THE INHIBITION OF SURFACE PHAGOCYTOSIS BY THE CAPSULAR "SLIME LAYER" OF PNEUMOCOCCUS TYPE III*

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PLATES 4 AND 5

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Surface phagocytosis has been shown to play an important rôle in the mechanism of recovery in acute bacterial pneumonia (1–5). Operating in the absence of specific antibodies, this form of phagocytosis acts as an immediate defense reaction of the host and causes prompt destruction of fully encapsulated bacteria. Highly virulent strains of type I pneumococci, Friedländer's bacilli, group A beta hemolytic streptococci, and staphylococci have all been found to be susceptible to surface phagocytosis.

The phagocytability of type III pneumococcus by the surface mechanism has been subjected to special study for the following reasons. (1) This organism is one of the most virulent encountered in human disease. (2) It possesses a particularly large capsule, the state of which determines its susceptibility to phagocytosis (6, 7). (3) Its capsule contains a polysaccharide with unique physicochemical properties (8).

The present study indicates that type III pneumococcus is more resistant to surface phagocytosis than any organism thus far studied. Its exceptional resistance is related to the presence of an outer "slime layer" (9) which is prominent only on highly virulent strains of the organism. The slime layer, which can be visualized under the electron microscope, stains metachromatically with methylene blue and is demonstrable only on the surfaces of rapidly multiplying cells. When the slime layer is lost with the aging of the bacterial population, the organism is readily phagocyted by the surface mechanism.

Resistance of Type III Pneumococcus to Surface Phagocytosis

Type III pneumococci (strain 8HCC[1]) recovered by centrifugation from 4 hour broth cultures were washed in gelatin-Locke's solution and added to heavy suspensions of leucocytes. The methods used in handling the bacteria, harvesting the leucocytes, and performing the phagocytic tests have previously

* This study was supported by the Commonwealth Fund.

[1] This strain, obtained from the Department of Bacteriology of the Washington University Medical School, was originally isolated in the bacteriological laboratories of Barnes Hospital from a patient with pneumococcal pneumonia. Virulence was maintained by frequent mouse passage and storage at 4°C. in rabbit's blood under vaseline.

been described (1, 2). When such leucocyte-pneumococcus mixtures were incubated for 30 to 60 minutes on moist filter paper, little if any phagocytosis resulted. Likewise, no appreciable phagocytosis was observed after incubation on the surfaces of freshly prepared tissues and on sections of formalin-fixed lung. Even when the mixtures were inoculated intrabronchially in living rats, only minimal phagocytosis was observed in the alveoli when the animals were killed an hour later. These findings are in marked contrast to the results previously obtained with other encapsulated organisms (Figs. 1 and 2). It is of interest that the few type III pneumococci that were phagocyted were found almost exclusively in macrophages (Fig. 3).

Presence of Capsular "Slime Layer"

During the preceding experiments it was noted that the capsules of the type III pneumococci stained metachromatically with methylene blue[2] (Fig. 10). The large envelope surrounding each organism from the 4 hour culture was of a bright pink color in contrast to the somatic portion which stained a deep blue (Fig. 11). The margins of the pink capsules appeared to have fuzzy outlines. Although at first the metachromatic staining was not uniform in all parts of the smears, the metachromasia was found to be relatively constant whenever a loopful of rat or rabbit serum was added to the mixture before the stain was applied. Similar results were obtained with toluidine blue.[3]

Repeated examinations of wet preparations in the absence of stain were made under darkfield illumination, with a phase difference microscope, and with an ordinary light microscope. Except for their relatively large size, the capsules under these conditions appeared no different from those of other pneumococci. However, when contained within a macrophage, as was occasionally observed in the phagocytic tests, the unstained type III pneumococcus presented a singular appearance. A relatively clear capsule with a sharp outline could be seen surrounding the somatic portion. Outside of the capsule there was visible an indefinite fuzz which displaced the cytoplasm of the macrophage and made the phagocyted organism appear extremely large (Fig. 4). Under bright illumination each phagocyted pneumococcus was faintly pink in contrast to the colorless cytoplasm surrounding it.

When homologous antiserum was added to wet preparations of the 4 hour culture, the usual quellung reaction occurred. In addition there was seen repeatedly on the outer surface of the swollen capsule a highly refractile zone of irregular outline (Fig. 5) with a greenish hue.

