STUDIES ON THE MECHANISM OF RECOVERY IN PNEUMONIA DUE TO FRIEDLÄNDER'S BACILLUS

III. The Rôle of "Surface Phagocytosis" in the Destruction of the Microorganisms in the Lung*

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Plates 27 and 28

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Evidence has been presented that encapsulated Friedländer's bacilli are destroyed by leucocytes in the pneumonic lungs of rats treated with sulfonamides, even when the animals possess no detectable opsonins in the blood (1). Similar results were reported previously in a study of experimental pneumococcal pneumonia (2, 3) and the destruction of organisms in the absence of antibody was shown to be due to a physical phenomenon designated "surface phagocytosis" (4). The experiments reported in the present paper demonstrate that under similar conditions the same surface mechanism is responsible for the phagocytosis of Friedländer's bacilli and results in rapid killing of the organisms.

(*A*) *The Resistance of Encapsulated Friedländer's Bacilli to Phagocytosis in a Fluid Medium*

Friedländer's bacillus in its virulent form, like pneumococcus, possesses a capsule which protects it against phagocytic cells. When suspended in a fluid medium with active leucocytes, Friedländer's bacilli are not phagocyted unless previously coated with specific opsonin. The ability of the fully encapsulated organism to resist phagocytosis in a fluid medium is shown by the following experiment.

Experiment 1.—Friedländer's bacilli from 2 ml. of a 4 hour culture in tryptose phosphate broth were washed twice with iced gelatin-Locke's solution and suspended with washed leucocytes harvested from the peritoneal cavities of four rats injected intraperitoneally 24 hours previously with starch-aleuronat (4). The cells were washed from the peritoneal cavity of each rat with cold gelatin-Locke's solution containing liquaemin[1] (1:1000 concentration) and after centrifugation were rewashed at least once before being added to the bacteria. All supernatant fluid was poured off the final centrifugates of the bacterial and leucocytic suspensions before they were mixed. The final cell-bacillus mixture contained approximately ten phagocytes and twenty-five bacilli per oil immersion field when placed beneath a

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[1] A commercial preparation of the sodium salt of heparin—1 ml. = 10 mg. (Roche Organon, Inc.)

coverslip. Such mixtures, incubated at 37°C. in hanging drop preparations or between coverslips, uniformly failed to show appreciable phagocytosis (Fig. 1). Direct observation of the phagocytes in the hanging drop mixtures[2] revealed that bacilli were frequently pushed aside by advancing leucocytes and were not phagocyted in spite of repeated contacts with the cells (Figs. 2 to 5).

(*B*) *Demonstration that Phagocytosis of Friedländer's Bacilli Occurs in the Lung in the Absence of Local as Well as Circulating Antibody*

Although phagocytosis of Friedländer's bacilli occurs in the lungs of rats whose serum contains no detectable opsonin (1), it may be argued that the observed phagocytic reaction is due to the local accumulation of antibody in the pulmonary tissues. The result of the following experiment would appear to rule out this possibility.

Experiment 2.—Normal rat lungs were fixed for 24 hours in 10 per cent formalin to eliminate all living cells and unprecipitated proteins. The fixed lungs were washed for at least 5 days in tap water and were rinsed with gelatin-Locke's solution before being used. Into the left main bronchus of each formalin-fixed lung was injected a suspension of leucocytes and Friedländer's bacilli in gelatin-Locke's solution. The bacteria used in the suspension were obtained by centrifugation from 3.5 ml. of a 4 hour culture in tryptose phosphate broth containing 1 per cent rabbit serum. The harvested organisms were washed twice in cold gelatin-Locke's solution before being added to washed leucocytes obtained from the peritoneal cavities of six rats (see above). To insure penetration of the leucocyte-bacillus mixture into the alveoli, each lung was expanded gently under negative pressure at the time the mixture was introduced by cannula into the bronchus. The injected lungs were incubated for 2 hours at 37°C. and then fixed in Zenker-formol solution, sectioned, and stained by the Gram-Weigert technique (5).

As shown in Fig. 6 phagocytosis of the Friedländer bacilli occurred even in the formalin-fixed lungs, which contained no possible source of opsonin other than the phagocytic cells themselves. It can be concluded, therefore, that the ability of leucocytes to attack virulent Friedländer's bacilli in the lung is not dependent upon the accumulation of local antibody in the pulmonary tissue.

