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# Morphology and Reproductive Processes of the L Forms of Bacteria
# I. Streptococci and Straphylocci

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ABSTRACT

The production of L forms from cocci, the conditions necessary for their multiplication, and their morphology have been studied for several years. In each strain studied, only a few organisms produced L forms. Transplants from these grew poorly at first, and growth on agar and in broth became abundant only after long cultivation. Multiplication in the form of small granules was observed only when the organisms were embedded in agar and occasionally in coagulated blood serum. On the surface of hard agar, the organisms increased in size but did not multiply. Abundant growth developed on membrane filters of appropriate size, extending into the filters as branching irregular masses. On gelatin, on most samples of coagulated serum, and on silica gel, the organisms grew to a very large size, and occasionally colonies developed by multiplication of large bodies. This multiplication occurred by irregular enlargement and separation into fragments. Growth in broth and in semisolid agar also occurred by multiplication of large bodies, but, in addition, the development of viable granules was observed inside the large bodies in broth culture. After the L forms began to grow abundantly, their nutritional requirements were simple; they no longer required animal serum. Their ability to multiply and their morphological characteristics depended to a large extent on the physical properties of the environment.

During many years of work with the L forms of bacteria, much information has accumulated on the conditions that permit their growth and on the factors that influence their morphology and their manner of multiplication. This information has been systematically re-examined and extended during the past 3 years by the study of L forms of gram-positive cocci and of *Proteus*. The observations on group A β-hemolytic streptococci and a strain of *Staphylococcus* are presented in this paper. Those on enterococci and on *Proteus*, and the study of several strains of L forms with an electron microscope, will be described in subsequent publications.

These observations were made by use of simple techniques of cultivation and of examination. They were readily reproduced, and the behavior of the L forms of different strains of cocci was similar. For this reason, detailed descriptions of individual observations have not been given. Rather, an effort has been made to illustrate the types of growth and variations of morphology with photographs, and only summary descriptions of the cultures and the conditions under which they were grown are presented.

## MATERIALS AND METHODS

Most observations have been made with the L forms of three strains of group A β-hemolytic streptococci (AED, GL8, and ADA) and of one strain of *Staphylococcus* (ATCC 6538). They were isolated more than 10 years ago by exposure of the cocci to penicillin or glycine on agar media containing 3% NaCl and 10% horse serum (8, 15). Their properties changed markedly during long cultivation. Many of the observations to be described could not be repeated with L forms immediately after isolation from the cocci.

The old L forms grew abundantly on the routinely used broth and agar media with or without the addition of animal serum. In most experiments Field's Tryptic Digest or Trypticase Soy media (BBL) were used. Good growth was also obtained when the nutrients were diluted to one-fifth of the usual concentration, or on agar media containing 1.5% Agar (Difco), 3% NaCl, 0.1% peptone, and 0.1% yeast extract. A medium poor in nutrients retarded autolysis of the cultures. In most experiments, 10% horse serum was added to the medium because it helped to make prep-

arations appropriate for microscopy. L forms of some strains of α-hemolytic streptococci grew well with the usual concentration (0.5%) of NaCl (4). However, the L forms with which most of the observations were made required increased osmotic protection for optimal growth, and 3% NaCl was used regularly.

In addition to agar and broth media, the development of the cultures was studied on membrane filters placed on appropriate agar media, on coagulated horse serum, and on broth solidified with gelatin and with silica gel. The latter were prepared according to standard methods with broth containing 3% NaCl and 10% horse serum.

The morphology and reproduction of the L forms were studied with a light microscope by examination of wet and dry stained agar preparations. The techniques of both methods have been described previously (6, 13). These observations could not have been made without techniques that required little time and were applicable to almost any medium. The parallel use of wet and dried stained agar preparations was of considerable advantage. In wet preparations, the natural tridimensional arrangement of the organisms growing on the surface and within the medium was preserved, and the interconnection between the organisms, both large and small, was more clearly visible. Also, the empty or autolyzed organisms that did not take the stain could be seen. The dry preparations, besides having the advantage of being permanent, showed the size and shape of the organisms better and were more satisfactory for photography. Observation of growth in slide cultures was often tried, but was not helpful in these studies. In a culture in which many organisms pass through a similar development, such as growth of small granules from the large bodies, observations at successive intervals gave as clear an indication of the process as observation in slide culture.

## RESULTS

*Development of L forms from the cocci.* Recently, L forms were again isolated from the four strains of cocci with the technique used previously to compare the properties of the old with those of the freshly isolated L forms. Large bodies and L forms developed only from a very few cocci. For this reason, the way in which L forms developed from the cocci could not be as easily observed as in some strains of gram-negative bacteria, pneumococci (14), or enterococci (Madoff et al., Ann. N.Y. Acad. Sci., *in press*). However, various observations suggested that it occurred in a manner similar to that observed in other bacteria. The L forms developed only after enlargement of a few cocci to large bodies (Fig. 1), and the growth of the granules from the large bodies was occasionally apparent. In transplants made from the old L cultures, growth started predominantly from the large bodies (Fig. 10, 11).

