THE PROCESS OF PHAGOCYTOSIS

The Agreement between Direct Observation and Deductions from Theory*

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Plates 1 and 2

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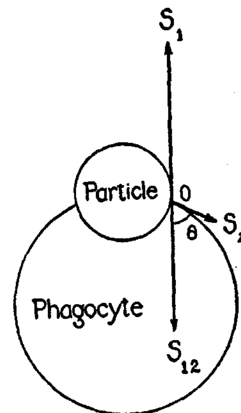
The importance of surface forces in phagocytosis has long been recognized. Rhumbler (1) successfully imitated many of the features of phagocytosis by simple physical models, and formulated his conception of its mechanism in terms of surface forces. Tait (2) subsequently treated the mechanism of phagocytosis in terms of the interfacial tensions involved. Fenn (3) recognized the earlier treatments as incomplete, and was the first to work out a satisfactory formulation of the surface forces concerned in phagocytosis. He showed that the same formulation could be derived either from considerations of free surface energy or of interfacial tension. Fenn's treatment has recently been elaborated by Ponder (4).



Text-Fig. 1

The surface forces involved in the ingestion of a particle are the interfacial tensions between the three phases in contact. Let Text-fig. 1 be a section through suspending medium, phagocyte, and partially ingested particle. Let O be a representative point in the line of contact between the three phases; let the vectors S_1, S_2, and S_{12} be the interfacial tensions,

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respectively, in the particle-fluid, phagocyte-fluid, and phagocyte-particle interfaces. If $S_1 > S_{12} + S_2$ the surface of the phagocyte would be drawn completely around the particle and ingestion would occur, provided viscosity or other forces did not interfere with the action of the surface forces. If $S_{12} > S_1 + S_2$ neither ingestion nor adhesion of particle and phagocyte would occur under the action of surface forces. When $S_1 < S_{12} + S_2$ and $S_{12} < S_1 + S_2$, the surface forces are in equilibrium with the particle in a position of partial ingestion, as shown in the figure; the position taken by the particle at equilibrium is such that $S_1 = S_{12} + S_2.\cos\theta$.

Unfortunately the treatment of surface forces in phagocytosis as formulated mathematically by Fenn and Ponder is not susceptible of rigorous experimental test, for the reason that two[1] of the interfacial tensions involved are not measurable. It is possible, however, to determine qualitatively whether or not the behavior of phagocytic cells, as directly observed and as analyzed by experiment, is in agreement with the implications of the mathematical theory.

In the present communication we propose to examine three deductions from the theory of Fenn and Ponder. These are (1) that a quantitative correlation should exist between phagocytosis and the surface properties of the particles ingested; (2) that phagocytosis is essentially a phenomenon of spreading of the phagocyte surface over the surface ingested; and (3) that partial ingestion should occur under certain circumstances.

Correlation between Phagocytosis and Surface Properties.—The first obvious deduction from a theory which assigns to surface forces a principal part in phagocytosis is that phagocytosis should be related in some orderly way with the surface properties of the particles phagocytized. This relation has been verified over a very considerable range of experimental conditions (7–14). Various bacteria, erythrocytes, and protein-coated collodion particles have been treated with graded concentrations of the phagocytosis-promoting substances of sera. The electric charge, isoelectric point, wetting properties, and cohesiveness of such series of sensitized particles have been estimated in

[1] It appears possible that at least an upper limit might be assigned to the phagocyte-liquid interfacial tension by application of the method of Harvey (5) or of a modification of that of Cole (6).

independent tests, and the phagocytosis of the particles has been quantitatively determined, using mammalian phagocytes both of the polymorphonuclear and large mononuclear types. A remarkably close correlation between phagocytosis and the surface properties of the particles undergoing ingestion has been regularly found. As the surface properties of the test particles were altered step by step in the series of serum dilutions, phagocytosis was increased in close parallelism. The conclusion drawn from this work is that the phagocytosis-promoting substances of immune sera form on the particles with which they interact a surface deposit upon which phagocytes can spread (12).