Organisms from 4 hour cultures were also examined with the electron microscope.[4] As is seen in the electron micrograph (Fig. 6) a wide halo with fuzzy

[2] Loeffler's alkaline methylene blue. The powdered stain "for bacilli" contained 86 per cent dye and was purchased from the Coleman Bell Co. of Norwood, Ohio.

[3] 1 per cent aqueous toluidine blue (52 per cent dye, Coleman Bell Co.).

[4] The following method was used in preparing the bacteria for electron microscopy. The

margins extended far beyond the borders of the type III capsule. When type I pneumococcus was photographed under similar conditions (Fig. 7), the analogous capsular fuzz was so slight as to be barely visible. Repeated observations with the electron microscope revealed a direct correlation between the presence of the wide capsular "slime layer" and the metachromatic staining with methylene blue.

The presence of the wide slime layer could also be detected by centrifugation of the organisms. Whereas pneumococci lacking the wide slime layer were thrown down in a small firm disc, the slime-covered organisms failed to pack and formed a flocculent centrifugate of about ten times as large a volume.[5]

Factors Determining Production of Slime Layer

The state of the outer capsule was found to depend upon the conditions under which the type III pneumococcus was grown. The slime layer in general was most prominent in liquid media that supported optimal growth. Limitation of the glucose content lessened its prominence as did omission of serum from the medium. The most favorable results were obtained with beef infusion broth at pH 7.8 containing 0.2 per cent dextrose and 10 per cent rabbit serum. The slime layer could be well demonstrated, however, in undiluted rabbit serum, in beef infusion broth with 0.05 per cent dextrose and 1 per cent serum, and in a "synthetic" medium containing casein hydrolysate (10).

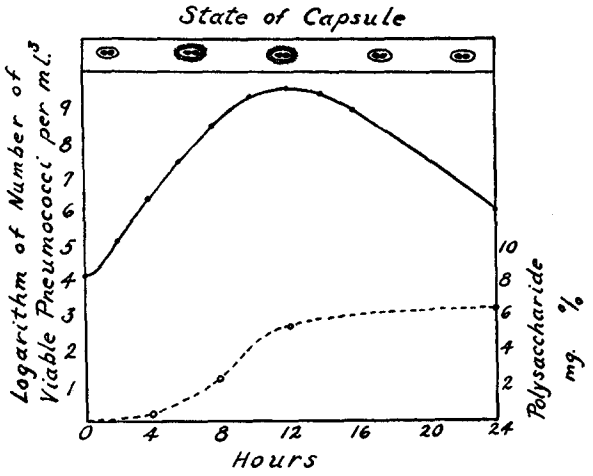
Metachromasia of the capsule, indicating the presence of the slime layer, was likewise noted *in vivo*. The prominent capsules of type III pneumococci, contained in peritoneal exudates aspirated from mice within 24 hours of inoculation, consistently stained metachromatically with methylene blue. Similar results were obtained with type III pneumococci recovered from early lesions of experimental pneumonia (11).

The production of the slime layer by type III pneumococcus was shown to vary with the growth phase of the organism. During the lag phase in serum-broth no slime layer could be demonstrated. After 4 to 8 hours' incubation the capsules of all the rapidly multiplying organisms exhibited a striking metachromasia. With further incubation, as the multiplication rate diminished, the slime layer became less definite until at 24 hours practically all the pneumococci were devoid of pink capsules when stained with methylene blue (Fig. 12). Organisms incubated for 24 hours on blood agar and examined by

organisms from 3 ml. of a 4 hour broth culture were suspended in 1 ml. of broth which was spread evenly over the surface of a blood agar plate and incubated for 4 hours at 37°C. A selected 1 ml. square was cut from the agar and placed upright on a glass slide. Several collodion screens, prepared in the usual manner, were mounted on a second glass slide and were touched, one at a time, to the area of growth on the agar block. In this way a range of bacterial density was obtained on the different screens, and by trial and error the screen with the optimum number and distribution of bacteria was selected for photography.

[5] All contrifugations were performed in a refrigerated (4°C.) angle centrifuge at 3,000 R.P.M. for 30 minutes.

electronic microscopy (Fig. 8) were also found to have only a minimal slime layer. In broth cultures incubated for more than 12 hours an amorphous pink-staining material was consistently noted in the medium (Fig. 13), suggesting that the same capsular substance present in the slime layer accumulated in the broth during bacterial growth. The relation of the slime layer to the growth phase of the organism and the synthesis of polysaccharide is roughly indicated in Text-fig. 1.[6]



TEXT-FIG. 1. Relation of capsular slime layer of pneumococcus III to phase of growth and production of type-specific polysaccharide. Upper curve, growth; lower curve, concentration of polysaccharide in medium (see Bukantz, S. C., Cooper, A., and Bullowa, J. G. M., *J. Bact.*, 1941, **42**, 29).