(*C*) *Phagocytosis of Friedländer's Bacilli on Tissue and Other Surfaces*

From the foregoing observations it appeared likely that the non-antibody phagocytosis of Friedländer's bacilli is due to the same type of surface mechanism that has been shown to operate in the case of pneumococci (4). To test this hypothesis experiments were performed in which mixtures of leucocytes and Friedländer's bacilli were placed on various solid surfaces and incubated in a moist atmosphere at 37°C. for 60 minutes. At the end of incubation impression

[2] Unstained organisms from 4 hour cultures in tryptose phosphate broth were used in all experiments except when preparations were made for photography, in which case the bacteria were stained with carbolfuchsin as previously described (4). Repeated control experiments demonstrated that the stained organisms behaved toward the leucocytes exactly as did the unstained organisms.

smears were made from the test surfaces and were stained with methylene blue. The exact techniques employed in carrying out the experiments have already been described (4).

As shown in Table I, leucocytes having access to the surfaces of normal or boiled tissues, or to such rough inert material as moistened filter paper are able to phagocyte the encapsulated Friedländer bacilli in the absence of antibody (Fig. 7). No such phagocytosis occurs on the smooth surface of a glass coverslip[3] (Fig. 1). Leucocytes suspended in the fluid medium of a hanging or suspended drop (4) likewise fail to phagocyte the organisms. Thus, fully encapsulated Friedländer's bacilli, like pneumoccocci, are phagocyted in the absence of opsonins only when the leucocytes have access to a suitable surface upon which to operate.

TABLE I

Tests for Phagocytosis on the Surfaces of Fresh Rat Tissues, Boiled Rat Tissues, Moistened Filter Paper, and on Glass*

Lung	+
Liver	+
Spleen	+
Boiled lung	+
Boiled liver	+
Boiled spleen	+
Filter paper	+
Glass coverslip	−

* Boiled for 30 minutes.

(*D*) *Direct Visualization of Surface Phagocytosis*

The technique employed to visualize directly the surface phagocytosis of Friedländer's bacilli was similar to that used in the study of pneumococcal infections (4).

Normal rat lungs, fixed for 24 hours in 10 per cent formalin, were washed for several days in tap water, embedded in paraffin, and cut in sections of 5 microns thickness. The cut sections were floated onto "O" coverslips and allowed to dry before being washed in xylol to remove the paraffin. The mounted tissues were finally run through alcohol, washed repeatedly in distilled water, and allowed to dry. A small drop of bacillus-leucocyte mixture in gelatin-Locke's solution was placed on each mounted lung section, which was then inverted as a hanging drop preparation in the cavity of a hollow ground slide and rimmed with vaseline to prevent drying. Each preparation was placed in the warm stage of the microscope (37 C.) and was observed under the oil immersion lens. The phagocytic process could thus be watched directly in the alveoli close to the margin of the drop where the layer of fluid was sufficiently thin to confine the leucocytes and the bacteria to the alveolar cavities.

[3] If the leucocyte-bacillus mixture is sufficiently concentrated, some phagocytosis will occur on the glass surface due at least in part, to "intercellular surface phagocytosis." (See below.)

As is illustrated by the photographs in Figs. 8–15, phagocytosis of the Friedländer bacilli resulted when the organisms were pinned against the alveolar walls by the migrating leucocytes. It should be emphasized that the leucocytes appeared to wander about purely at random, there being no evidence of either positive or negative chemotaxis. The sequences photographed were selected to illustrate the phagocytic process, but frequently the leucocytes singled out for observation moved about the alveolus without trapping any of the bacteria against the alveolar walls. In such cases no phagocytosis resulted. Occasionally bacteria were caught between the surfaces of two or more leucocytes that happened to collide with one another. Bacteria so trapped between the surfaces of contiguous cells were often phagocyted. This type of phagocytosis referred to as "inter-cellular surface phagocytosis," will be described in more detail elsewhere (6).