In the present communication we shall compare the other deductions from the physical theory with the behavior of phagocytes under direct observation.

Experimental Methods

Phagocytes.—Exudative polymorphonuclear leucocytes (7) and large mononuclear phagocytes (macrophages) (13) have been obtained from the peritoneal cavity of rabbits by methods elsewhere described. These were washed in 0.85 per cent NaCl or Ringer's solution and suspended in slightly diluted rabbit serum. In the major part of the work the cells used were samples from the same lots used in quantitative phagocytosis experiments (13, 14). Human polymorphonuclear leucocytes were used in a number of experiments. A platinum loopful of leucocyte suspension, a loopful of the suspension of particles to be phagocytized, and a loopful of specific immune rabbit serum were placed on a carefully cleaned slide, mixed, and a clean cover-slip was gently lowered on top. The edges of the cover-slip were sealed with Salvoline. In such films some leucocytes were freely suspended, some were spread out on the slide, and some on the cover-slip; only rarely was a single cell in contact both with slide and cover-slip. The preparations were put immediately under microscopic observation in a warm-box kept near 37°C.

Particles Phagocytized.—Suspensions of washed sheep and washed chicken erythrocytes, *Bacterium typhosum*, *Bacillus subtilis*, and *Monilia albicans* were used. Specific rabbit antisera were prepared for the sensitization of each type of cell. For observation and photography of the bacteria with the bright-field, they were first stained with carbolfuchsin and then washed four to five times. Erythrocytes and monilia were not stained. With the dark-field no staining was necessary.

Optical Apparatus.—For transmitted light, Zeiss aplanatic N. A. 1.4 condenser. For dark-field, Zeiss cardioid condenser. Zeiss apochromatic 60 × objective with iris diaphragm. Zeiss 20 × compensating ocular. Zeiss microscope incandescent lamp No. 1 with 165 watt Mazda projection bulb. Zeiss Phoku camera. Hypersensitive panchromatic plates. For transmitted light, yellow G filter No. 15.

Exposure time with transmitted light, 3 seconds; with dark-field, 30–60 seconds. Developer D 11 contrast (Eastman). Developed 5 minutes, room temperature. The superficial protoplasm of the phagocyte and the multiform processes and membranes to which it gives rise can be seen more clearly with the cardioid condenser than with any other optical arrangement with which we are familiar.

EXPERIMENTAL RESULTS

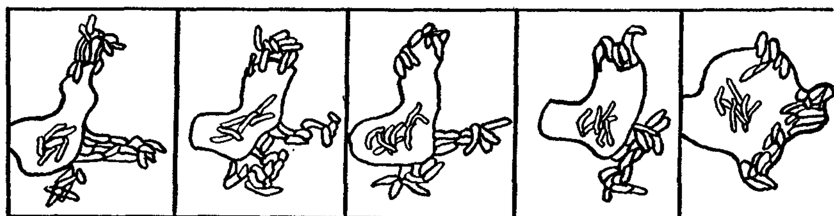
Phagocytosis a Phenomenon of Spreading.—It follows both from the mathematical formulation of Fenn (3) and Ponder (4) and from the experimental analysis (7–14) that the capacity of the phagocyte to spread over the surface of the particle undergoing ingestion is a principal factor in determining phagocytosis. Is this deduction in agreement with the process of phagocytosis as directly observed? This question we have examined with especial care. Prediction and observation have been found to be uniformly in agreement.

In mixing the phagocytes, erythrocytes or bacteria and serum as described, many collisions between phagocytes and test particles are brought about. Additional contacts between particle and phagocytes may later be made by the locomotion of the latter. In the absence of sensitizing serum the test particles typically neither stick to one another nor to the phagocytes (Figs. 1 and 2) and the particles are not ingested. In the presence of dilute sensitizing serum, agglutination of the particles and adhesion to the phagocytes may be much in evidence with little complete ingestion occurring (Figs. 5, 6, and 11–14). In the presence of more concentrated sensitizing serum, the test particles adhere to the phagocytes and are drawn into their cytoplasm in great numbers (Figs. 7–10, 29, and 30).