Four additional strains of pneumococcus type III[7] were studied by the same methods and all were found to be similar to the original strain in their capacities to produce capsular slime layers. A barely perceptible zone of pink at the capsular margins was noted in methylene blue preparations of type I pneumococci (A-5 strain (1)) and type A Friedländer's bacilli (Chic strain (2)) from peritoneal exudates. The amount of pink-staining material in the capsules of these latter organisms, however, was insignificant as compared to that observed with all five strains of type III pneumococcus.

[6] It might be postulated that the absence of the slime layer in older cultures is due to enzymatic destruction of the outer capsule in the later stages of bacterial growth. No such enzymatic activity could be demonstrated, however, either in the supernatnat fluid from 24 hour cultures or in 72 hour autolysates.

[7] These additional strains were obtained from Dr. J. Enders, Dr. G. Rake, Dr. C. MacLeod (A66), and from the bacteriological laboratory of the Barnes Hospital (McGeery).

Spontaneous Mutation Involving Slime Layer

During the routine handling of one of the strains of type III pneumococcus (8HCC) a 4 hour broth culture was encountered from which two types of centrifugate were obtained. In addition to the large volume of flocculent material that characterized the centrifugate of organisms possessing the wide slime layer, a closely packed disc was observed at the bottom of the centrifuge tube. The bacteria in the disc possessed a barely discernible slime layer when studied under the electron microscope (Fig. 9) and their capsules, though demonstrable by the quellung reaction, were relatively small and failed to stain metachromatically (Fig. 14). Furthermore, pneumococci from the disc, when incubated for 24 hours on blood agar, grew in small colonies with slightly depressed centers. The small colonies were easily distinguished from the large mucoid colonies of the parent strain. When the uncentrifuged 4 hour broth culture was streaked on blood agar plates, both types of colonies formed on incubation, although the mucoid variety predominated. Repeated mouse passage of the variant producing small colonies failed to revert it to the mucoid type. The distinguishing characteristics of the parent and mutant[8] strains are summarized in Table I.

TABLE I

Distinguishing Characteristics of Parent Strain and Mutant of Type III Pneumococcus (8HCC)

Characteristic	Parent strain	Mutant
Colony type on blood agar	Large, mucoid	Small, smooth, with depressed center
Centrifugate from 4 hr. broth culture	Flocculent, large volume	Small, firm disc
Staining of capsule with methylene blue	Metachromatic	Unstained
Quellung reaction to type III antiserum	Large capsule with irregular greenish margin	Capsule small, no outer greenish zone

Physical Properties of Slime Layer

The pneumococcal cells possessing the wide slime layer were found to have very different physical properties from those without it. Mention has already

[8] This type III mutant appears to be analogous to the "intermediate" variant of type II pneumococcus recently described by MacLeod and Krauss (12). Their intermediate strain produces type II polysaccharide in relatively small amounts and forms rough colonies on solid media. The fact that it does not give a positive quellung reaction, however, indicates that its capsule is even smaller than that of the present type III mutant. Other intermediate variants of both type I and type II pneumococcus have previously been described (13–17).

been made of the large volume occupied by the flocculent centrifugates containing slime-covered organisms. When type III pneumococci from 4 hour broth cultures were suspended in undiluted rabbit serum, most of the cells remained in suspension after centrifugation. The few cells thrown down from the serum formed a small, closely packed disc in the bottom of the tube. The organisms in the disc possessed no stainable slime layer, whereas those remaining in suspension stained metachromatically in the usual manner. Thus the physical properties of cells possessing the wide slime layer are sufficiently different from those lacking it, to enable separation of the two forms by centrifugation from serum.

The amorphous substance which accumulates in the medium during the growth of type III pneumococcus and stains in the same manner as the slime layer, remains in suspension during centrifugation even in relatively nonviscous fluids such as 1 per cent serum broth. The capsular material, therefore, that composes the slime layer appears to behave like a colloid and is brought down by centrifugation only when attached to the relatively heavy bacterial cell.