(*E*) *Evidence that Surface Phagocytosis Brings about Destruction of Friedländer's Bacilli*

Apparent intracellular digestion of Friedländer's bacilli following phagocytosis by the surface mechanism was demonstrated as follows:—

Leucocyte-Friedländer's bacillus mixtures prepared as described above (section A) were incubated on the surface of moistened filter paper for 45 minutes (see section C) and were then washed from the filter paper with 10 ml. of iced gelatin-Locke's solution. The cells, many of which contained intracellular organisms, were twice centrifuged in iced gelatin-Locke's solution at 800 R.P.M. for 5 minutes to eliminate extracellular bacilli. The centrifuged cells were finally suspended in approximately 10 volumes of normal rat serum containing liquaemin in a concentration of 1:100 and were incubated at 37°C. The final suspensions were made in normal rat serum in order to provide an optimal environment for the leucocytes. (It should be reemphasized that normal rat serum contains no detectable antibody against this strain of Friedländer's bacillus (1).) Smears of the incubating cell suspension were made every 15 to 30 minutes and were stained with methylene blue. The numbers of intracellular and extracellular bacilli noted per 500 polymorphonuclear leucocytes are recorded in Table II.

The results of the bacterial counts indicate a pronounced decrease in the number of intracellular organisms during the period of incubation following phagocytosis. The decrease in intracellular organisms appears to be due to digestion of the phagocyted bacteria. However, because of the concomitant increase in the number of extracellular bacilli during incubation, it is possible that the decrease in intracellular organisms was due merely to the escape of bacteria from the cytoplasm of the phagocytes rather than to digestion. A method was devised, therefore, to observe directly the fate of the phagocyted bacteria during the period of incubation.

As in the preceding experiment phagocytosis was brought about on moistened filter paper, and leucocytes containing phagocyted Friedländer's bacilli were suspended in normal rat serum after the extracellular organisms had been removed by centrifugation. A drop of the serum-cell mixture was spread, in as thin a layer as possible, on a mounted section of fixed rat

lung (see section D) and was observed in the warm stage of the microscope under oil immersion lens.

In each experiment a single selected leucocyte containing two or more phagocyted bacilli was watched for several hours. Repeatedly the outlines of the bacilli were seen to become faint as if from partial digestion, and occasionally the organisms disappeared completely from the cytoplasm of the leucocyte. Only once was intracellular multiplication of the bacilli observed, and in this instance the leucocyte when first observed was non-motile and apparently dead.

Because of these last observations additional experiments were carried out to test further the ability of phagocyted organisms to multiply in the cytoplasm of the cells.

TABLE II

Data Indicating Intracellular Digestion of Friedländer's Bacilli Following Surface Phagocytosis

Time of incubation (37°C.) following surface phagocytosis	No. of intracellular bacilli per 500 leucocytes	No. of extracellular bacilli per 500 leucocytes
min.		
0	755	102
45	632	341
90	468	>1000
150	356	>1000

Leucocytes that had phagocyted Friedländer's bacilli on moistened filter paper, and from which most of the extracellular organisms had been removed by centrifugation were smeared on a glass slide and stained with methylene blue. A direct count on the stained preparation was made to determine the number of leucocytes containing Friedländer's bacilli. A second portion of the centrifuged cells was spread gently over the surface of a thin coverslip and before the preparation could dry, the coverslip was inverted onto a warm glass slide on the surface of which had been placed a drop of melted agar (45°C.) containing trypan blue. The trypan blue-agar mixture was made by mixing equal parts of 3 per cent agar (in double strength tryptose phosphate broth) and 1 per cent trypan blue in Locke's solution. Repeated control experiments showed that this concentration of trypan blue in the agar exerted no demonstrable effect upon the growth of unphagocyted Friedländer's bacilli. When the inverted coverslip was placed upon the drop of trypan blue-agar, capillary action caused the agar to spread out rapidly between the two glass surfaces, embedding the cells in the upper plane just beneath the coverslip. After the agar had solidified, the coverslip was rimmed with paraffin, and the preparation was incubated at 37°C. for 2 hours before being examined under the microscope.

In repeated experiments only about 10 per cent of the leucocytes containing Friedländer's bacilli exhibited colonies of bacteria,[4] and most of the intracellular colonies were observed to be in leucocytes, the nuclei of which were stained with trypan blue. Since trypan blue, in the concentrations employed, stains the nuclei of dead cells and is not taken up by living cells (7), it was concluded that

[4] These data were later confirmed by using the more direct agar-slide method. (See below.)

intracellular multiplication occurred primarily in the cytoplasm of non-viable leucocytes.

