# THE MECHANISMS BY WHICH MACROPHAGES PHAGOCYTE ENCAPSULATED BACTERIA IN THE ABSENCE OF ANTIBODY*

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Plates 46 and 47

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It has long been held that virulent microorganisms with protective capsules are resistant to phagocytosis in the absence of specific antibody (1). That polymorphonuclear leucocytes, under conditions simulating those which obtain in the tissues of the host, can ingest and destroy such organisms without the assistance of antibody has recently been demonstrated (2–8). The mechanisms involved are those of *surface phagocytosis*. The cells have been shown to phagocyte encapsulated bacteria by: (*a*) trapping them against the surfaces of immovable tissue structures, *e.g.* the walls of alveoli (2), (*b*) by pinning them against the surfaces of other leucocytes (6), or (*c*) by catching them in the meshes of fibrin clots (7). These same mechanisms have also been demonstrated to operate *in vivo* (9, 10). The present studies deal with the ability of macrophages[1] to phagocyte fully encapsulated bacteria in the absence of antibody.

## *Methods*

Macrophages were obtained from sterile peritoneal exudates produced in adult albino rats by intraperitoneal injection of 5 ml. of beef infusion broth.[2] After 48 hours each animal was killed with ether, the peritoneal cavity was opened, and the exudate was removed by washing the peritoneum with Locke's solution containing 1 per cent gelatin and heparin in a concentration of 1 mg. per 100 ml. The cells of the exudate were concentrated by centrifugation in an angle head centrifuge at 4°C. (1000 r.p.m. for 5 minutes), were washed with cold gelatin-Locke's solution containing heparin, were concentrated a second time, and were stored on ice. Approximately 0.15 ml. of concentrated cells was obtained from two rats. The type of exudate harvested is indicated in Table I.

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[1] The term macrophage is here used to designate the large, mononuclear, phagocytic cell which usually predominates in late inflammatory exudates.

[2] Proteose-peptone No. 3 obtained from Difco Laboratories, Detroit, was used in preparing the beef infusion broth (11).

Methylene blue, Wright, and Giemsa stains were used to differentiate the cells of the exudate. The viability of cells was tested by the trypan blue method (12).

By means of phagocytic techniques, the details of which have previously been described (2, 6, 7), macrophages were tested for their ability to phagocyte and destroy type I pneumococci and group A Friedländer's bacilli.

TABLE I

*Experimental Production of Macrophage Exudate*

| No. of Experiments | Macrophages in Exudate | | Macrophages Viable* | |
|---|---|---|---|---|
| | Range | Mean | Range | Mean |
| | *per cent* | *per cent* | *per cent* | *per cent* |
| 10 | 76–92 | 83 | 75–92 | 83 |

* As determined by uptake of trypan blue (8).

## RESULTS

*The Antiphagocytic Effect of the Capsules of Pneumococcus I and Friedlander's Bacillus.*—Macrophages suspended in gelatin-Locke's solution were mixed with the fully encapsulated bacteria and incubated for 30 minutes at 37°C. on the surfaces of glass slides. Following the incubation smears were made and stained with methylene blue. Examination of the stained preparations revealed no evidence of phagocytosis. As shown in Fig. 8 the macrophages were surrounded by bacteria, but there were no intracellular organisms.

The general behavior of the macrophages toward the encapsulated bacteria under such conditions was observed directly in hanging drop preparations on the warm stage of the microscope. The pseudopods of the cells were seen to push aside the microorganism with which they came in contact, and none of the bacteria were phagocyted (Figs. 1 to 3).

*The Opsonizing Action of Type-Specific Antibody.*—When homologous type-specific antipneumococcal serum was added to similar mixtures of macrophages and pneumococci, the cells promptly phagocyted the bacteria. Direct observation of the phagocytic process in hanging drop preparations revealed that the opsonized bacteria, instead of being pushed aside by the pseudopods, stuck to their surfaces and were eventually ingested (Figs. 4 to 7).

