phagocytosis on glass, the effect observed is not sufficient to explain the phagocytic reaction which occurs on filter paper. Furthermore, surface phagocytosis has been repeatedly demonstrated under experimental conditions in which the presence of parahydroxybenzoic acid was presumably excluded (1–5). Finally, direct microscopic study of the non-antibody phagocytic process has revealed that the cells ingest the encapsulated bacteria either by trapping them against the surfaces of adjacent phagocytic cells (3, 4, 11) or by pinning them against other stationary surfaces provided by tissues (2, 4, 11) or fibrinous exudate (5).

The one condition most frequently ignored in phagocytic tests performed *in vitro* is that relating to the presence of surfaces comparable to those which exist in body tissues. The importance of such surfaces in determining whether or not fully encapsulated pneumococci are phagocyted in the absence of antibody has been conclusively demonstrated (1–9). Emphasis has been placed on the fact, however, that *surface phagocytosis is of significance only in the destruction of encapsulated bacteria which are relatively resistant to phagocytosis* (9, 25).

In discussing the mechanism of phagocytosis in the absence of antibody, Lerner makes no distinction between *Escherichia coli*, which is highly susceptible to phagocytosis, and *Diplococcus pneumoniae*, type I which when fully encapsulated is resistant to phagocytosis except in the presence of a suitable surface or a sufficient concentration of opsonin. It is well known that phagocytosis *per se* does not necessarily lead to the destruction of such virulent bacteria as *Mycobacterium tuberculosis*, *Salmonella typhosa*, and *Brucella abortus*, all of which are readily ingested by phagocytic cells. Their ultimate fate depends upon factors of intracellular immunity which are at present not fully understood (32). Pneumococci and Friedlander's bacilli, on the other hand, are usually destroyed promptly once they have been engulfed by polymorphonuclear leucocytes (2, 4, 9) or by macrophages (12). Because of their capsules, however, they are ingested in the absence of antibody only as a result of surface phagocytosis. It has been concluded, therefore, that surface phagocytosis plays an important defensive role in the pre-antibody stage of infections caused by these organisms (9, 25).

Doubt has been expressed by Francis (19) as to the significance of surface phagocytosis in pneumococcal infections primarily because of its "limited efficiency." Although the bactericidal effect of surface phagocytosis in a given infection cannot be directly measured, there is abundant indirect evidence that it plays a dominant role in the pre-antibody stages of pneumococcal disease.

The degree of phagocytosis observed, for example, in the pneumonic exudate

shown in Fig. 2 strongly suggests that polymorphonuclear leucocytes in infected alveoli provide a relatively efficient defense against the invading organisms. Since such phagocytosis has been shown to occur in the absence of demonstrable opsonins, and since the only known mechanism by which fully encapsulated pneumococci can be ingested in their absence is that of surface phagocytosis, it appears logical to conclude that the surface mechanism is responsible for the phagocytic properties of the exudate. More direct evidence of the protective role of surface phagocytosis has been obtained in recent experiments dealing with the curative action of penicillin (13). In a study of experimental myositis produced with types I and III pneumococci, comparative bacterial counts made serially from the lesions have revealed that type III pneumococci, which, unlike type I, are resistant to surface phagocytosis during their logarithmic phase of growth, reach maximum population densities about 100 times greater than those attained by type I pneumococci. The significantly lower counts reached in the type I lesions appear to be due to the bactericidal effect of surface phagocytosis. Similarly, when the rate of destruction of type I pneumococci during penicillin therapy is measured in myositis lesions heavily infiltrated with leucocytes, it is found to be considerably more rapid than that which takes place in analogous acellular lesions produced in granulocytopenic animals. Serial histologic observations indicate that this difference is likewise due to the bactericidal action of surface phagocytosis.

From all these findings it appears justifiable to conclude that surface phagocytosis plays a major role in natural antipneumococcal defense except under conditions in which the leucocytic exudate is so dilute that phagocytosis cannot readily occur (9, 25, 33). Strong evidence in support of this conclusion has been obtained by direct visualization of surface phagocytosis *in vivo* during pneumococcal and Friedlander's bacillus bacteriemia (11).