The foregoing results indicated that phagocyted Friedländer's bacilli were eventually killed by the leucocytes, provided the latter were still viable. Since the process of digestion appeared to be relatively slow, sometimes lasting for several hours, a technique was devised to determine the approximate speed with which the phagocyted bacilli were *killed* by the leucocytes. The method used was based upon a test for the viability of organisms previously exposed to the intracellular environment of phagocytic cells.

Concentrated suspensions of Friedländer's bacilli and rat leucocytes in gelatin-Locke's solution containing 10 per cent rat serum[5] were incubated for 30 minutes on moistened filter paper as in previous experiments. The leucocytes were then washed from the surface of the paper with iced gelatin-Locke's solution and were rewashed three times to remove extracellular bacteria.[6] The final centrifugation was carried out in cold tryptose phosphate broth, rather than Locke's solution, and the centrifugate was transferred to an iced mortar containing 0.25 gm. of sterile sand. The sand-cell mixture was ground for 15 seconds with a mortar to break up the leucocytes and free the phagocyted bacteria. The ground mixture was taken up in 5 ml. of cold tryptose broth, and the sand and cellular debris were separated from the bacteria by slow centrifugation. The bacilli remaining in the supernatant were finally concentrated by centrifugation in the cold (20 minutes, at 2000 R.P.M.), and the supernatant was discarded. The centrifuged bacteria thus freed from the leucocytes, were finally suspended in 0.5 ml. of 2 per cent liquid agar (45°C.) and were incubated between two thin layers of solid agar prepared as follows:—

Melted 2 per cent agar at 45°C. was smeared onto the clean surface of a coverslip in a very thin layer. This first layer of agar was allowed to solidify but not dry. A drop of the agar containing the bacteria was then spread in a similar manner over the first layer and was allowed to solidify. A third thin layer of plain agar was immediately spread over the surface of the second, and as soon as the agar had solidified, the preparation was incubated for 2 hours in a sealed Petri dish lined with moistened filter paper. Upon being removed from the Petri dish, the agar surface was stained for 1 minute with methylene blue and was washed in tap water. The wet preparation was finally inverted, mounted on a glass slide, and rimmed with melted paraffin to prevent drying of the agar. Ready diffusion of the methylene blue through the top layer of agar caused clear staining of the bacteria in the middle agar layer. It was found necessary to use three layers of agar, as described, in order to prevent disruption of colonies during the staining procedure.

In such agar-slide preparations each viable organism was easily identified since it formed a definite colony in the solid medium (Fig. 16). Dead organisms, on the other hand, remained as single isolated bacilli (Fig. 17). When non-phagocyted Friedländer's bacilli[7] were tested by the same method, 99 to 100 per cent of them multiplied to form colonies. In contrast, more than one-third of the phagocyted bacteria freed from the cytoplasm of the leucocytes remained as single organisms in the agar. Their failure to multiply indicated that they had been killed by the leucocytes apparently during the 30 minute period of incubation on filter paper. Ex-

[5] To preserve maximum activity of leucocytes (4).

[6] Cells separated from bacteria by slow centrifugation (800 R.P.M., for 5 minutes).

[7] The usual proportion of phagocytes to bacteria was included in these control mixtures, but no phagocytosis was allowed to take place, since the incubation was carried out on glass slides rather than on filter paper. The control mixtures were ground with sand in the same manner as the test mixtures.

posure of Friedländer's bacilli at 37°C. to the intracellular environment for periods longer than 30 minutes (up to 2 hours) did not materially influence the results of the experiment.