L forms starting to develop from the cocci often autolyzed and did not grow in transplant. When

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FIG. 1. *Strain GL8 of group A β-hemolytic Streptococcus exposed to penicillin on the surface of agar containing horse serum and 3% NaCl (1 day of incubation). Small coccus colony with large bodies. Wet stained agar preparation.* × *2,250.*

FIG. 2. *Similar plate and preparation as in Fig. 1, after 2 days of incubation. Small and large granules of the L form grow in several directions into the agar.* × *2,250.*

FIG. 3. *L colonies of strain D58 group A β-hemolytic Streptococcus. They are small and embedded almost entirely inside the medium.* × *250.*

FIG. 4. *Colonies of an 18-hr-old culture of L form of strain GL8 carried for many years in the laboratory. Extension on the surface of agar is composed of large bodies, and a few secondary L colonies start to grow.* × *250.*

FIG. 5. *A colony as in Fig. 4, after 7 days of incubation, extending by growth of secondary colonies embedded in the medium.* × *100.*

FIG. 6. *Young secondary colonies consisting of small granules at the periphery of a large colony as in Fig. 5.* × *2,250.*

FIG. 7–12. *Development of L colonies of strain GL8 on horse serum-agar with 3% NaCl, inoculated from a similar plate. All photographs were made from dried stained agar preparations. Fig. 8,* × *250; all others,* × *2,250.*

FIG. 7. *Transplant on agar from 1-day-old agar culture. Before incubation.*

FIG. 8. *After 6 hr of incubation, tiny colonies developed on lightly inoculated areas.*

FIG. 9. *Heavily inoculated area after 1.5 hr of incubation. Large bodies increased in size but no granules started to grow.*

FIG. 10 and 11. *Lightly inoculated area after 1.5 hr of incubation. Small darkly stained granules grow from large bodies and embed themselves in the medium.*

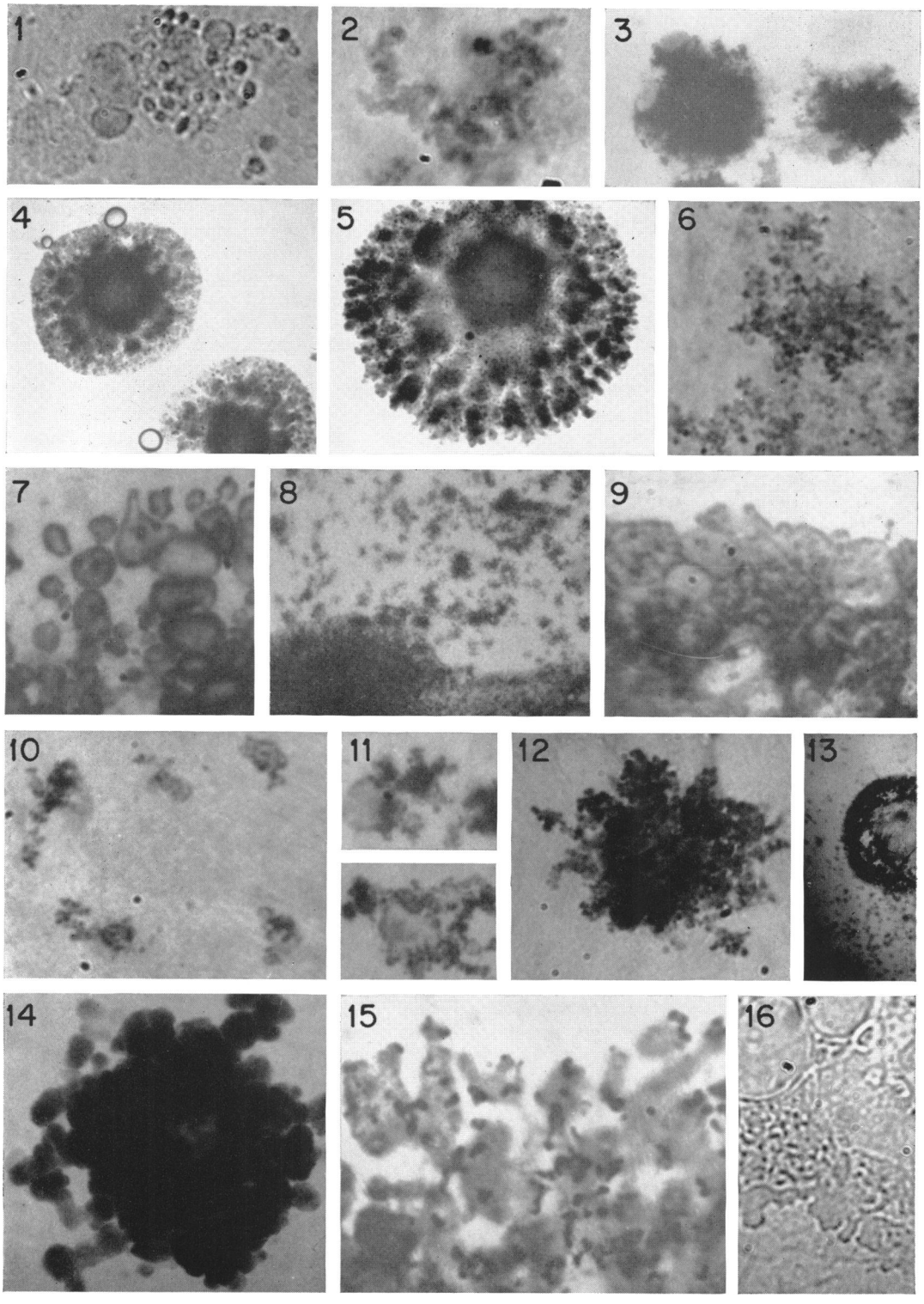
FIG. 12. *Small colony extending inside the medium after 12 hr of incubation.*