Chemical Properties of Slime Layer

The capsular slime layer is a labile structure of the type III cell. Not only does it disappear rapidly in aging cultures but it is also readily destroyed by heat, weak alkali, and ethyl alcohol. When cells from 4 hour broth cultures were suspended in gelatin-Locke's solution and heated to 100°C. for 5 minutes, the slime layer could not be identified by staining with methylene blue. Heating at 52°C. for 10 minutes, on the other hand, had no effect upon the staining reaction. Organisms incubated for 30 minutes in N/20 sodium hydroxide, in 13 per cent sodium salicylate, and in saturated solutions of sodium bicarbonate lost their slime layers. Similar results were obtained with 95 per cent alcohol.

From serological experiments evidence was obtained that the principal constituent of the slime layer combined with type-specific antibody. Cells from young cultures failed to stain metachromatically when previously exposed to type III antiserum, indicating a probable combination of antibody with the slime layer. Similarly, the pink-staining material that accumulated in the culture medium during growth, and appeared to be of capsular origin, was found to be completely removed by precipitation with type III antibody. Because of the known specificity of such serological reactions, it was concluded that the slime layer contained the same polysaccharide that occurs in the rest of the capsule.

Further and more conclusive evidence that type III polysaccharide is present in the slime layer was derived from enzymatic studies employing a polysaccharidase known to depolymerize the specific capsular polysaccharide of the type III pneumococcus (18). A lyophilized preparation of this enzyme[9] was

[9] Obtained through the kindness of Dr. R. J. Dubos

dissolved in Locke's solution (pH 7.4) in a concentration of 0.1 mg. per ml. Type III pneumococci harvested from 4 hour broth cultures and possessing definite capsular slime layers were incubated at 37°C. for 1 hour in the enzyme solution. At the end of incubation the cells were devoid of slime layers as indicated by the absence of metachromasia, and their capsules gave a smaller quellung reaction than those of cells exposed to heat-inactivated enzyme.

Relation of Slime Layer to Surface Phagocytosis

Whereas pneumococci without stainable slime layers are susceptible to surface phagocytosis (Fig. 15), those possessing them are highly resistant (Fig. 10). Procedures which injure the slime layer destroy this resistance. When the slime layer is altered by heat, by alkali, by exposure to alcohol, or by the enzymatic action of type III polysaccharidase, the type III pneumococcus behaves no differently from other encapsulated bacteria (1, 2, 4) and is readily phagocyted on tissue surfaces and on moistened filter paper. Similarly, the type III cells that have lost their prominent slime layers, either through aging of the culture or as a result of mutation,[10] are readily attacked by phagocytes on suitable surfaces. Even the few cells that lack the stainable slime layer in 4 hour broth cultures and can be recovered by centrifugation from serum are phagocyted on filter paper. From these findings it is clear that the ability of pneumococcus type III to resist surface phagocytosis is due to the presence of the large slime layer that covers the outer surface of the capsule.

Bactericidal Effect of Surface Phagocytosis

When fully encapsulated type I pneumococci or Friedländer's bacilli are phagocyted by the mechanism of surface phagocytosis, they are destroyed by the phagocytes (1, 2). That type III pneumococci are also killed by this form of phagocytosis is indicated by the following experiment.

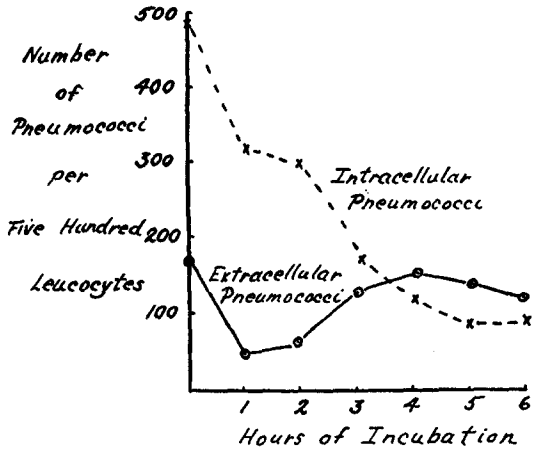
Concentrated suspensions of rat leucocytes and type III pneumococci harvested from 18 hour broth cultures were incubated at 37° on moistened filter paper (1, 2). After surface phagocytosis had taken place (30 minutes' incubation), the leucocytes were washed from the filter paper and resuspended in gelatin-Locke's solution containing a small amount of fresh rat serum to preserve the maximum activity of the leucocytes (1). This suspension was then incubated for an additional period of 6 hours. Smears were made of the mixture at hourly intervals and stained with methylene blue.