Although the rapid bactericidal action of surface phagocytosis was thus clearly demonstrated, it should be emphasized that the agar-slide method, as used in these experiments, was relatively crude and did not yield quantitatively accurate data. All errors involved, however, were such as to minimize rather than exaggerate the actual bactericidal effect of the phagocytosis.[8]

DISCUSSION

Surface phagocytosis has been shown to be of prime importance in the mechanism of recovery in pneumococcal pneumonia since it brings about destruction of pneumococci in the lung in the absence of antibody. In the present studies of experimental Friedländer's bacillus pneumonia phagocytosis of encapsulated bacilli has likewise been observed in the lung in the absence of both circulating and local opsonins. Analysis of the non-antibody phagocytic reaction has revealed that the same surface factors are involved as in the case of pneumococcus. Only by pinning the encapsulated Friedländer bacilli against the alveolar or bronchial surfaces, or against the surfaces of adjacent phagocytes, can the leucocytes engulf the unopsonized organisms. The bacilli floating freely in a fluid medium are only pushed about by the leucocytes and are not taken into the cells.

Since Friedländer's organism exerts no apparent chemotactic effect upon the leucocytes, the surface mechanism of phagocytosis would be relatively inefficient in ridding the lung of bacteria were it not for the extremely dense population of leucocytes which eventually accumulates in each infected alveolus.[9] When the spread of the pneumonia has been stopped by chemotherapy (1), the crowding of phagocytic cells into the infected alveolar spaces and bronchi finally results in all of the bacteria being pinned against tissue surfaces or trapped between two or more viable leucocytes. Thus all of the bacteria remaining in the lung may in time be phagocyted by the surface mechanism.

Once the bacilli enter the cytoplasm of the phagocyte they are rapidly killed. Although digestion of the phagocyted Friedländer organisms appears to be slower than that of pneumococci and may not be complete for several hours,

[8] The principal sources of error in the method were as follows: (1) Killed organisms that had been partially digested were often difficult to identify as bacilli and therefore were not included in the final counts. (2) The relatively large number of viable bacteria released from each of the dead leucocytes in which intracellular multiplication occurred, tended to distort the final count in favor of the living organisms. (3) All bacilli that remained outside of the leucocytes in the filter paper preparations and were not removed by the differential centrifugation appeared in the final counts as viable bacilli. (4) Whereas, occasional single bacilli (killed) may have been missed in the counting process, no viable organisms could have been overlooked because of the obvious colonies formed.

[9] The density of the leucocytic population in typical lesions of bacterial pneumonia is apparent from routine tissue sections taken from areas of advanced consolidation (5, 9).

many of the bacilli are rendered non-viable in less than 30 minutes. Intracellular multiplication occurs only rarely and appears to result from premature death of the phagocyte. Even under the conditions of *in vitro* experiments approximately 90 per cent of the phagocytes survived long enough to kill all phagocyted organisms and thus prevent intracellular multiplication. Since surface phagocytosis of Friedländer's bacilli can be demonstrated in the lung, and since the ingested bacilli are rapidly killed by the leucocytes, it is only logical to conclude that this non-antibody form of phagocytosis plays just as important a part in the mechanism of recovery in Friedländer's pneumonia as it does in pneumococcal pneumonia.

It is not implied by the foregoing statement that all Friedländer's bacilli that invade the lung are destroyed by surface phagocytosis. Some of the bacilli are carried away from the alveoli and bronchi by lymphatic drainage, and some eventually enter the blood stream (9). How those organisms that escape from the lung are ultimately destroyed remains to be determined. It can be safely said, however, that of those Friedländer's bacilli that remain in the lung, the great majority appear to be killed by the surface mechanism of phagocytosis.

As has already been pointed out (1), pneumonia caused by Friedländer's bacillus differs from Type I pneumococcal pneumonia in one important respect. Whereas Type I pneumococcus rarely causes permanent damage to pulmonary tissue, the Friedländer bacillus not infrequently multiplies to such an extent in certain alveoli that necrosis results and a lung abscess is formed. In such abscessed areas the phagocytes are unable to destroy all of the bacilli, even when the animal is treated intensively for many days with sulfonamide (1). The failure of the leucocytes to rid abscesses of bacteria would appear to be due to at least two factors. First, the absence of the normal alveolar walls in the abscessed areas deprives the leucocytes of much of the tissue surface upon which they operate in the intact lung. Thus surface phagocytosis is rendered relatively inefficient. Secondly, most of the leucocytes, particularly in the center of a lung abscess are either non-viable or so sluggish that they cannot attack the bacteria. Leucocytes deprived of oxygen soon become non-motile and lose their phagocytic properties (8). Since the principal source of oxygen for leucocytes in a lung abscess is the intact alveolar capillaries at the periphery of the lesion, it is not surprising that the phagocytes in the central portion of the abscess fail to destroy the bacteria. These same two factors would appear to account at least in part for the failure of systemic chemotherapy to cure empyema and other pyogenic complications of pneumococcal as well as Friedländer's pneumonia (4).