*Surface Phagocytosis.*—To test the phagocytic properties of macrophages in the absence of antibody, under conditions simulating those in the body, the cells were mixed with encapsulated bacteria in gelatin-Locke's solution and were incubated for 30 minutes at 37°C. on various tissues obtained from freshly killed rats. It was found that the surfaces of lung, liver, spleen, and abdominal muscle promoted phagocytosis. Similar results were obtained when the mixture was incubated on moistened filter paper (Fig. 9). In each experiment tests were also made on glass slides as described above. Failure of phagocytosis

to occur in such control preparations indicated the absence of opsonins in the test mixture.

The mechanism of the phagocytosis was studied in sections of formalin-fixed lung mounted on glass coverslips. The mixture of cells and bacteria, suspended in Locke's solution, was spread over the section, and inverted as a hanging drop over the well of a hollow ground slide. Rimmed with vaseline to prevent drying, the preparation was incubated at 37°C. in the warm stage of the microscope. In such preparations the macrophages, which moved about relatively little, could be seen to extend large, broad pseudopods which occasionally trapped one or more pneumococci against the alveolar walls. Bacteria thus caught against the immovable tissue surfaces were often successfully phagocyted (Figs. 12 to 15).

*Intercellular Mechanism of Surface Phagocytosis.*—During direct observations of the phagocytic mechanism it was noted that in areas where the macrophages were crowded together the pseudopod of one cell would often trap an organism against the surface of an adjacent cell. Bacteria thus caught between the surfaces of two or more cells were usually phagocyted (Figs. 18 to 21).

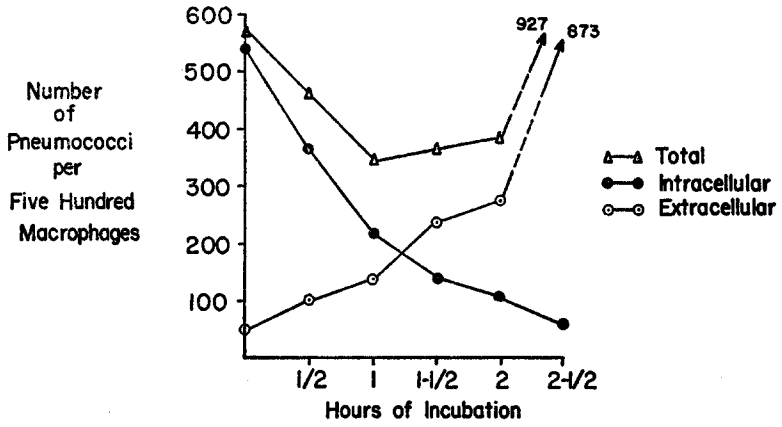
Since concentration of the cells seemed to promote intercellular phagocytosis, the following experiment was performed. A dilute suspension of cells and bacteria in gelatin-Locke's solution was incubated for 30 minutes at 37°C. along with a similar preparation in which the cells and bacteria had been concentrated in the bottom of a tube by centrifugation at 2000 R.P.M. for 5 minutes in an angle head centrifuge. Following incubation, smears were made from each preparation and were stained with methylene blue. Examination of smears from the concentrated mixture revealed many intracellular bacteria (Fig. 16), whereas smears from the dilute suspension showed no evidence of phagocytosis (Fig. 17).

*Phagocytosis in the Presence of Fibrin.*—In order to test the effect of fibrin upon the phagocytic mechanism, a mixture of macrophages and pneumococci was suspended in citrated rat plasma.[3] Thrombin was added producing prompt clotting and retraction. To the same suspension in a control tube saline was added instead of thrombin, and no clot formed. The two preparations were then incubated for 60 minutes at 37°C., and smears were made from each and were stained with methylene blue. Only in the preparation containing the clot, in which fibrin strands provided a suitable surface against which the macrophages could trap the bacteria, did phagocytosis occur (Figs. 10, 11).