As is shown in Text-fig. 2, the number of intracellular pneumococci decreased markedly during the 6 hours of incubation, whereas the number of extracellular pneumococci remained relatively constant. The failure of the extracellular count to increase during the period when the intracellular or-

[10] The type III mutant, though susceptible to phagocytosis on tisue surfaces and filter paper, possessed sufficient capsule to render it resistant to phagocytosis in the ordinary test on glass (1).

ganisms were decreasing indicates that the ingested pneumococci were actually digested by the phagocytes and not merely freed from their cytoplasm.

Further evidence that the phagocyted type III pneumococci were digested was obtained from direct observation (1) of individual leucocytes containing bacteria. When such cells were watched under the microscope for 6 to 8 hours in the warm stage, the ingested organisms could be seen gradually to undergo lysis within the cytoplasm of the cell. As has been previously mentioned, type III organisms harvested from 4 hour broth cultures and possessing stainable slime layers were occasionally phagocyted by macrophages. Digestion of these organisms was likewise noted on direct observation. Thus it



TEXT-FIG. 2. Data indicating intracellular digestion of type III pneumococci following surface phagocytosis.

appears that phagocytic cells are capable of killing type III pneumococci, regardless of the state of their capsules, once the organisms have been ingested.

DISCUSSION

The capsules of most bacteria are composed of mucoid substances of high molecular weight that are secreted by the cells and tend to concentrate upon their surfaces (19). In the case of pneumococcus the capsular substance determines the type specificity of the organism (18). The highly viscous capsular material of type III pneumococcus is known to consist essentially of long chained carbohydrate polymers made up of units of 4-β-glucuronosido-glucose (20). The viscosity of the intact polysaccharide is apparently due to the length of its complex thread-like molecule (21). Whether it is bound to protein in the capsule or is present as an unbound carbohydrate is not known.

The capsular slime layer of type III pneumococcus was first differentiated from the capsule proper by Etinger-Tulczynska in 1933 (9). Although its

existence has recently been corroborated both by staining methods (22) and by electron microscopy (23), no biological significance has been attached to its presence on the surface of the cell. The present studies have shown that the slime layer contains type III polysaccharide, that it stains metachromatically with methylene blue, that it is present only on the surfaces of actively multiplying cells, and that when present it protects the organisms against surface phagocytosis.

Whether the slime layer should be considered to be a structure distinct from the capsule may be seriously questioned. Since it is present only on the surfaces of those cells that are producing polysaccharide at a maximum rate, it appears to represent an extension of the capsule, in the outer portion of which the polysaccharide is less densely packed. In keeping with this hypothesis is the fuzzy appearance of the slime layer under the electron microscope, and the presence of the same type-specific polysaccharide in both the inner and outermost portions of the envelope.

The metachromatic staining of the capsular slime layer is of particular significance. The polychromatic effect of basic dyes such as toluidine blue and methylene blue is due to the tendency of these dyes to polymerize on the surfaces of substances containing high molecular carbohydrates (24). Structures that stain metachromatically adsorb polymolecular layers of dye, in contrast to normally stained substrates that adsorb unimolecular layers. The fact that the inner capsule of the type III pneumococcus fails to take the methylene blue stain in contrast to the metachromatically stained slime layer indicates that the polysaccharide in the inner capsule is in a different physicochemical state from that in the slime layer. The nature of this difference and its relation to the antiphagocytic properties of the pneumococcal capsule are at present under investigation.

Aside from indicating that the slime layer is physicochemically different from the rest of the capsule, the metachromatic staining reaction of the type III pneumococcus is of additional immunological interest because of the fact that it is nullified by the presence of type-specific antibody. Under properly controlled conditions this staining reaction may be used to test for the presence of type-specific immune bodies in both intra- and extracellular sites. An analogous test based upon the staining properties of toxoplasma has recently been used by Sabin and Feldman to demonstrate that antibody is unable to penetrate phagocytic cells containing intracellular parasites (25).

The manner in which the slime layer interferes with surface phagocytosis is revealed in part by microscopic study of phagocytic preparations. When encapsulated bacteria such as type I pneumococci or Friedländer's bacilli are trapped between immovable tissue structures and the surfaces of motile leucocytes, they are readily phagocyted. The slime-covered pneumococcus III, on the other hand, appears to be less readily trapped against the tissues,

and when it is caught in this manner, it fails to be ingested. Only macrophages succeed in phagocyting an occasional type III organism, and their limited success may be due to the fact that their pseudopods extend over an appreciably larger area than those of the granulocytes.