SUMMARY

Phagocytosis of encapsulated Friedländer's bacilli has been demonstrated in the lungs of rats in the absence of both circulating and local antibody. The

mechanism of phagocytosis independent of antibody has been shown to be due to the same surface factors that operate in the phagocytosis of Type I pneumococcus under similar conditions. Direct observation of the phagocytic process reveals that leucocytes in the lung can phagocyte unopsonized Friedländer's bacilli only by trapping them against the surfaces of alveolar walls or bronchi, or by pinning them against the surfaces of adjacent leucocytes. Evidence is presented that Friedländer's bacilli thus phagocyted are rapidly killed in the cytoplasm of the phagocytic cells. Reasons are discussed for the failure of prolonged chemotherapy to cure lung abscesses that not infrequently complicate the pneumonia due to Friedländer's bacillus.

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EXPLANATION OF PLATES

Fixed smears and agar-slides stained with methylene blue (Figs. 1, 7, 16, and 17), lung section stained by the Gram-Weigert technique (Fig. 6), and bacteria in vital preparations stained with carbolfuchsin (Figs. 2 to 5 and 8 to 15). Fixed smears, agar-slides, and lung section photographed by Mr. Cramer Lewis.

PLATE 27

FIG. 1. Failure of leucocytes to phagocyte Friedländer's bacilli on surface of glass slide. Although in intimate contact with the cells the bacilli have not been phagocyted. × 1800.

FIGS. 2 to 5. Series of photomicrographs showing failure of leucocyte to phagocyte Friedländer's bacilli in hanging drop.

FIG. 2. Advancing pseudopods of cell are just coming into contact with two clumps of bacilli. Time, 1:05½ p.m. × 1250.

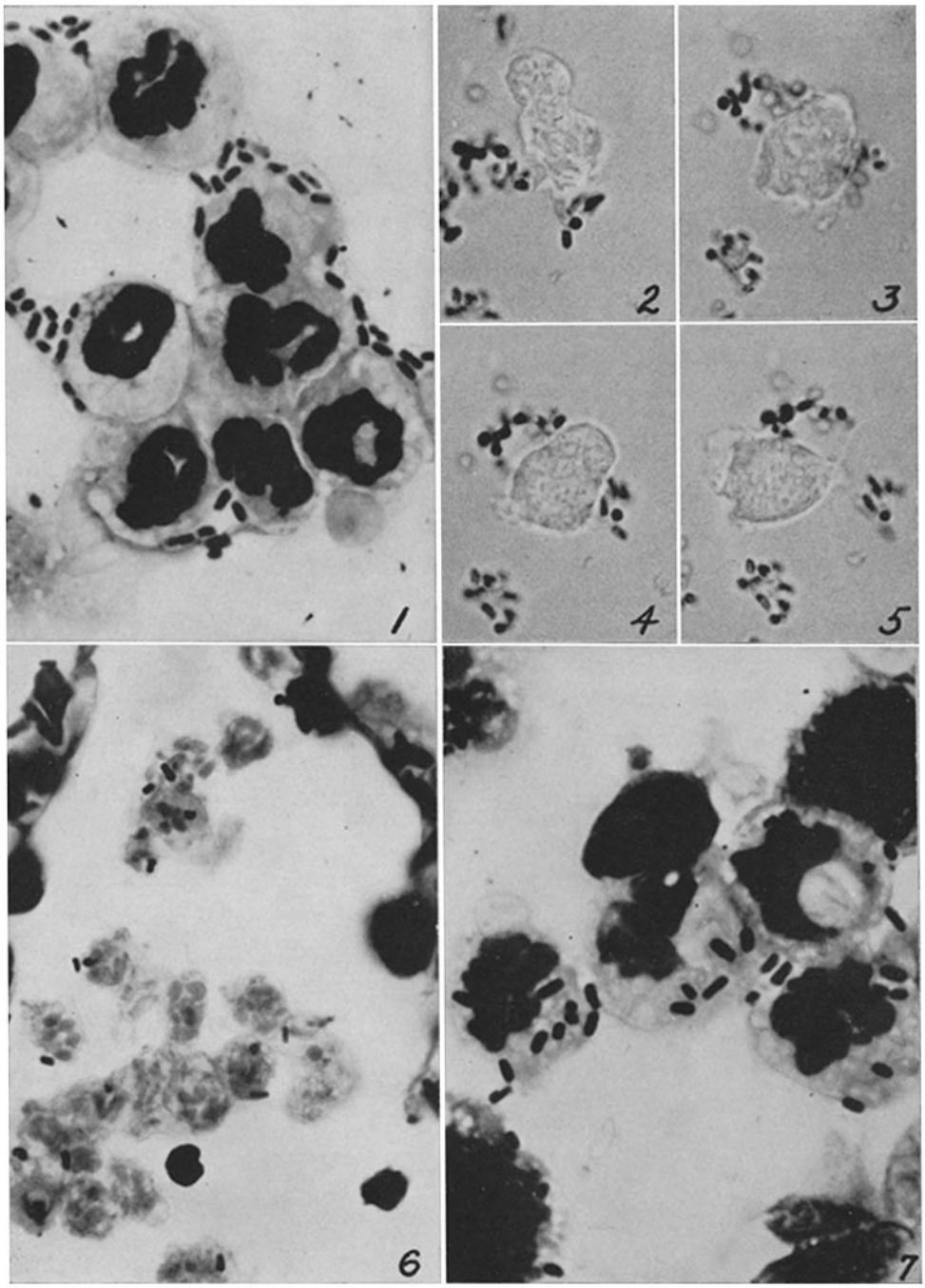
FIG. 3. Advancing cell has pushed clumps of bacilli apart and is in intimate contact with the bacteria. Time, 1:06 p.m. × 1250.