*Bactericidal Effect of Surface Phagocytosis.*—In order to test the bactericidal effect of phagocytosis accomplished by the above mechanisms, mixtures of macrophages and pneumococci were incubated on moistened filter paper. Following incubation the mixture of cells and bacteria was washed from the

[3] Rat plasma has been shown to contain no antibody for Type I pneumococcus (2).

filter paper and placed in the incubator for a period of 2½ hours. In stained smears made at 30 minute intervals the number of intracellular and extracellular pneumococci per 500 macrophages was counted. As shown by the data depicted in Text-fig. 1, the number of extracellular pneumococci increased at approximately the same rate as would be expected from the normal growth curve of the organism. In contrast, during the 1st hour of incubation there was a marked decrease in the number of intracellular pneumococci. That a net loss of bacteria occurred during the 1st hour is indicated by the curve for the total number of organisms in the preparation. This net loss can only be explained by the destruction of a large proportion of the phagocyted bacteria.




TEXT-FIG. 1. Data indicating intracellular destruction of pneumococci by macrophages following surface phagocytosis.

Further evidence for destruction of the phagocyted pneumococci was obtained by direct observation of individual macrophages containing ingested bacteria. When such cells were observed continuously under the microscope over a period of several hours, gradual lysis of the phagocyted organisms was seen to take place.

## DISCUSSION

The mechanism by which macrophages phagocyte encapsulated microorganisms in the absence of antibody has been demonstrated to be the same as that previously described in similar studies with polymorphonuclear leucocytes (2, 6, 7, 9, 10).[4] As regards the general behavior of the two types of cells, however,

[4] Lerner and Victor (13) have questioned the validity of the previous studies relating to surface phagocytosis on the grounds that they were carried out in the presence of filter paper, which contains a "phagocytosis-promoting substance," parahydroxybenzoic acid. These authors apparently disregarded not only the *in vivo* studies (9, 10), but also the fact that

one important difference was noted. Whereas the polymorphonuclear leucocytes moved actively about in the phagocytic preparations, the macrophages were considerably less motile and therefore made fewer contacts with the bacteria. Neither type of cell showed evidence of chemotaxis in any of the preparations studied.

Wilson has recently described the egestion by polymorphonuclear leucocytes of previously phagocyted group A streptococci (14). Although this phenomenon was carefully looked for in the present studies, it was not observed to occur. If egestion of pneumococci by macrophages occurs at all, it must be rare. Certainly, egestion cannot account for the disappearance of the intracellular pneumococci observed in the present experiments dealing with the bactericidal effect of surface phagocytosis.

As to the relative importance of polymorphonuclear leucocytes and macrophages in acute bacterial infections, conflicting views have been expressed. Gay, in studies of experimental streptococcal infections, ascribed prime importance to the "macrophage reaction" (15). He concluded that the macrophage exudate played the principal role in destroying the invading organisms. He stressed, however, the dependence of the macrophages upon the presence of opsonins. Robertson and Van Sant have also emphasized the importance of the "macrophage reaction" in the recovery process in experimental pneumococcal pneumonia (16). They, too, on the basis of *in vitro* studies made on glass slides, concluded that the phagocytosis of pneumococci by macrophages was dependent upon opsonins.