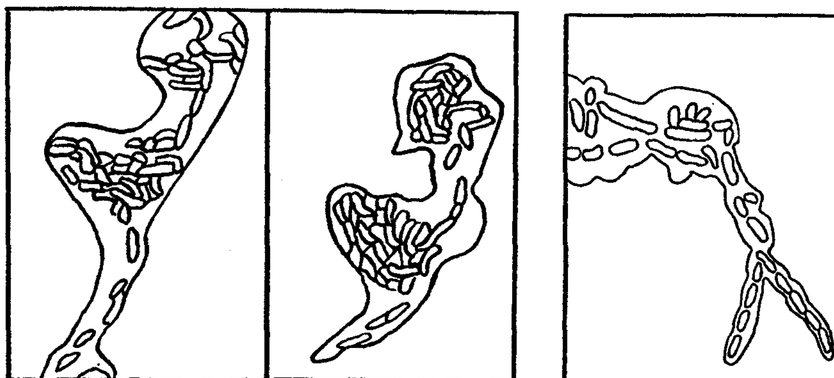
The test particles may be drawn into the phagocytes with comparatively little distortion of the latter (Figs. 29 and 30). Or a process composed of the hyaline superficial protoplasm may flow out over the surface of the particle undergoing phagocytosis (Figs. 3, 4, 15–19, 28, and 34–37). A semidiagrammatic tracing of Figs. 15–19 is shown in Text-fig. 2. Or the spreading of the leucocytes over the sensitized particles may cause marked deformation of the leucocytes (Figs. 20–24 and 25–27). A semidiagrammatic tracing of Figs. 25–27 is shown in Text-fig. 3.

The types of ingestion described of course merge into one another. For instance four chains of sensitized *subtilis* bacilli were seen arranged

in a diamond-shaped figure with a suspended spherical macrophage in their center. When first observed the chains were merely tangent and adherent to the macrophage surface. Gradually the areas of contact between *subtilis* chains and phagocyte surface increased, the adherent tangents becoming arcs of circles, which were slowly drawn into the macrophage protoplasm. The ends of the chains projected for a time beyond the macrophage surface, but these eventually were



TEXT-FIG. 2



TEXT-FIG. 3

drawn in also; in several instances hyaline processes were observed to flow out over the projecting end of the *subtilis* chain as the last step in its ingestion.

For purposes of comparison between observation and deduction from theory the essential point is that in all instances observed the particles were not taken up in vacuoles of the suspending medium; on the contrary *the protoplasm of the phagocytes was in immediate contact with the surface undergoing phagocytosis.* Phagocytosis as observed,

then, is primarily a phenomenon of surface spreading—the spreading of the phagocyte surface over the surface of the object undergoing ingestion. Prediction from theory and from experimental analysis is thus in agreement with observation on this second essential point.

Before leaving this point, however, two possible sources of confusion should be mentioned. Phagocytes of the large mononuclear type are able to form delicate petal-like extensions of their peripheral hyaline protoplasm—the "sheet-like pseudopods" of Smith, Willis, and Lewis (15), the "undulating membranes" of Carrel and Ebeling (16), the "*pseudopodes pétaloïdes*" of Fauré-Fremiet (17) (Figs. 33 and 35). W. H. Lewis (18) has described under the term "pinocytosis," and shown in moving pictures, the engulfing of tiny vacuoles of the fluid medium by these processes. Should such a vacuole contain a minute particle it would of course be engulfed also. What may have been such an instance has been described by Chambers and Borquist (19). However, although we have seen the phagocytosis of a large number of bacteria and erythrocytes by direct extension of the phagocyte surface over the surface of the particle ingested, we have never observed ingestion in a vacuole. Phagocytosis and pinocytosis we believe to be quite different phenomena.