FIG. 13. *Very small colonies of Streptococcus L form among large ones after several days of incubation.* × *100.*

FIG. 14. *Small colony of Staphylococcus L form in 0.4% agar with 3% NaCl. The colony consists of large bodies.* × *2,250.*

FIG. 15. *Growth (6-hr) of GL8 L form on membrane filter. The large bodies extend with irregular protrusions on the surface. The dark irregular filaments start to penetrate the filter.* × *2,250.*

FIG. 16. *Vacuolized large body of L form of enterococcus, similar to those of Fig. 1 after 5 hr of incubation on horse serum-agar containing 3% NaCl. The stained periphery extending irregularly on the surface is broken into small granules. Wet stained agar.* × *2,250.*




FIGS. 1–16

they grew, the colonies usually developed slowly and remained small. Such colonies were situated almost entirely within the agar, and only a few large bodies developed on the surface (Fig. 3). The granules that had penetrated the agar tended to be large, or a mixture of small and large (Fig. 2). Adaptation to abundant growth usually required long cultivation. At present, L cultures isolated 6 months ago from the four strains of cocci grow moderately well on agar and very poorly in broth.

*Growth of the old L forms on agar and in broth.* Well-spaced colonies of the old well-established L strains grew to a large size, 1 to 2 mm or more, on agar media with a 1 to 2% concentration of agar containing 3% NaCl (Fig. 4, 5). As long as growth was abundant, the concentration of NaCl did not appear to affect the appearance of the colonies. They had the characteristic structure and appearance of L colonies, with the center consisting of small granules embedded in the medium and a periphery on the surface of the agar consisting of large bodies. The colonies in densely inoculated areas remained small and after a few days were largely autolyzed. At the periphery of inoculated areas and of isolated colonies, consecutive crops of small secondary colonies developed under the edges of the original colonies (Fig. 5). These secondary colonies consisted of granules embedded in the agar (Fig. 6). In this way some colonies continued to increase in size for several months and to induce growth in transplants.

In transplants made from 1- or 2-day-old cultures by streaking inverted blocks of agar containing the colonies on fresh agar plates, large bodies as well as smaller forms of decreasing size, could be seen on the surface of the agar (Fig. 7). After 1 to 2 hr of incubation at 36 C, the large bodies increased in size and their shape became irregular. At the periphery of large bodies that lay isolated on the surface, condensations appeared, and small granules started to grow embedded in the agar This was apparent after 1 hr of incubation (Fig. 10, 11). Growth starting from the small granules present in the transplants has not been observed. Where the inoculum was dense and the large bodies were crowded together (Fig. 9), they increased in size, but the appearance of small granules was delayed by several hours.

It was apparent that the granules grew from the large bodies and were not accidentally attached to them. Large bodies transplanted from a 24-hr culture were refractile, had sharp boundaries, and took the stain well. After some growth, they became irregular and condensations appeared at their periphery, which was the first indication of the growth of granules. Multiplication of the granules produced a small colony embedded in the agar within a few hours (Fig. 12). The origin of granules from large bodies was even more clearly apparent in transplants from broth cultures.