SUMMARY

Experiments recently reported (18) have been interpreted to indicate that surface phagocytosis plays no significant part in natural antipneumococcal defense. A repetition of these experiments has revealed: (*a*) that the cellular content of the leucocytic suspensions used in the phagocytic tests was of a different order of magnitude from that of the exudates which usually exist in infected tissues, (*b*) that the suspensions were too dilute to allow surface phagocytosis of pneumococci to occur, and (*c*) that the ratio of bacteria to leucocytes was such that, when a sufficiently concentrated exudate was employed, the pneumococci injured the leucocytes and thus prevented phagocytosis from taking place.

When conditions of the tests were suitably controlled, and conventional quantitative methods were employed to measure the end results of the phagocytic reaction, the essential observations relating to surface phagocytosis were fully confirmed. The significance of this non-antibody mechanism of defense in pneumococcal infections was thus further substantiated.

BIBLIOGRAPHY

1. Wood, W. B., Jr., Smith, M. R., and Watson, B., Surface phagocytosis—its relation to the mechanism of recovery in pneumococcal pneumonia, *Science*, 1946, **104**, 28.
2. Wood, W. B., Jr., Smith, M. R., and Watson, B., Studies on the mechanism of recovery in pneumococcal pneumonia. IV. The mechanism of phagocytosis in the absence of antibody, *J. Exp. Med.*, 1946, **84**, 387.
3. Wood, W. B., Jr., and Smith, M. R., Intercellular surface phagocytosis, *Science*, 1947, **106**, 86.
4. Smith, M. R., and Wood, W. B., Jr., Studies on the mechanism of recovery in pneumonia due to Friedlander's bacillus. III. The role of "surface phagocytosis" in the destruction of the microorganisms in the lung, *J. Exp. Med.*, 1947, **86**, 257.
5. Smith, M. R., and Wood, W. B., Jr., Relation of surface phagocytosis to the fibrinous character of acute bacterial exudates, *Science*, 1949, **110**, 187.
6. Smith, R. O., and Wood, W. B., Jr., Cellular mechanisms of antibacterial defense in lymph nodes. I. Pathogenesis of acute bacterial lymphadenitis, *J. Exp. Med.*, 1949, **90**, 555.
7. Wood, W. B., Jr., and Smith, M. R., The inhibition of surface phagocytosis by the capsular "slime layer" of pneumococcus Type III, *J. Exp. Med.*, 1949, **90**, 85.
8. Wood, W. B., Jr., and Smith, M. R., Host-parasite relationships in experimental pneumonia due to pneumococcus Type III, *J. Exp. Med.*, 1950, **92**, 85.
9. Wood, W. B., Jr., Studies on the cellular immunology of acute bacterial infections, *Harvey Lectures*, 1951, **47**, 72.
10. Smith, M. R., Perry, W. D., Berry, J. W., and Wood, W. B., Jr., Surface phagocytosis *in vivo*, *J. Immunol.*, 1951, **67**, 71.
11. Wood, W. B., Jr., Smith, M. R., Perry, W. D., and Berry, J. W., Studies on the cellular immunology of acute bacteriemia. I. Intravascular leucocytic reaction and surface phagocytosis, *J. Exp. Med.*, 1951, **94**, 521.
12. Sawyer, W. D., Smith, M. R., and Wood, W. B., Jr., The mechanisms by which macrophages phagocyte encapsulated bacteria in the absence of antibody, *J. Exp. Med.*, 1954, **100**, 417.
13. Smith, M. R., and Wood, W. B., Jr., An experimental analysis of the curative action of penicillin in acute bacterial infections. II. The role of phagocytic cells in the process of recovery, *J. Exp. Med.*, 1956, **103**, 499.
14. Haemmerli, M., Spielt de "Surface Phagocytosis" en der Sulfonamidtherapie die ihr von Wood zergedachte Rolle?, *Schweiz. Z. Path. u. Bakt.*, 1949, **12**, 289.
15. Roth, W., Immun-und Oberflachen phagocytose von bekapselten pathogenen Bakterien, *Schweiz. Z. allgem. Path. u. Bakt.*, 1950, **13**, 1.
16. Balch, H. H., and Spencer, M. T., Phagocytosis by human leucocytes. I. Effect of fibrin on phagocytosis of staphylococci and of encapsulated pneumococci by normal human leucocytes, *J. Clin. Inv.*, 1954, **33**, 1314.
17. Rutenberg, S. H., and Fine, J., Resistance to bacteria in hemorrhagic shock.