Another possible source of confusion is the fact that in stained films showing phagocytosis bacteria can often be seen to lie in little vacuoles in the cytoplasm (15, 20). These digestive vacuoles are seen especially about the bacteria which have been ingested for some minutes and have been moved in toward the center of the cell. These vacuoles are a phenomenon not of ingestion but of intracellular digestion.

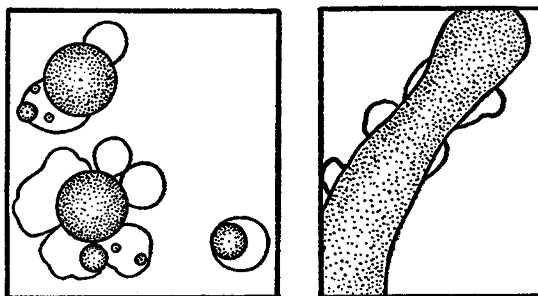
Partial Ingestion.—Fenn's formulation of surface forces in phagocytosis predicts that under certain conditions partial ingestion should occur; this is an important point of departure from the formulations of Rhumbler and Tait. Fenn recognizes two conditions:

(*a*) The free surface energy is at a minimum when the particle is partially ingested; surface forces are therefore in equilibrium and satisfy the equation $S_1 = S_{12} + S_2.\cos\theta$.

(*b*) Surface forces would tend to bring about complete ingestion, but this is prevented by the resistance to deformation of the phagocyte; surface forces are therefore not in equilibrium but are held in check by viscosity.

The second condition has certainly been realized in our experiments, and to the best of our belief also the first. Figs. 31 and 32 show fields in which macrophages were mixed with an emulsion of light California mineral oil. A tracing of Figs. 31 and 32 is shown in Text-fig. 4. Emulsion droplets of small size are readily and completely ingested by the macrophages. On the larger drops the macrophages spread (Figs. 31 and 32) to positions determined by the balance between surface forces and their own resistance to deformation.

Incidentally it may be mentioned that such small emulsion droplets are very readily ingested by macrophages, but ordinarily not by polymorphonuclear leucocytes. Such a difference cannot be explained by differences in resistance to deformation, since the polymorphonuclears are on the average more fluid cells than the macrophages. This is evidently an instance in which a difference in the surfaces of the two types of phagocyte is a critical factor in determining phagocytosis. Another such instance was found in the quantitative phagocytosis study;—collodion particles are readily ingested by macrophages but not by polymorphonuclear leucocytes (13).



TEXT-FIG. 4. Tracing of Figs. 31 and 32. Macrophages white with black outlines; mineral oil stippled.

Partial ingestion with surface forces in equilibrium is more difficult to demonstrate conclusively. When weakly sensitized erythrocytes are mixed with phagocytes partial ingestion often occurs (Figs. 5, 6, and 11–14). Such partially ingested cells are not completely ingested during the time they are kept under observation even though this may be far longer than is required for complete ingestion of more strongly sensitized erythrocytes. It is difficult to believe that the

partial ingestion by such a fluid cell as is shown in Figs. 11–14 could represent anything other than equilibrium under surface forces. Moreover in stained preparations (20) it has very frequently been observed that strongly sensitized bacteria were completely ingested whereas weakly sensitized bacteria under otherwise similar conditions were merely adherent to the surfaces of the phagocytes. Although we realize that such observations fall somewhat short of rigorous proof that the surface forces are in equilibrium, we believe that this is by far the most probable interpretation. The third deduction from theory, namely the occurrence of partial ingestion, is thus likewise in agreement with observation.