Decreasing or increasing the concentration of agar markedly influenced growth. Large colonies developed on the surface of soft media containing about 0.7% agar. The structure of these colonies was similar to those on harder agar, but the organisms embedded in the medium were considerably larger. However, small granules were present at the extending edge of the deep growth, and the arrangement of the granules was similar to that seen in harder agar. This suggested that the way in which they multiplied was similar to that in harder agar, but the tendency to grow to larger size was greater. On the surface of hard medium with 3.5% agar, no multiplication was observed, but the organisms grew to very large bodies.

After inoculation within agar of the usual consistency (1 to 2%), fewer L colonies developed than in transplants made on the surface. Growth consisted of organisms similar to those in the embedded part of surface colonies. When inoculated into softer media with 0.4 to 0.7% agar, many colonies started to develop, but most of them remained small and consisted of a few large bodies. These did not develop to the size of those seen in broth or gelatin (Fig. 17). In 0.2 and 0.4% agar, large colonies developed, consisting of large bodies of moderate and uniform size with some admixture of granules (Fig. 14). The way in which multiplication occurred was not established.

It was difficult to obtain growth of the L forms in broth after their isolation (8). The old L strains started to grow immediately in broth inoculated either from broth or from agar. They grew equally well with and without animal serum. A swirling mucoid mass was produced more abundantly in a shallow layer of broth in a flask than in a tube. The mucoid mass consisted of large bodies ranging in size from a few microns to 50 $\mu$ or more, most of them in various stages of vacuolization and degeneration (Fig. 17). In a well-grown 24-hr broth culture, there were many medium-sized and larger bodies, which were nonvacuolated and refractile, and which took a deep stain. After several days, most large bodies were highly vacuolated, foamlike, and not stained. A few nonvacuolated large bodies remained in such autolyzed masses, and on transfer to agar growth was obtained from broth cultures several months old. Multiplication of small granules similar to that observed within agar medium was not seen during the cycle of development in broth cultures. How-

ever, vacuoles within the large bodies often contained a few or masses of small or quite large granules (Fig. 19, 21). These were highly refractile and were stained like the full part of the large bodies. In some large bodies, the granules were not in the vacuoles, but the nonvacuolated areas or the whole large bodies were transformed to a dense, darkly stained group of granules of various sizes (Fig. 18). These structures appeared to be single large bodies in which the granules developed, and not colonies of granules. They had the smooth boundary and the shape of large bodies, and the structure of a partly vacuolated large body was retained. The granules were inside the cell envelope. Transplanted to agar, the granules either started to grow immediately into the agar and to produce a large colony, or they grew at first on the surface to a dense group of large bodies. Growth into large bodies occurred also when such structures were transferred to fresh broth. This process, the transformation of a large body into a mass of viable granules within the cell envelope, probably occurrs also under other conditions and with other L forms. It has been observed most clearly in broth cultures of the L forms of gram-positive cocci.

When transplanted from broth to agar, the foamlike large bodies that did not contain full and stainable parts produced no growth. The large bodies that were full or were only partially vacuolated produced two types of growth. In most cases, small granules started to grow within 1 or 2 hr of incubation from several sites or occasionally from the whole periphery of the large bodies, penetrating into the agar (Figs. 22, 23). Typical colonies on agar resulted from this form of growth. In some instances, there was an increase in size of the fully stained periphery of large bodies, with division or fractionation into several smaller large bodies. Later, growth of small granules started from these.

In transfers from broth to broth, multiplication of small granules has not been observed, in contrast to results observed on agar. Only the development of granules inside the large bodies and the enlargement of the large bodies followed by their vacuolation and fractionation into smaller ones were observed (Fig. 20). This process was well advanced within a few hours of incubation at 36 C, and continuation of growth was rarely observed after 1 day.

Multiplication of small granules within the agar differed, insofar as it was visible with the light microscope, from the division of bacteria. Growth in the agar seemed to occur as small elongated forms at the ends of which condensations developed, as at the periphery of large bodies. Between these condensations, the structure disappeared and double granules were produced which are characteristic of both L forms and mycoplasmas (12, 17).

*Growth on solid media other than agar.* Coagulated serum, gelatin, starch, silica gel, and membrane filters were tested. Growth occurred on all these media. The structure and development of colonies, except for those on membrane filters, differed considerably from those of colonies on agar. The transferred organisms grew to large bodies, sometimes of very large size. Very few of these multiplied, and their mode of multiplication was similar to that seen in broth and in the S2 L type colonies observed with *Serratia* on agar (1). The large bodies became irregularly larger with protrusions and they broke up into fragments. Multiplication of small granules was not seen, and the medium was not invaded. Colonies grew slowly, but after incubation for several weeks occasional colonies were quite large and consisted of enormous large bodies, mostly vacuolated, as in broth cultures. Exceptionally, in a few batches of coagulated sera, the medium under a few colonies was invaded with small granules as on agar, and these colonies assumed the appearance of agar colonies. In these cases, also, multiplication started in the form of large bodies, and the embedded center developed only in a few colonies.