VII. Demonstration of leucotoxin in plasma of shocked rabbit, *Proc. Soc. Exp. Biol. and Med.*, 1956, **93**, 484.

18. Lerner, E. M., Phagocytosis of bacteria in the absence of antibody and the effect of physical surface—reinvestigation of surface phagocytosis, *J. Exp. Med.*, 1956, **104**, 233.
19. Francis, T., Response of the host to the parasite, *in* Bacterial and Mycotic Infections of Man (R. J. Dubos, editor), Philadelphia, J. B. Lippincott Co., 1952, 98.
20. Wood, W. B., Jr., Studies on the mechanism of recovery in pneumococcal pneumonia. I. The action of type-specific antibody upon the pulmonary lesion of experimental pneumonia, *J. Exp. Med.*, 1941, **73**, 201.
21. Robertson, O. H., and Van Sant, H., A comparative study of phagocytosis and digestion of pneumococci by macrophages and polymorphonuclear leucocytes in normal and immune dogs, *J. Immunol.*, 1939, **37**, 571.
22. Robertson, O. H., and Sia, R. H. P., Studies on pneumococcus growth inhibition. II. A method for demonstrating the growth-inhibitory and bactericidal action of normal serum-leucocyte mixtures, *J. Exp. Med.*, 1924, **39**, 219.
23. Hanks, J. H., Quantitative aspects of phagocytosis as influenced by the number of bacteria and leucocytes, *J. Immunol.*, 1940, **38**, 159.
24. Rous, P., and Jones, F. S., The protection of pathogenic microorganisms by living tissue cells, *J. Exp. Med.*, 1916, **23**, 601.
25. Wood, W. B., Jr., The role of surface phagocytosis in acute bacterial infections, 1950 Year Book of Pathology, Chicago, Year Book Publishing Co., 1950, 13.
26. Wood, W. B., Jr., and Irons, E. N., Studies on the mechanism of recovery in pneumococcal pneumonia. II. The effect of sulfonamide therapy upon the pulmonary lesion of experimental pneumonia, *J. Exp. Med.*, 1946, **84**, 365.
27. Wood, W. B., Jr., McLeod, C., and Irons, E. N., Studies on the mechanism of recovery in pneumococcal pneumonia. III. Factors influencing the phagocytosis of pneumococci in the lung during sulfonamide therapy, *J. Exp. Med.*, 1946, **84**, 377.
28. Lerner, E. M., and Victor, J., Effect of filter paper, para-hydroxybenzoic acid, and fixed tissue on phagocytosis, *Proc. Soc. Exp. Biol. and Med.*, 1952, **80**, 110.
29. Dubos, R. J., The Bacterial Cell, Cambridge, Harvard University Press, 1945.
30. Wilson, G. S., and Miles, A. A., *in* Topley and Wilson's Principles of Bacteriology and Immunity, Baltimore, Williams & Wilkins Co., 1955, 360.
31. Mudd, S., McCutcheon, M., and Lucke, B., Phagocytosis, *Physiol. Rev.*, 1934, **14**, 210.
32. Suter, E., Interaction between phagocytes and pathogenic microorganisms, *Bact. Rev.*, 1956, **20**, 94.
33. Wood, W. B., Jr., Pneumococcal pneumonia, *in* A Text Book of Medicine (R. L. Cecil and R. F. Loeb, editors), Philadelphia, Saunders and Co., 1955, 126.

EXPLANATION OF PLATE 1

FIG. 1. Early phase of inflammatory response in experimental pneumococcal pneumonia (20). Leucocytic exudate has not yet had time to accumulate in infected alveoli, and phagocytosis is unimpressive. Gram-Weigert stain. × 1000.