Viscosity.—L. Loeb (21) has related the ameboid motion of the amebocytes of *Limulus* to "1) changes in consistency in the ectoplasmic layer as well as in the granuloplasm, 2) phenomena of contraction and 3) surface tension changes." Loeb in 1927 sought to carry over these conceptions to the explanation of phagocytosis by mammalian cells, assigning a primary importance to softening of certain parts of the surface layer of the cell in contact with a foreign body. Whether or not such local softening occurs on contact of phagocytes with foreign particles, it is evident that the quantitative correlation which has since been demonstrated between phagocytosis and the surface properties of the particles phagocytized (7–14) is not explained by viscosity changes and is explainable in terms of interfacial tension relations.

The resistance of the protoplasm to deformation is, on the other hand, a modifying factor in phagocytosis which, under certain conditions, may reach critical importance. Fenn (22) for instance found very high temperature coefficients for phagocytosis below 30°C. as compared with those above 30°. He interpreted his data as indicating that below 30° the viscosity of the phagocytes was so high as to become the limiting factor for phagocytosis. Ponder (4) has treated mathematically the retarding influence of viscosity on the rate of phagocytosis. He has shown, moreover, that when ingestion occurs in a moving current such as the blood stream, in which forces may act to dislodge the particle undergoing ingestion from the phagocyte surface, the rate of ingestion as determined by viscosity may become a critical factor.

An average difference in viscosity between phagocytes of the large mononuclear and the polymorphonuclear types has been observed by E. R. and E. L. Clark (23), by Goss (24), and by ourselves (25). The macrophages offer on the average more resistance to deformation than the polymorphonuclears. This difference has been evidenced in the present study in two ways. In the first place the act of ingestion is on the average more quickly accomplished by the polymorphonuclears, and in the second the polymorphonuclears are more readily distorted to all manner of bizarre shapes in spreading over the larger bodies phagocytized (Figs. 25 and 27).

Unformulated Factors.—It seems clear then that surface forces are a principal factor in determining ingestion, and that viscosity is an important factor in controlling its rate. It is perhaps worth emphasizing, however, that a complete explanation of the behavior of phagocytes is not afforded by these factors alone. A particle phagocytized under the action of surface forces does not enter a homogeneous liquid, but a system possessing internal organization in high degree. The process which has spread over the particles undergoing phagocytosis is frequently retracted (Figs. 15–26). The protoplasm of the phagocytes possesses elastic properties (Figs. 11–14). The ingested particles are commonly moved in toward the center of the cell. They frequently undergo rapid intracellular digestion. The formation and retraction of pseudopods appears to be the consequence of internal changes within the cell as well as of the tendency of the cell surface to spread upon external surfaces. Reversible changes in viscosity, as evidenced by the appearance and disappearance of Brownian movement, may be seen to occur in local areas within the cell.

CONCLUSIONS

The phagocyte, then, is a complex system delicately responsive to internal and external influences. Interfacial tensions, and under certain conditions viscosity, are critical factors in determining the ingestion of particles with which the phagocyte has come into contact. Deductions from the formulation of these factors by Fenn and Ponder are in agreement with observation and with experimental analysis. However, other and still unformulated forces also enter into the behavior of these remarkable cells.

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EXPLANATION OF PLATES

Plate 1

All figures are unretouched photographs of living phagocytes.

Figs. 1 and 2. Macrophages (*m*), polymorphonuclear leucocytes (*p*), and sheep erythrocytes. The large macrophage in Fig. 1 contains three ingested oil droplets. No sensitizing immune serum present; little or no agglutination or phagocytosis of erythrocytes.

Fig. 3. A macrophage ingesting a sensitized cell of *Monilia albicans* (*mo*). Note process of macrophage (*ps*) spreading around monilia.

Fig. 4. A macrophage ingesting sensitized sheep erythrocytes. At top erythrocytes being drawn into macrophage. On right a process spreading over two erythrocytes.

Figs. 5 and 6. Partial ingestion of weakly sensitized sheep erythrocytes by macrophages and polymorphonuclear leucocytes (*p*). The shadows marked (*d*) are dust on the camera lens.

Fig. 7. A cluster of strongly sensitized erythrocytes surrounding and being drawn into a macrophage.