FIG. 4. Cell passes between the two clumps of bacilli. Time, 1:06½ p.m. × 1250.

FIG. 5. Cell continues on its way leaving bacilli behind without having phagocyted any of them. Time, 1:07 p.m. × 1250.

FIG. 6. Phagocytosis of Friedländer's bacilli following injection of bacillus-leucocyte mixture into formalin-fixed rat lung. × 1300.

FIG. 7. Phagocytosis of Friedländer's bacilli on surface of moistened filter paper. × 1800.



(Smith and Wood: Mechanism of recovery in pneumonia. III)

PLATE 28

FIGS. 8 to 12. Series showing surface phagocytosis in section of normal rat lung.

FIG. 8. Leucocyte is seen approaching Friedländer's bacilli near alveolar wall. Time, 12:30 p.m. × 1250.

FIG. 9. Leucocyte has reached alveolar wall and is about to trap bacilli against the tissue surface. Time, 12:31 p.m. × 1250.

FIG. 10. Cell has trapped some of the bacilli against the wall and is in process of phagocyting them. Time, 12:32 p.m. × 1250.

FIG. 11. Having phagocyted several of the bacilli, the leucocyte is moving along the alveolar wall. Time, 12:35 p.m. × 1250.

FIG. 12. The cell continues to migrate up the alveolar wall; the bacilli can still be seen clearly in the cytoplasm of the leucocyte. Time, 12:40 p.m. × 1250.

FIGS. 13 to 15. Series showing surface phagocytosis of Friedländer's bacilli in another section of rat lung.