In the present studies, macrophages have been shown to be capable of ingesting and destroying encapsulated bacteria in the absence of antibody. It must be concluded therefore, that they are capable of taking part in the cellular defenses of the host during the preantibody stages of pneumococcal and Friedländer's bacillus infections. Nevertheless, it seems doubtful that they play a dominant role in recovery for, as has been demonstrated in previous studies, phagocytosis by polymorphonuclear leucocytes predominates in such infections (17, 18). On the other hand, the macrophages in acute bacterial exudates appear to constitute an important second line of cellular defense which is called into play in the later stages of infection or more particularly in patients suffering from granulocytopenia. Experiments with irradiated animals clearly show that

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all the direct observations on the phagocytic mechanism were done in the absence of filter paper; *i.e.*, in formalin-fixed tissue sections mounted on glass coverslips (2, 3, 6, 7). In addition many of the phagocytic tests in filter paper–lined Petri dishes (2) were carried out with test tissues, fiberglass, etc., placed on coverglasses and thus clearly out of contact with the filter paper. It is evident, therefore, that surface phagocytosis cannot be explained by the action of a "phagocytosis-promoting substance" in filter paper. The surface mechanisms which do account for its occurrence have been defined and photographed (2, 3, 6, 7, 9, 10).

the "macrophage reaction" may provide the primary defense in agranulocytosis (19).

Finally it should be pointed out that this study has dealt only with the "wandering" macrophages of inflammatory exudates. No comparable data are as yet available on the mechanism of phagocytosis by "fixed" macrophages such as those in the sinusoids of the liver and the spleen.

SUMMARY

Evidence has been presented: (1) that macrophages from experimentally produced inflammatory exudates are capable of phagocyting fully encapsulated Type I pneumococci and group A Friedländer's bacilli in the absence of antibody, (2) that the principal mechanisms involved are those of surface phagocytosis, and (3) that the majority of pneumococci ingested by macrophages in antibody-free preparations are ultimately destroyed.

The relationship of these phenomena to the mechanism of recovery in pneumococcal and Friedländer's bacillus infections has been briefly discussed.

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BIBLIOGRAPHY

1. Dubos, R. J., The Bacterial Cell, Cambridge, Harvard University Press, 1945, 205.
2. Wood, W. B., Jr., Smith, M. R., and Watson, B., *J. Exp. Med.*, **84,** 1946, 387.
3. Smith, M. R., and Wood, W. B., Jr., *J. Exp. Med.*, **86,** 1947, 257.
4. Haemmerli, M., *Schweiz. Z. Path. u. Bact.*, **12,** 1949, 281.
5. Balch, H. H., *J. Clin. Inv.*, in press.
6. Wood, W. B., Jr., and Smith, M. R., *Science*, **106,** 1947, 86.
7. Wood, W. B., Jr., and Smith, M. R., *Science*, **110,** 1949, 187.
8. Wood, W. B., Jr., *Harvey Lectures*, **47,** 1952, 72.
9. Smith, M. R., Perry, W. D., Berry J. W., and Wood, W. B., Jr., *J. Immunol.*, **67,** 1951, 71.
10. Wood, W. B., Jr., Smith, M. R., Perry, W. D., and Berry, J. W., *J. Exp. Med.*, **94,** 1951, 521.
11. Smith, D. T., and Martin, D. S., Zinsser's Textbook of Bacteriology, New York, Appleton-Century-Crofts, Inc., 9th edition, 1948, 916.
12. Rous, P., and Jones, F. S., *J. Exp. Med.*, **23,** 1916, 601.
13. Lerner, E. M., and Victor, J., *Proc. Soc. Exp. Biol. and Med.*, **80,** 1952, 110.
14. Wilson, A. T., *J. Exp. Med.*, **98,** 1953, 305.
15. Gay, F. P., and Morrison, L. F., *J. Infect. Dis.*, **33,** 1923, 338.
16. Robertson, O. H., and Van Sant, J., *J. Immunol.*, **37,** 1939, 571.
17. Wood, W. B., Jr., *J. Exp. Med.*, **73,** 1941, 201.
18. Sale, L., Jr., and Wood, W. B., Jr., *J. Exp. Med.*, **86,** 1947, 239.
19. Wood, W. B., Jr., and Irons, E. N., *J. Exp. Med.*, **84,** 1946, 365.