The resistance of type III pneumococcus to phagocytosis in general has long been recognized. Enders *et al.* (6, 7) clearly demonstrated that loss of capsule with aging goes *pari passu* with shrinkage of cell volume and increase in susceptibility to phagocytosis both *in vitro* and *in vivo*. Dubos describing the behavior of type III pneumococci in peritoneal exudates of unimmunized mice, states that "no phagocytosis is observed and in fact leucocytes appear to be maintained at a definite distance from the infective agent by a sort of negative chemotactic effect." It is apparent from the present studies that the ability of the type III pneumococcus to maintain attacking leucocytes at a distance is due to the presence of the wide slime layer which extends well beyond the margin of the capsule as ordinarily seen in wet preparations. It is of significance that repeated tests under various conditions have failed to demonstrate that type III polysaccharide is toxic to leucocytes either in purified form or in the state in which it exists in the animal body (26). Thus, it appears that the polysaccharide acts merely as a protective envelope exerting its antiphagocytic effect when present on the surface of the cell.

Although type III pneumococcus is the only bacterial species thus far studied that possesses in the logarithmic phase of growth a sufficiently large slime layer to prevent surface phagocytosis, it is not implied that pneumococcus III is necessarily unique in this respect. It seems not unlikely that other organisms, particularly when growing at a maximum rate within the animal body, may produce sufficient quantities of mucoid capsular material to form analogous slime layers which will temporarily protect them from surface phagocytosis. If this hypothesis proves to be correct, it will change but little the general significance of surface phagocytosis as a mechanism of natural immunity. It has already been emphasized that the presence of the slime layer is a transient phenomenon. Evidence will be presented in a subsequent report (11) that even the type III pneumococcus becomes susceptible to phagocytosis by the surface mechanism after a relatively few hours of growth in the animal body. Although the slime layer undoubtedly contributes to virulence by influencing the initial struggle between phagocytes and bacteria, it does not prevent the organisms from ultimately being ingested and destroyed by surface phagocytosis long before the development of humoral immunity.

SUMMARY

Five strains of type III pneumococcus have been shown to possess wide capsular slime layers during the logarithmic phase of growth in serum broth.

The slime layer stains metachromatically with methylene blue and can be

visualized under the electron microscope as a fuzzy halo which extends well beyond the surace of the capsule proper and causes centrifugates of the organism to be of extremely large volume.

This outer capsular structure is most readily demonstrated *in vivo* and in nutrient broth containing glucose and serum. It disappears from the surface of the cell with aging of the culture, and is easily removed by dilute alkali, alcohol, and heat.

Exposure of slime-covered type III pneumococci to homologous antibody and to type III polysaccharidase reveals that the slime layer contains the same type-specific polysaccharide that is present in the rest of the capsule.

From a type III strain producing a prominent slime layer an intermediate mutant has been isolated which forms small non-mucoid colonies on blood agar and possesses a relatively small capsule with a barely discernible slime layer.

The wide slime layer protects virulent type III pneumococci from surface phagocytosis. Whenever the type III cells lose their broad slime layer, whether from aging of the culture, from mutation, from exposure to injurious chemicals, or from the action of type III polysaccharidase, they become susceptible to phagocytosis by the surface mechanism. Once phagocyted the type III pneumococci are promptly destroyed, even in the absence of antibodies.

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EXPLANATION OF PLATES

Dry smears were stained with methylene blue (Figs. 1, 2, 3, 10 to 15). Wet preparation in Fig. 4 was unstained; that in Fig. 5 was stained with methylene blue. The electron micrographs (Figs. 6 to 9) were taken on an RCA microscope (Type E.M.U.) by Mr. Harry U. Rhoads of the Research Laboratories of the Lambert Pharmaceutical Company. Valuable advice concerning the preparation of specimens for electronic microscopy was obtained from Dr. Geoffrey Rake of the Squibb Institute. All other photographs were made by Mr. Cramer Lewis, Director of Division of Illustration, Washington University School of Medicine.

PLATE 4

FIG. 1. Surface phagocytosis of fully encapsulated type I pneumococcus from 4 hour broth culture. Pneumococcus-leucocyte mixture suspended in gelatin-Locke's solution was incubated at 37°C. for 30 minutes on moist filter paper. × 1,440.

FIG. 2. Failure of type III pneumococcus from 4 hour culture to be ingested by surface phagocytosis. Contrast with result shown in Fig. 1, where experimental conditions were identical. × 1,440.