Media solidified with 10 to 30% gelatin were studied. The transferred organisms grew to very large size, extending on the surface in several directions. The edges of the extensions were often very thin and finely divided (Fig. 25, 26). The medium was not penetrated. On 30% gelatin, a few of the large bodies divided, and colonies were produced which enlarged for several weeks (Fig. 24).

Observations with various L forms on membrane filters have been previously described (7). Multiplication of L forms of the cocci has been observed on filters with pore size of 0.2 $\mu$ or larger placed on appropriate agar media. In these, the organisms grew into the pores of the filter and produced colonies as on agar, with an embedded center and a periphery on the surface consisting of large bodies. This growth differed from that on agar in that the embedded growth did not extend as small granules, but as irregular, interconnected masses, or irregular, branching filaments. On membrane filters (Millipore Filter Corp., Bedford, Mass.) with a pore size of 0.1 $\mu$ or smaller, the transferred organisms did not multiply, but increased in size, with some growing up, as on gelatin, to very large irregular forms with multiple extensions. Examination of young cultures on filters with a larger pore size showed that condensations were produced at the irregular

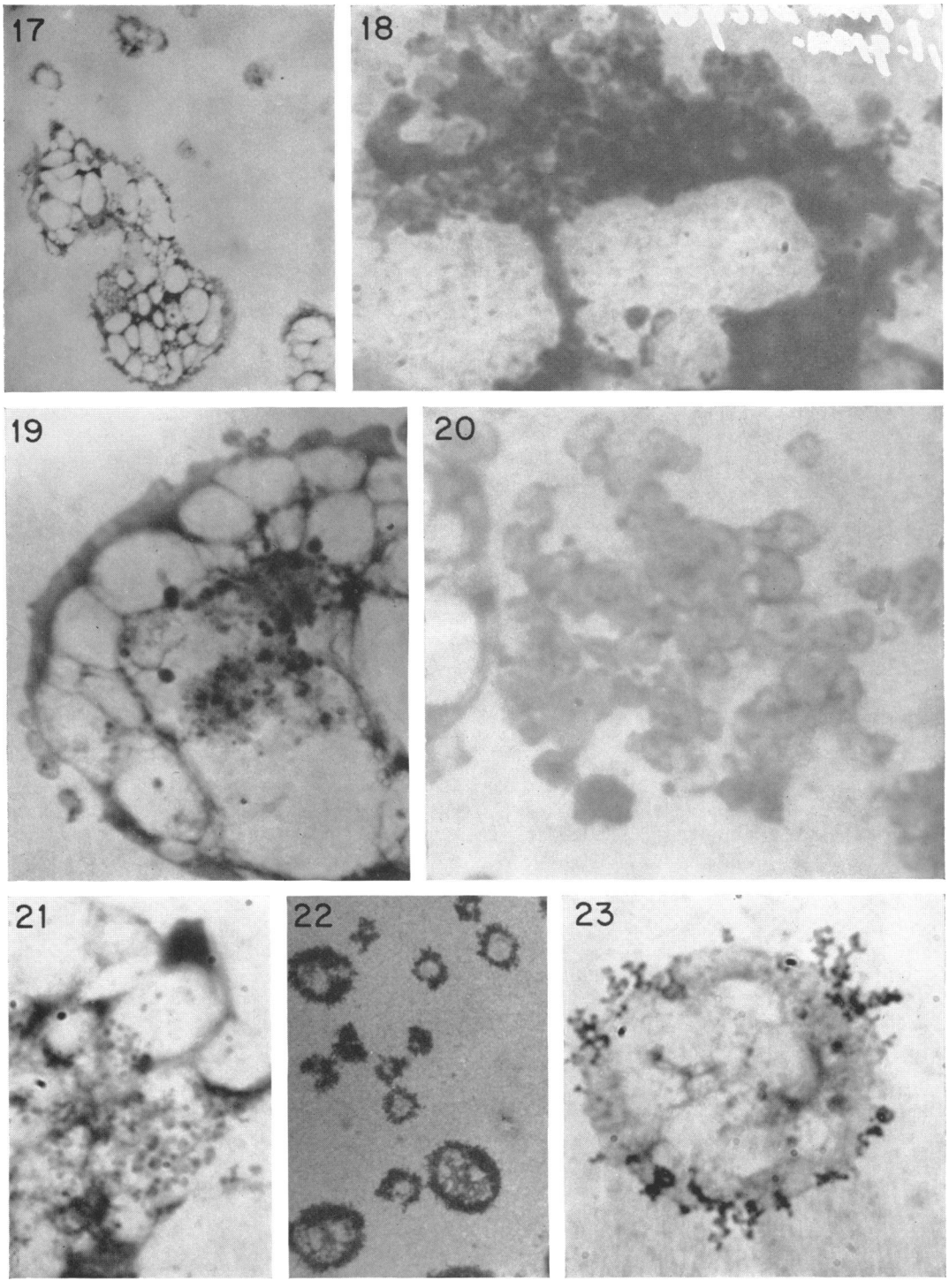



FIG. 17–23. *Large bodies of strain GL8 L form grown in broth containing 3% NaCl and their development after transfer to fresh broth or agar. All photographs made from dried stained agar preparations.* × *2,250, except for Fig. 17 and 22.*