## EXPLANATION OF PLATES

Dried smears (Figs. 8 to 11, 16 and 17) were stained with methylene blue. For purposes of photography the bacteria in wet preparations were previously stained with carbol fuchsin (3) (Figs. 1 to 7, 12 to 15, and 18 to 21). Repeated control experiments demonstrated that the stained organisms behaved toward the macrophages exactly as did unstained organisms. All the photomicrographs were taken at a magnification of 1450 diameters.

## PLATE 46

FIGS. 1 to 3. Failure of macrophages to phagocyte pneumococci on a glass surface in the absence of antibody.

FIG. 1. Macrophage extending a broad, blunt pseudopod into the midst of a group of pneumococci. Time, 9:58 a.m.

FIG. 2. None of the pneumococci with which the pseudopod has come in contact have become adherent to the cell. Time, 10:04 a.m.

FIG. 3. Although the bacteria can be seen to have been pushed aside by the pseudopod of the macrophage, none have been phagocyted. Time, 10:10 a.m.

FIGS. 4 to 7. Phagocytosis of Type I pneumococci in the presence of homologous type-specific antibody.

FIG. 4. A macrophage already containing one organism has extended a pseudopod toward a group of agglutinated pneumococci. Note "quellung" reaction. Time, 2:01 p.m.

FIG. 5. The pneumococcus first reached by the pseudopod has become adherent to its surface and is in the process of being phagocyted. Time, 2:03 p.m.

FIG. 6. The pneumococcus can now be seen to be well within the cytoplasm of the pseudopod. Time, 2:04 p.m.

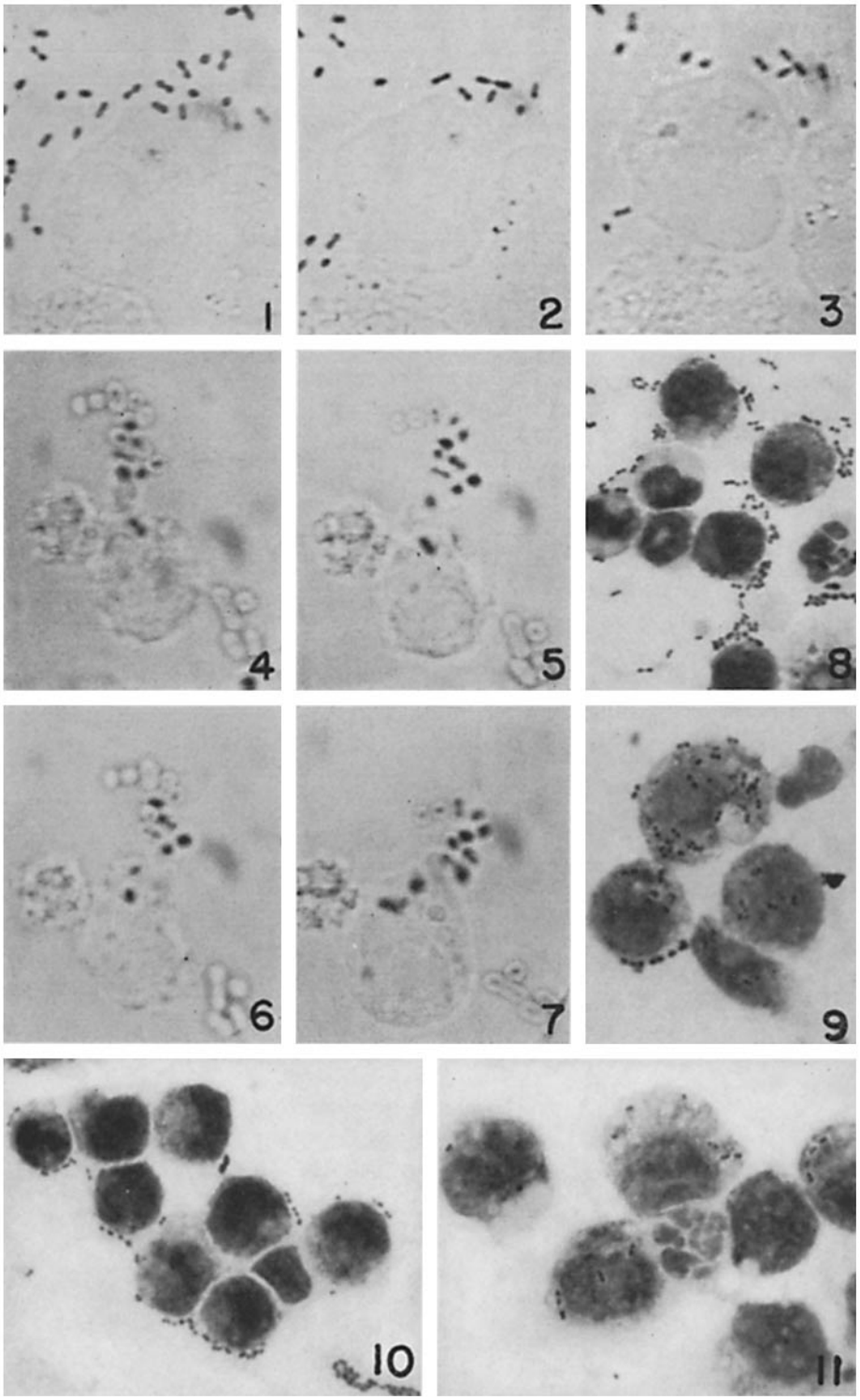
FIG. 7. The macrophage is beginning to ingest a third pneumococcus. Time, 2:15 p.m.