FIG. 3. Phagocytosis by macrophages of occasional type III pneumococci from 4 hour culture. Conditions as in Figs. 1 and 2. × 1,620.

FIG. 4. Phagocyted type III organism from 4 hour culture (wet preparation). Outline of wide capsule can be clearly seen in cytoplasm of unstained macrophage. Note outer "fuzz" surrounding capsule proper. × 1,575.

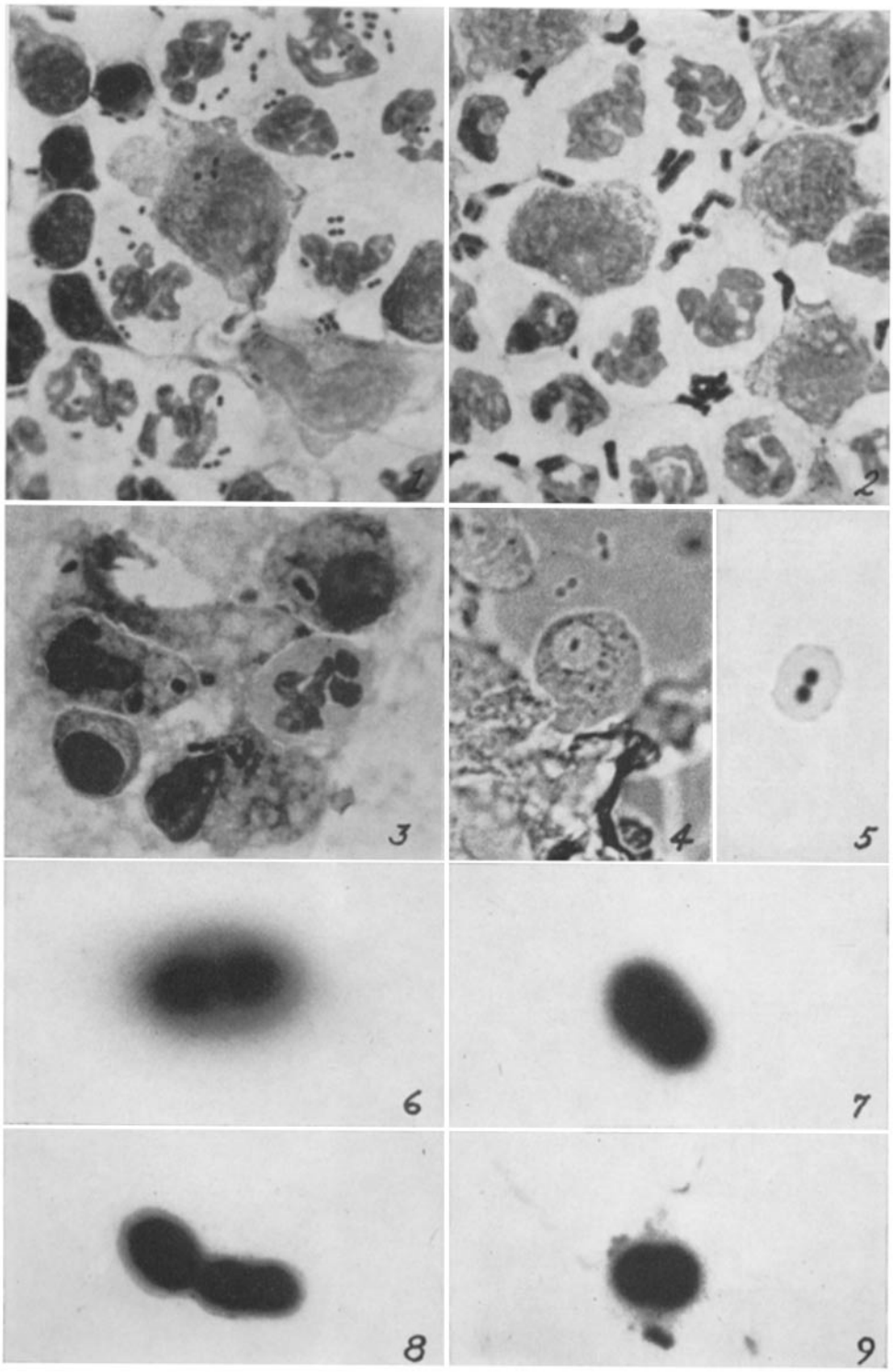
FIG. 5. Quellung reaction of pneumococcus III (4 hour culture) to type-specific antiserum. Irregular refractile zone can be seen at periphery of swollen capsule. × 2,900.