Figs. 8 and 9. Successive stages in ingestion of a mass of strongly sensitized sheep erythrocytes by a macrophage; the macrophage contains an oil droplet (*o*).

Fig. 10. A macrophage embedded in and ingesting strongly sensitized sheep erythrocytes. The macrophage contains two previously ingested oil droplets.

Figs. 11–14. Successive stages in migration of polymorphonuclear leucocyte which has partially ingested weakly sensitized sheep erythrocytes. That portion (*a*) of the leucocyte to the left of the figure has partially ingested three erythrocytes which remain adherent to the glass slide; the portion (*b*) of the leucocyte which contains one partially ingested erythrocyte continues to migrate toward the lower right hand corner of the field until the protoplasm of the leucocytes is stretched into a thin filament. While under direct observation the adherent erythrocytes in the upper left hand corner were pulled loose from the glass and the protoplasmic filament contracted like a stretched rubber band.

Plate 2

Figs. 15–19. Successive stages in ingestion of clumps of strongly sensitized typhoid bacilli by a polymorphonuclear leucocyte. In Figs. 15, 16, and 17 a process of the leucocyte spread over a clump of sensitized bacteria shown above the leucocyte. In Figs. 18 and 19 this process contracted, drawing the ingested bacteria toward the center of the cell. In Fig. 19 a second process began to spread over a clump of bacteria to the right of the leucocyte.

Figs. 20–24. Successive stages in contraction of the process of a polymorphonuclear leucocyte which has spread over a clump of sensitized typhoid bacilli. In Fig. 24 the process has contracted and the bacteria have moved in toward the center of the cell.

FIGS. 25 and 26. A polymorphonuclear leucocyte spread over agglutinated *B. subtilis*. In Fig. 26 the cell is tending to round up and some bacteria have moved toward the center.

FIG. 27. A polymorphonuclear leucocyte spread over a λ-shaped chain of *subtilis* bacilli.

FIG. 28. Two polymorphonuclear leucocytes each filled with monilia cells. A process (*ps*) of the upper leucocyte has just spread around a monilia cell, (*mo*), and the lower leucocyte is spreading over another half-ingested monilia cell.

FIG. 29. A macrophage ingesting *subtilis* bacilli. Two chains of bacilli are adherent to the macrophage surface; the right hand arm of the upper Y-shaped chain has been drawn into the macrophage.

FIG. 30. *Subtilis* bacilli being drawn into a macrophage.

FIG. 31. Macrophages ingesting or spreading on the droplets of an emulsion of mineral oil.

FIG. 32. Macrophages spread out on a peninsula of mineral oil.

FIGS. 33–37. Are dark-field photographs.