edges of the large bodies. From these, growth of short filaments started into the pores of the filters, and produced the colonies (Fig. 15). Continuation of growth and multiplication was possible only where the organisms were surrounded by the structure of the filter. When the organisms grew through the filter, they extended into the agar medium on which the filters were placed in the form of small granules, as usual.

*Variations of the cultures.* Alterations of the cultures after adaptation to abundant growth have already been described. Further alterations continued over the course of several years. One was characterized by the abundant development of secondary colonies under the periphery of well-developed colonies (Fig. 5). Recently, it was observed that in cultures after several days of incubation many very small colonies developed among the large colonies (Fig. 13). This could be seen best at the edge of the inoculated area. Transplants made from these small colonies in most cases grew in the same way as those from regular colonies, and in older cultures they underwent autolysis at the same time as the larger colonies. The larger colonies and most of the tiny ones were produced by similar organisms. A few of the tiny colonies in all four L strains of cocci were different in appearance and continued to increase in size for several weeks after the large colonies were autolyzed (Fig. 27). It was possible to obtain growth from them in transplants, and for 1.5 years they have remained unchanged in their properties (Fig. 28). *We have designated this form of growth as LX.* Agar blocks of the old L cultures of the cocci were transferred to broth containing 0.5% NaCl and high concentrations (10 to 30%) of gelatin, dextran, or polyethylene glycol. The usual L forms did not grow in these media, but, in transplant to agar, pure cultures corresponding to LX grew out. When isolated large colonies of streptococcal and staphylococcal L forms were subcultured, no LX could be obtained from them.

Several properties of the LX cultures resembled those of mycoplasmas. Growth was slow. Colonies often did not increase in size for several days and consisted of very small granules (Fig. 29). The periphery of the colonies usually was made up of small granules instead of large bodies. The similarity to PPLO was even more marked in sections of these organisms examined with an electron microscope (*unpublished data*). These organisms differed from mycoplasmas in that they required increased salt concentration for growth. In addition, in contrast to mycoplasmas, colonies became quite large (1 to 2 mm) after long incubation, and a thick, mucoid confluent growth developed in heavily inoculated areas.

When this mucoid growth was inoculated heavily on the surface of agar media containing 0.5% NaCl and 10% horse serum, tiny colonies of faintly stained granules grew under the inoculum into the agar. This form of growth could not be propagated in transplants. When the plates were incubated for several weeks, however, a few large colonies developed in almost all plates. Colonies reached 2 to 4 mm in size and consisted of large bodies with little or no central growth into the agar (Fig. 31). The large bodies multiplied on the surface of agar by irregular growth and segmentation (Fig. 32). When present, the central growth embedded in the agar consisted of quite large, deeply stained granules. A moderate growth of similar colonies was obtained in subculture on plates with 0.5% NaCl. If planted on agar with 3% NaCl, they produced large colonies within 24 hr with the same morphology as on agar with 0.5% NaCl (Fig. 30). The presence or absence of penicillin exerted no influence on the cultures. *We have designated this form of growth as LX 2.* During the year and a half that LX forms have been carried with 3% NaCl, LX 2 colonies have not been obtained on the plates, although they grew much better and faster with 3% than with 0.5% NaCl. In contrast, they developed in most transfers from LX to plates containing 0.5% NaCl. Observations made to date, including serological studies, have not established the origin of LX and LX 2 nor their derivation from the cocci. The properties of these cultures correspond to L forms and the resemblance of LX to myco-

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FIG. 17. *GL8 L form in broth containing 3% NaCl. Small and very large vacuolized bodies.* × *400.*

FIG. 18. *Broth culture as in Fig. 17. The stained part of the vacuolized large body is transformed to small and larger granules.*

FIG. 19. *Large vacuolized body with granules of variable size free in the vacuoles.*

FIG. 20. *Large bodies transferred from broth to fresh broth. After 3 hr of incubation, they are broken into groups of round large bodies of medium size.*

FIG. 21. *Part of a vacuolized large body with small granules in the vacuoles.*

FIG. 22. *Vacuolized large bodies transferred from broth to agar after 3 hr of incubation. The large bodies are surrounded by a thick growth of small darkly stained granules embedded in the agar.* × *250.*