FIG. 6. Electron micrograph of type III pneumococcus from 4 hour broth culture. Note fuzzy slime layer that extends far beyond margins of ordinary capsule (see Fig. 8) and obscures outline of somatic portion of cell. × 10,000.

FIG. 7. Electron micrograph of type I pneumococcus from 4 hour culture. Compare relatively narrow capsular slime layer with that of pneumococcus III (Fig. 6). × 10,000.

FIG. 8. Electron micrograph of type III pneumococcus from 24 hour broth culture. With aging of bacterial population the slime layer has disappeared from the surface of the capsule. × 10,000.

FIG. 9. Electron micrograph of type III mutant that has lost capacity to produce wide slime layer (4 hour broth culture). Compare with parent strain (Fig. 6). × 10,000.



(Wood and Smith: Inhibition of surface phagocytosis)

PLATE 5

FIG. 10. Failure of slime-covered type III pneumococci (from 4 hour broth culture) to be phagocyted by surface mechanism. Conditions as in Fig. 1. Note metachromasia of slime layer. × 2,200.

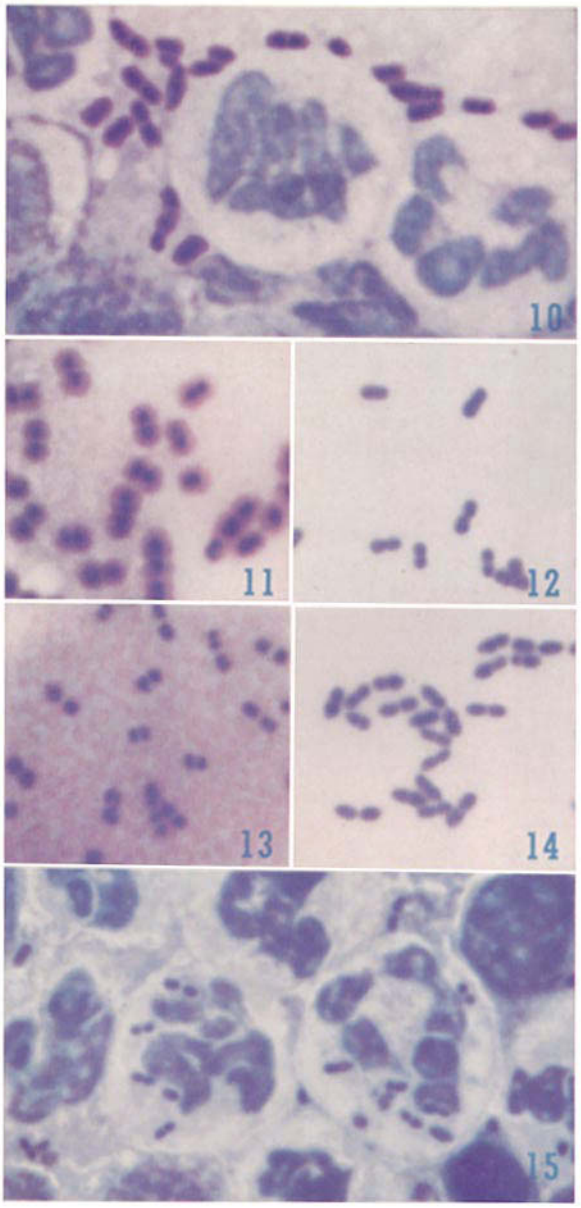
FIG. 11. Type III pneumococci with metachromatically stained slime layer. Note fuzzy outline of capsular margin. Organisms harvested from 4 hour broth culture. × 2,900.

FIG. 12. Absence of metachromatic slime layer on type III pneumococci from 24 hour culture. Contrast with Fig. 11. × 2,900.

FIG. 13. Amorphous material staining metachromatically in supernatant fluid of 24 hour broth culture of type III pneumococcus. × 2,900.

FIG. 14. Type III mutant which lacks the capacity of producing a prominent slime layer. Note absence of metachromatic staining as compared with Fig. 11. × 2,900.

FIG. 15. Surface phagocytosis of type III pneumococci from 24 hour broth culture. Pneumococcal cells fail to stain metachromatically indicating that they are devoid of wide slime layer (compare with Fig. 10). × 2,200.



(Wood and Smith: Inhibition of surface phagocytosis)