FIG. 8. Smear from phagocytic test showing the failure of macrophages to ingest pneumococci on a glass surface. Although the cells are surrounded by pneumococci, no intracellular organisms are seen.

FIG. 9. Phagocytosis of pneumococci incubated with macrophages on filter paper. Note the large number of intracellular bacteria.

FIG. 10. Failure of macrophages to phagocyte microorganisms during incubation in unclotted plasma.

FIG. 11. Phagocytosis of Type I pneumococci by macrophages in clotted plasma. (Compare with Fig. 10 in which conditions were identical except that plasma was unclotted.)




(Sawyer *et al.*: Phagocytosis of encapsulated bacteria by macrophages)

## PLATE 47

FIGS. 12 to 15. The mechanism of surface phagocytosis as observed in thin sections of formalin-fixed lung.

FIG. 12. A pneumococcus (arrow) may be seen lying between a macrophage and the surface of an alveolar wall. Time, 2:52 p.m.

FIG. 13. The cell has extended a pseudopod which has pinned the pneumococcus against the alveolar wall. Time, 2:56 p.m.

FIG. 14. The pneumococcus thus trapped is being ingested by the macrophage. Time, 3:00 p.m.

FIG. 15. The cell has moved a short distance along the alveolar wall, and the pneumococcus can be clearly seen within its cytoplasm. Time, 3:20 p.m.

FIG. 16. Smear made following incubation of a concentrated suspension of macrophages and bacteria. Many of the pneumococci have been phagocyted.

FIG. 17. Failure of macrophages to phagocyte pneumococci in an analogous dilute suspension.