FIG. 23. *Large body, as in Fig. 22, after 1.5 hr of incubation on agar. Small elongated granules start to grow from the full periphery and embed themselves into the agar.*

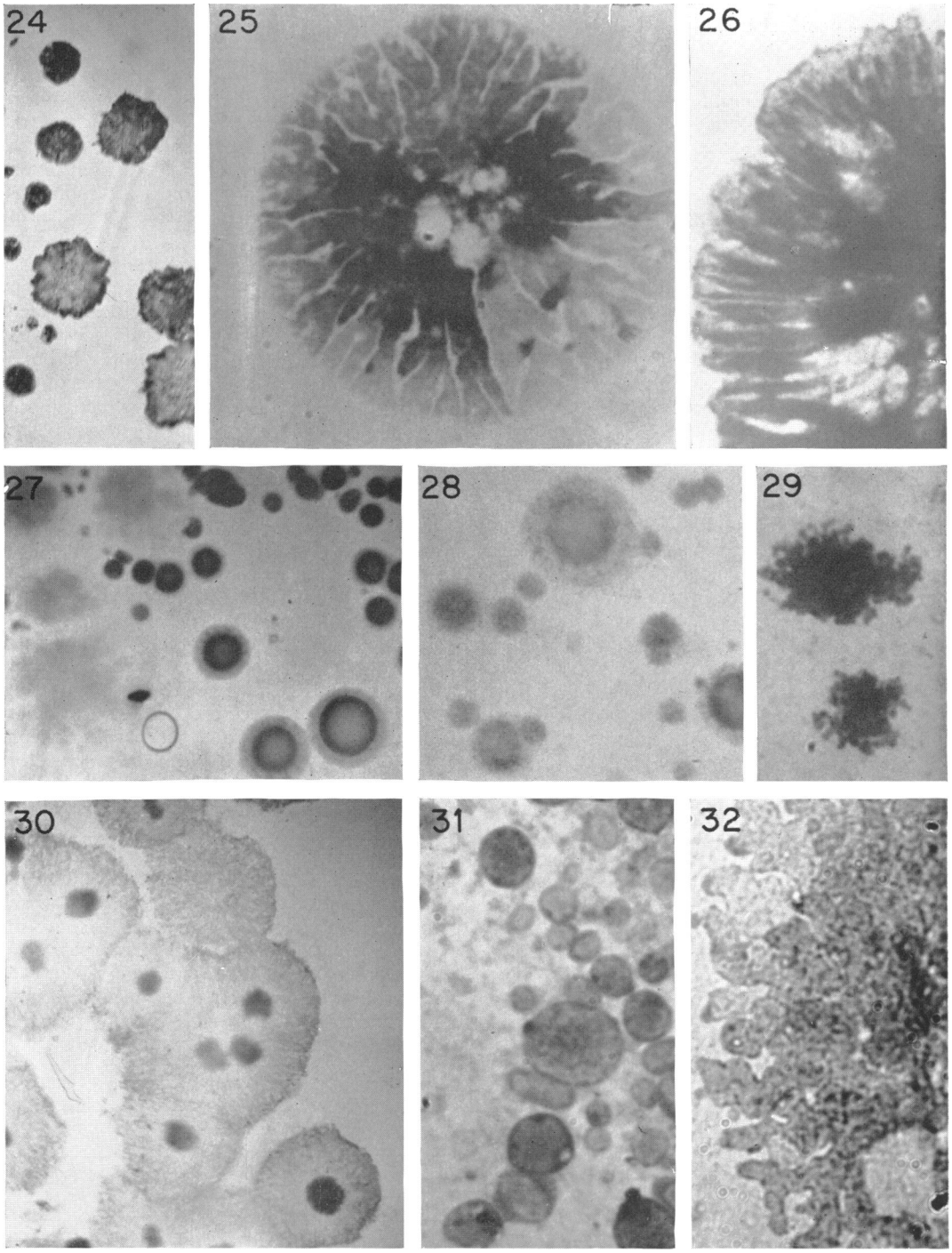


FIG. 24–32. *All photographs on this plate were made from L forms of Straphylococcus except Fig. 29, which is of Streptococcus GL8. Photographs of cultures on gelatin were made from stained impression preparations. Fig. 30, wet stained agar preparation; others, dry stained agar preparations.* × *2,250, unless otherwise indicated.*

FIG. 24. *Colonies of Staphylococcus L on 30% gelatin.* × *250.*

FIG. 25 and 26. *Growth of vacuolized large bodies on 30% gelatin transferred from broth, extending on the surface as a thin branching sheet with irregular edges with vacuoles and granules of different sizes.*

plasmas in some respects gives considerable interest to them.