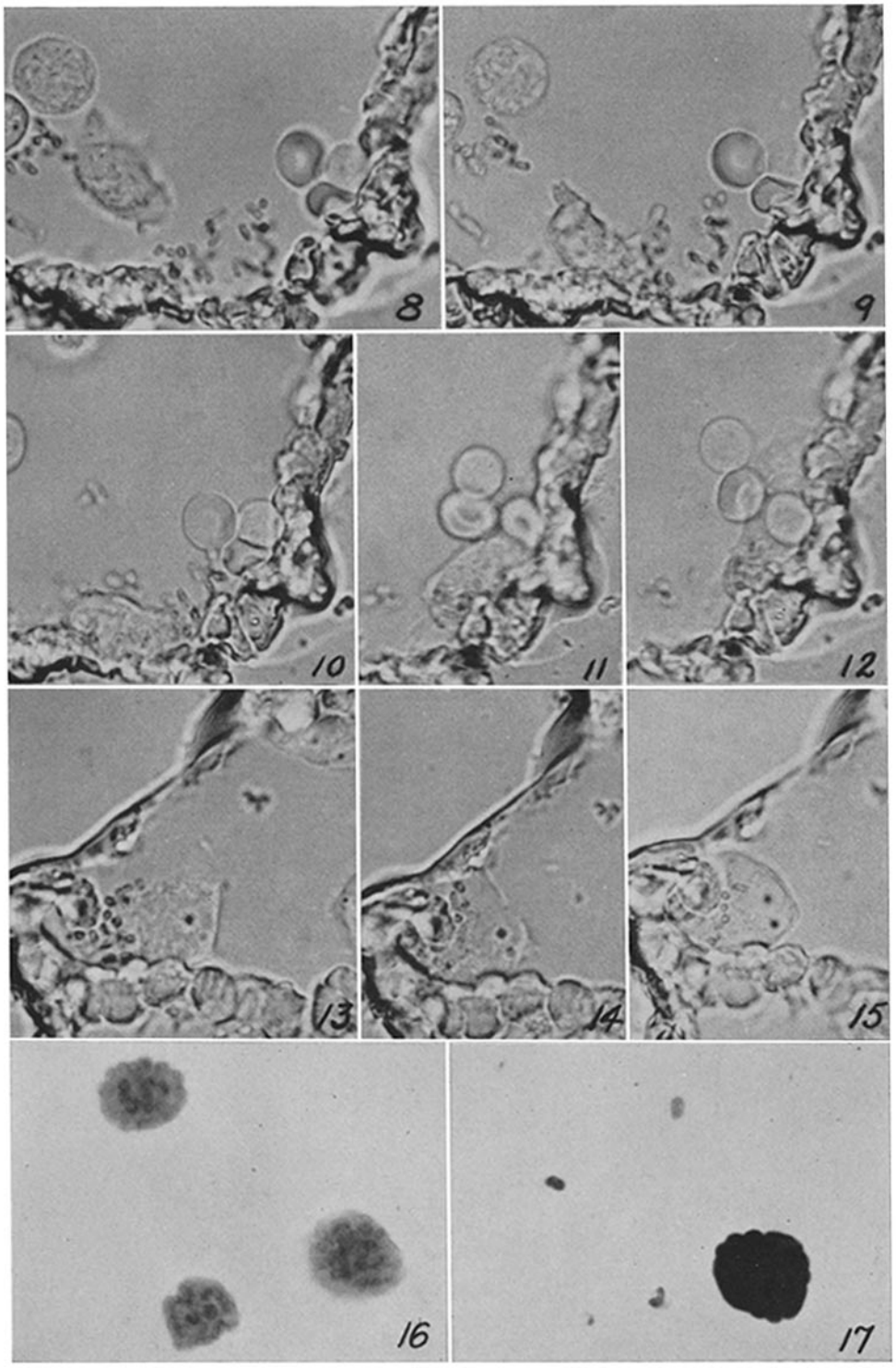
FIG. 13. Leucocyte is just making contact with bacilli trapped in corner of alveolus. Time, 12:35 p.m. × 1250.

FIG. 14. Leucocyte has pinned bacilli against alveolar wall and is phagocyting them. Some of the bacilli can already be seen in the phagocyte. Time, 12:37 p.m. × 1250.

FIG. 15. Phagocytosis complete with most of bacilli in cytoplasm of the leucocyte. Time, 12:40 p.m. × 1250.

FIG. 16. Multiplication of non-phagocyted Friedländer's bacilli in agar slide. Colony formation indicates that each of the three bacilli that were originally in the field was viable. × 1800.

FIG. 17. Failure of three out of four previously phagocyted Friedländer's bacilli to multiply in agar slide. The three single bacilli that failed to multiply in the agar had been killed by leucocytes, whereas the viable organism formed a typical colony. The bacillus nearest to the colony is partially obscured by an overlying bit of cellular debris. × 1800.



(Smith and Wood: Mechanism of recovery in pneumonia. III)