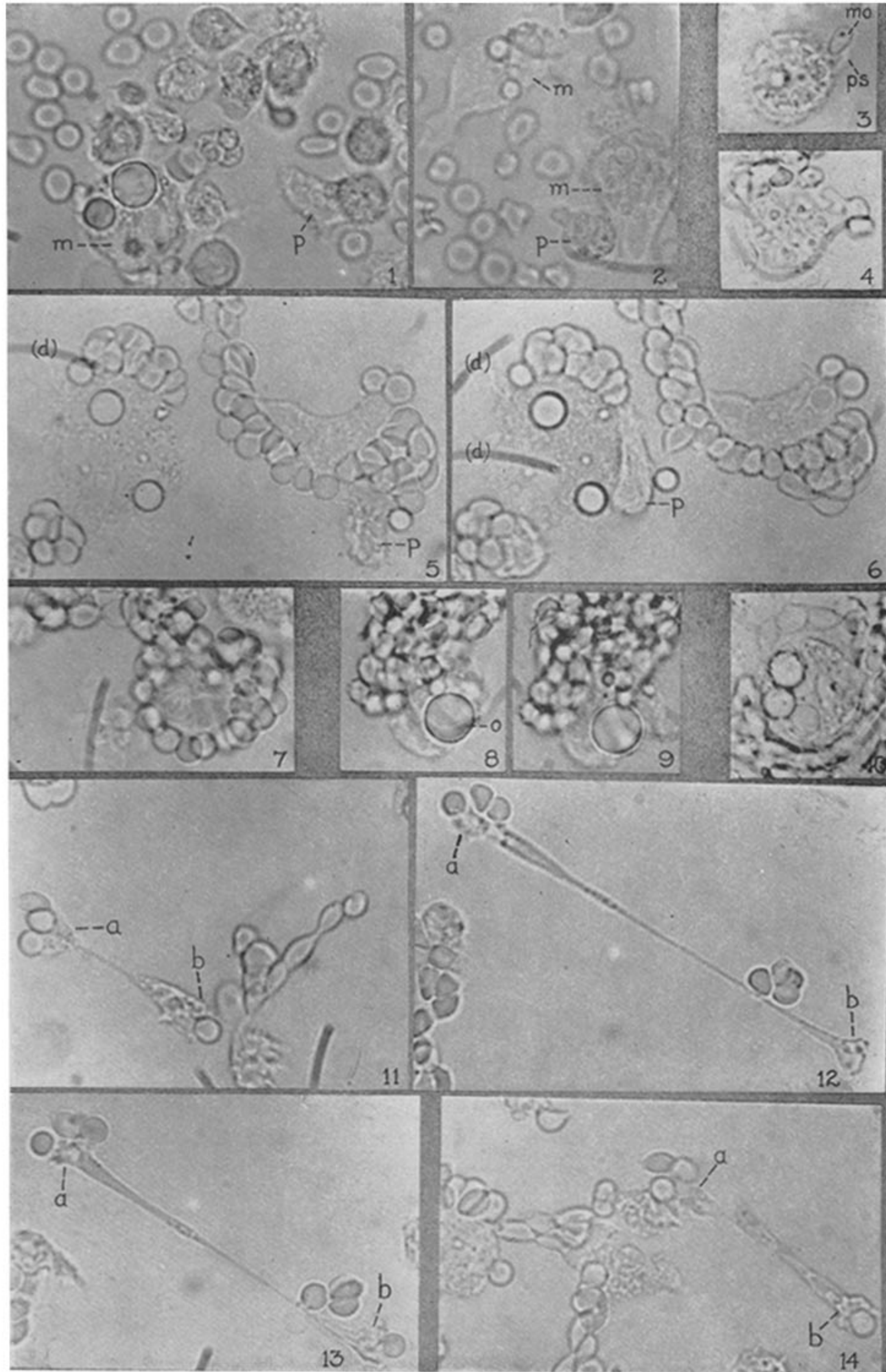
FIG. 33. Macrophage spread out on glass. In order to bring out the detail of the peripheral hyaline protoplasm, the detail of the central granular protoplasm has been lost by overexposure.

FIG. 34. Macrophage extended to a pear-shape by spreading over a *subtilis* chain. The *subtilis* chain (*s*) is the stem of the pear and the vague white around it (*ps*) a process of the macrophage.