## DISCUSSION

Since more observations on L forms pertinent to the problems studied in this paper are soon to be published, only a few remarks will be made here on certain properties of the L forms that were most clearly apparent in the L forms of cocci. Probably of most interest are those relating to the reproductive processes of the large bodies and the development of viable granules inside of them.

The occurrence of large bodies in bacterial cultures has been known since the beginning of bacteriology, but reproductive processes were first observed in them in 1930 (11). They have been regarded as dying organisms without biological significance. This is still the opinion of several authors concerning the large bodies developing in cultures of bacteria and of L forms (9, 16). However, their role in the multiplication of L forms is apparent. In old agar and broth cultures of the L forms of cocci, a few large bodies usually remain filled with cytoplasm and stainable. As long as such bodies are present in the cultures, growth will develop on transplant, and it can be seen that growth starts from the intact large bodies. In transplants of young cultures also, growth starts from the large and not from the small organisms. In addition to producing small granules in agar the large bodies may multiply in broth or on the surface of some solid media as such.

The development of bacteria inside large bodies produced from bacteria and in large bodies of B-type L colonies has been observed previously (2; *unpublished data*). This process occurred regularly in a strain of *Streptobacillus moniliformis* (3). That the bacteria in these instances and the granules in the L forms of cocci developed inside the large bodies was apparent, as no bacteria or granules were seen in the cultures outside of them. These observations, which may be important for the study of the structure of L forms and of bacteria, have thus far not been repeated by others.

The manner in which large bodies participate in multiplication allows speculation concerning their structure. When they produce other large bodies, they first extend irregularly in various directions and then segment. The extensions may be narrow and branching, as observed on gelatin or membrane filters. The detached pieces initially may be comparable in size to the bacteria, but they soon enlarge and reproduce bodies comparable to those from which they originated. On the surface of agar media, on the other hand, small granules start to form at multiple sites on the periphery of the large bodies. Sometimes the whole periphery breaks up into growing granules. This suggests that the large bodies have many small centers of growth which, when detached, are able to survive and multiply. The development of viable granules within large bodies suggests that under some conditions these foci acquire full structure, not only at the surface of the large bodies but also inside them.

The ready transition of large bodies into granules and vice versa, the presence of all gradations in size between the small and large forms in the cultures, and the influence of the physical state of the culture medium on morphology and mode of multiplication all suggest that the small and large forms are essentially similar organisms without specialized biological functions. On the other hand, the growth of granules starts usually from condensations developing on the periphery of large bodies. Similar condensations are often visible at the ends of small rodlike granules multiplying inside the agar. This suggests that multiplication may be introduced by certain structural changes which are similar in organisms of all sizes.

The great influence of the physical state of the medium on growth and morphology is not surprising, because the L forms lack the physical influence of the rigid cell wall of bacteria that regulates the size, shape, and probably also the mode of division. Adhering to a surface or being embedded in the gel structure of agar or in the pores of membrane filters serves as a substitute in some respects for the functions of the rigid cell wall.

Another factor that plays a role in the initiation of growth is the influence that the organisms exert on one another, probably by the diffusion of metabolic products. Multiplication of single

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FIG. 27. *Culture (1-week-old) of Staphylococcus L on horse serum-agar with 3% NaCl. The regular colonies are autolyzed and appear as shadows. A new crop of the different (LX) colonies have developed* × *100.*

FIG. 28. *LX colonies grown in transplant 2 days.* × *250.*

FIG. 29. *LX colonies (2-day-old) of Streptococcus GL8. Impression preparation; stained with Safranin.*

FIG. 30. *LX2 from Staphylococcus. Incubation for 1 day on agar containing 3% NaCl.* × *100.*

FIG. 31. *Organisms of LX2 immediately after transfer from agar culture to surface of agar.*

FIG. 32. *Edge of young colony of LX2. Irregular growth and segmentation.*

small granules has not been observed on the surface of agar, but small granules grow out from the large bodies and continue to grow in contact with others as they embed themselves in the agar to form the developing colonies. Hijmans (*personal communications*) estimated that the smallest single L form of streptococcus capable of producing progeny on the surface of agar has a diameter of 1.5 $\mu$, a size much larger than that of organisms growing in the young colonies. The development of consecutive crops of secondary colonies at the periphery of single large colonies suggests a similar influence. On the other hand, the crowding together of large bodies appears to retard the development of granules, and growth of transplants does not occur in a wide area around actively growing colonies. The influences that help or hinder growth may be similar to those observed with acid-fast bacteria (10).

The multiplication of small granules has not been observed in broth or on the surface of agar after adaptation of the L forms of cocci to abundant growth. This occurs in old cultures of *Proteus* and *Salmonella* L forms. The study of the variations of the L forms of the cocci has remained incomplete, as the relation of the cultures designated as LX and LX 2 to the cocci thus far could not be determined.

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