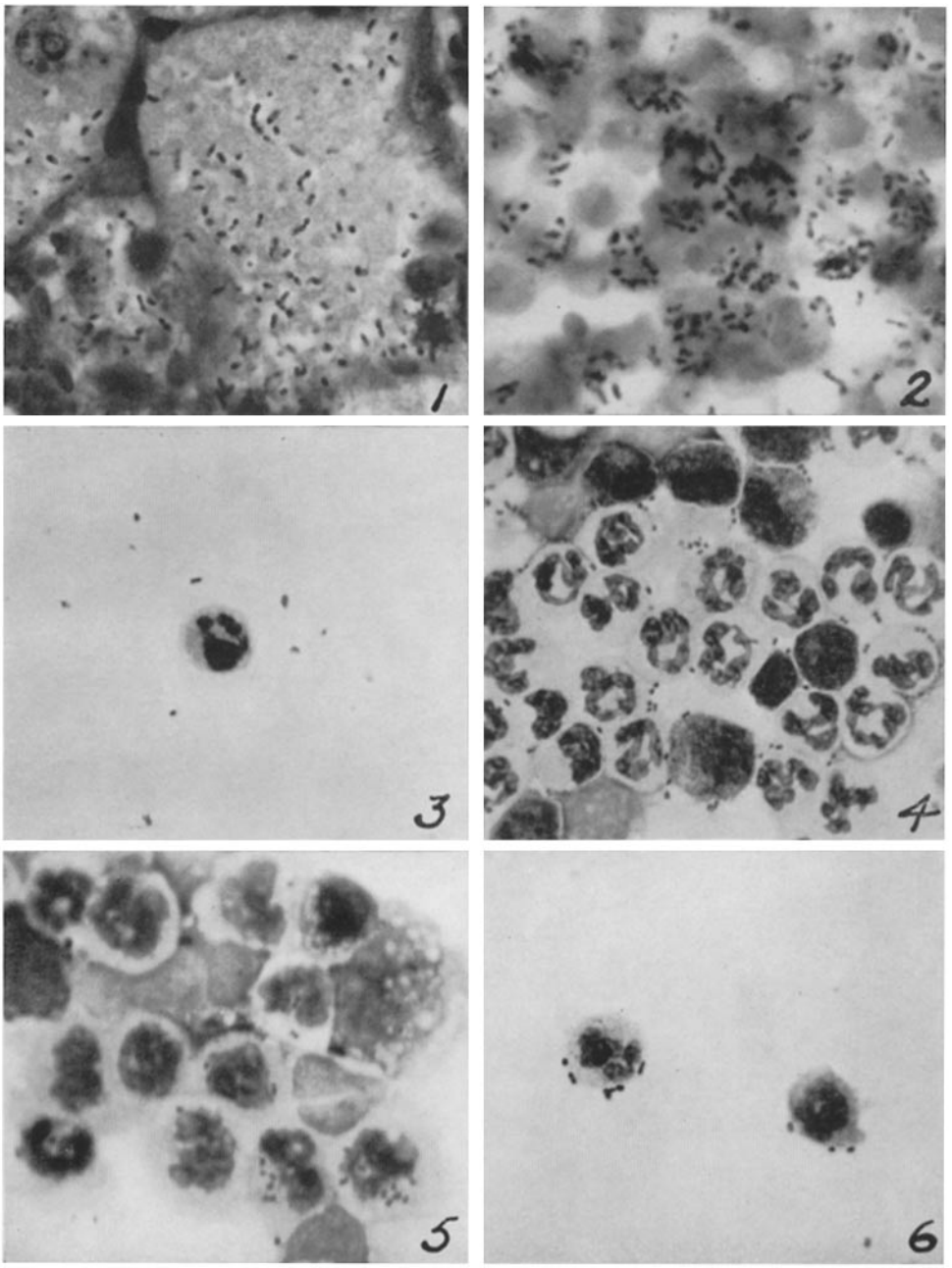
FIG. 2. Later stage of pneumococcal infection in which leucocytes are numerous in alveoli and phagocytosis is clearly demonstrable. Gram-Weigert strain. × 1000.

FIG. 3. Representative smear made from the dilute exudate employed by Lerner. Note relative sparseness of leucocytes. Smear made prior to incubation of leucocyte-pneumococcus mixture. Methylene blue stain. × 1000.

FIG. 4. More concentrated exudate employed in "standard" phagocytic test. Note that the concentration of leucocytes in contrast to preparation shown in Fig. 3, is roughly comparable to that of pneumonic exudate of Fig. 2. Smear made prior to incubation of mixture. Methylene blue stain. × 1000.

FIG. 5. Phagocytosis of pneumococci in relatively concentrated exudate incubated on filter paper. "Standard" technique was employed and following incubation mixture was centrifuged to eliminate extracellular bacteria. (See Methods). Methylene blue stain. × 1125.

FIG. 6. Failure of pneumococci to be phagocyted in dilute exudate incubated on filter paper. No centrifugation to eliminate extracellular bacteria. Methylene blue stain. × 1000.



(Smith and Wood: Surface phagocytosis)