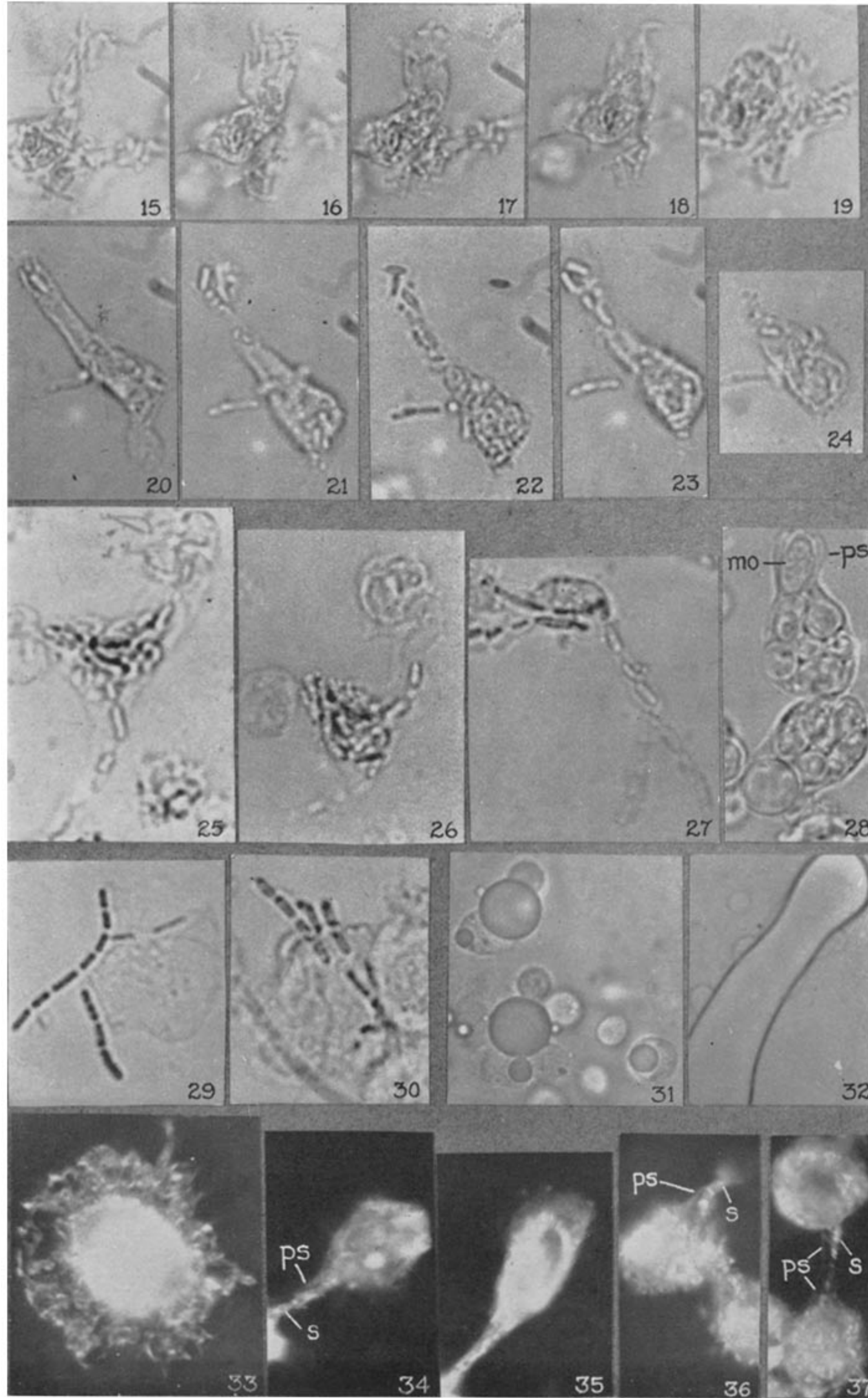
FIG. 35. The same macrophage a few minutes later. The cell has thrown out thin "veil-like processes" toward the top of the picture.

FIG. 36. Macrophage with hyaline protoplasmic process (*ps*) spreading over a *subtilis* chain (*s*). The latter becomes out of focus in the upper right hand corner of the picture.

FIG. 37. Two macrophages with *subtilis* chain (*s*) between them. Each cell has ingested one end of the bacterial chain and has extended a hyaline process (*ps*) on that portion which lies between the cells. The *subtilis* appears as a white chain and the two processes as delicate sheaths with dim outlines separated from the bacteria by dark spaces. The processes from the two cells met and remained approximated for some minutes; both were then withdrawn into their respective cells. One process was observed to extend again out over the *subtilis* chain before the field was lost to view.



(Mudd and Mudd: Process of phagocytosis)



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