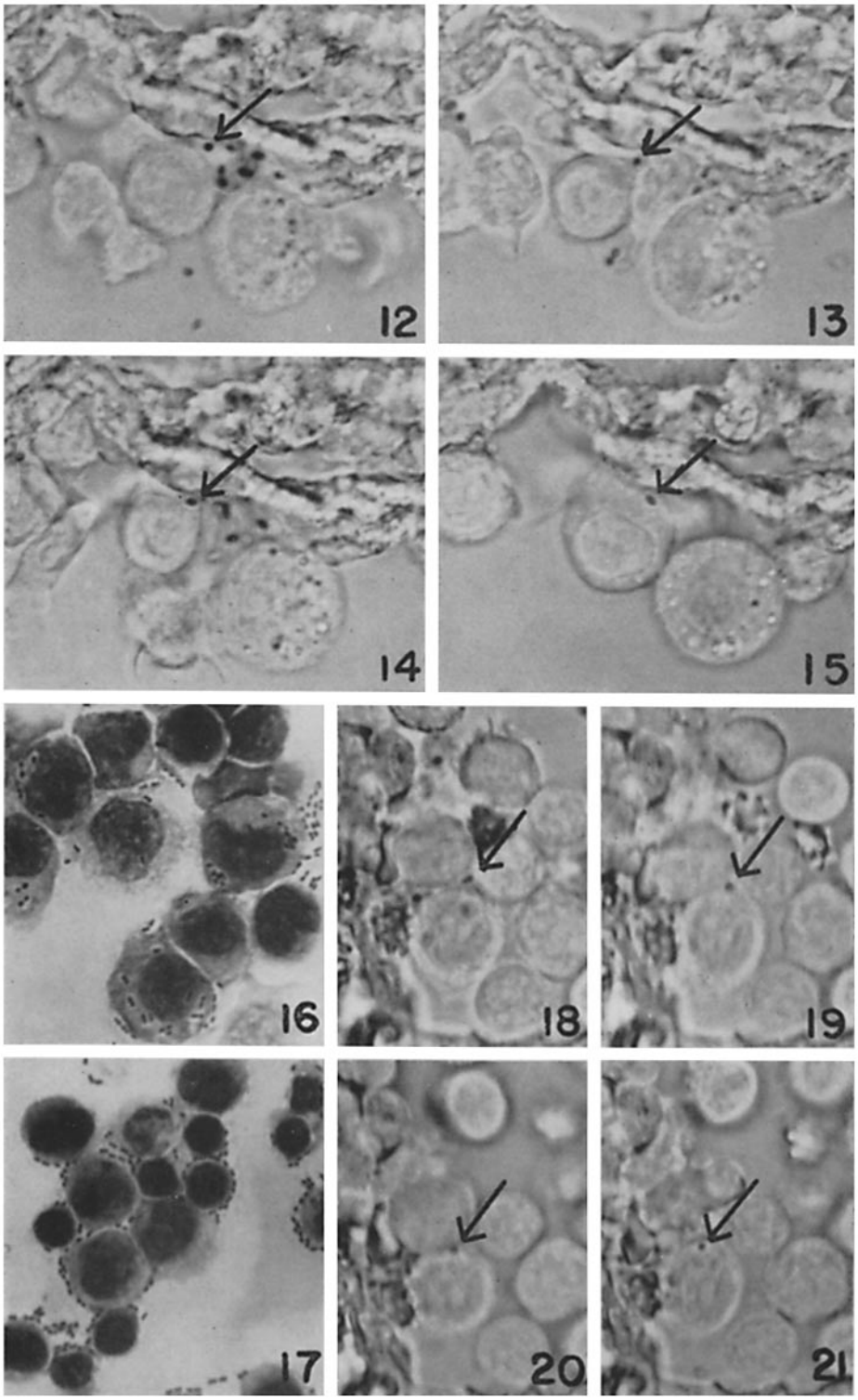
FIGS. 18 to 21. The mechanism of intercellular surface phagocytosis.

FIG. 18. A pneumococcus (arrow) is trapped in the space between three cells. Time, 11:28 a.m.

FIG. 19. The lowermost macrophage of the three can be seen to have pushed the organism against the surface of the uppermost cell of the group. Time, 11:30 a.m.

FIG. 20. The trapped pneumococcus is being ingested by the lower cell. Time, 11:31 a.m.

FIG. 21. As the upper cell moves away, the pneumococcus can be seen to be within the cytoplasm of the lower one. Time, 11:32 a.m.




(Sawyer *et al.*: Phagocytosis of encapsulated bacteria by macrophages)