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**OBSERVATIONS SUGGESTING THE DEVELOPMENT OF STREPTOCOCCI
FROM PLEOMORPHIC FILAMENTOUS GRAM NEGATIVE BACTERIA†**

In the course of studying a freshly isolated strain of *Mycoplasma laidlawii*, a single small bacterial colony developed on a plate among the mycoplasma colonies. It attracted attention as the mycoplasma grew to large satellite colonies around it. In transplants made from the bacterial colony, tiny colonies developed, consisting of thin Gram negative rods and filaments which soon became pleomorphic and autolyzed. This organism was designated 1GN. On a plate inoculated with this organism, a large white micrococcus colony developed, probably as a contaminant from the air. Around the coccus colony large satellite colonies developed, but most of these did not contain the Gram negative organisms or mycoplasma. They consisted of Gram positive streptococci varying considerably in morphology in different colonies. This paper contains the description of this observation and studies resulting from it. Since the 1GN was in many respects similar to filamentous Gram negative bacteria usually present in the human throat, studies were made with such organisms to explore whether some of these would produce phenomena similar to that observed accidentally. No connection was apparent between the mycoplasma and the 1GN.

MATERIALS AND METHODS

The bacterial strains studied were: The Gram negative bacterium (1GN) indicated in the introduction, and in addition, eighteen strains of Gram negative filamentous bacteria isolated from the human throat. The plates from which these strains were isolated were received from the routine bacteriology laboratory of the hospital, and were marked to contain *Hemophilus hemolyticus*. These strains are designated as H.H., but no attempt was made to check their classification. All strains were isolated by us and studied immediately after isolation.

The air coccus that produced satellite growth in the culture of 1GN consisted of medium-sized uniform round cocci which stained strongly Gram positive. These cultures were different from the Gram positive pleomorphic organisms that developed in the cultures of the Gram negative bacteria and no pleomorphism or alteration of

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morphology or staining was seen in them in successive transplants. Accidental contamination of microscopic preparations with the air coccus, which occasionally occurred, was therefore immediately apparent. Several other colonies of similar micrococci found as contaminants were also tested for their ability to produce satellite growth.

To assure the similarity of transplants to be examined under different conditions, the following procedure was used: one colony or a small amount of the confluent growth was picked up with the loop and smeared and thoroughly mixed on a small area of the plate. Different areas of the plates or different plates were inoculated with the same loop from the originally smeared area. This procedure was also useful in obtaining cultures of isolated colonies.

The media employed were those used routinely in the laboratory: nutrient broth and agar, either plain or with horse serum or horse blood added (BAP); in a few cases, 10% Dubos oleic albumin complex (Difco) was added to the medium¹; 30% gelatin in nutrient broth was also used. Several other media were tested but were not useful in these studies. These included media containing fresh yeast extract² and high concentration of NaCl or sucrose and media with low nutrient content that is more similar to the natural habitat of bacteria than the usual media. The plates were incubated at 30°-32°C.

For microscopic observation, both wet and dry permanent stained agar preparations were used^{3,4} and preparations made with Klieneberger's agar fixation technic.⁵ Slight modifications of these methods are described in a previous paper.⁶ When the cultures are examined, it is important to be able to make permanent preparations of the growth in its natural arrangement within a few minutes. Without such preparations comparison of cultures obtained at different times and on different media is not possible. Photographs offer no substitute in such pleomorphic cultures for the permanent preparations. With the method used, only a part of the culture, that present in the excised piece of agar, is examined, and it is conceivable that slight admixture to the culture might not be noticed. This difficulty is eliminated by making several preparations from different areas of the culture.

The Gram staining procedure of Hucker and Conn⁷ was used and the reliability of the method was tested in impression preparations made with Klieneberger's agar fixation technic.⁵ The centers of large-sized colonies were not decolorized, but at the periphery the Gram staining was clear-cut. In order to see in the impressions the organisms present at the center of the colonies, in making the preparations the large colonies were streaked on the surface of the agar. Autolyzed colonies were decolorized after several days of incubation.

To avoid errors in making and handling large numbers of cultures and preparations, the two authors used the same cultures and worked with similar technics, but independently. All experiments were made by one person, including the transplanting, examination, and preservation of the cultures and the making, staining, and examination of the microscopical preparations. The cultures were sealed and kept for long periods in the cold room to make later comparisons and tests possible. The 1GN was noticed and isolated from the original plate by Dr. Pachas, who also studied the influence of different Gram positive cocci on this organism. The observations described were made by Dr. Dienes, who also made the photographs.

RESULTS

A few small colonies of thin, pleomorphic Gram negative rods developed on blood agar plate (BAP) in the transplant from the original colony of

1GN. Single colonies were transferred to blood agar plate on April 2 (see Table 1); again, a few small colonies grew, some of which were streaked over the uninoculated surface of the same plate (April 6). The colonies consisted of small or somewhat longer thin rods and filaments, large round bodies, and small well-stained granules (Fig. 3). After 24 hours' incubation the center of the colonies was autolyzed, and growth progressed at the periphery. Growth in broth was poor, and was only slightly increased by the addition of hemolyzed blood. Anaerobiosis accelerated and increased pleomorphism and autolysis of the cultures.

On April 10, a large white colony consisting of usual-sized uniform round cocci was noted on the plate from April 6, among the small colonies of 1GN. The colonies grew to much larger size as satellites around the contaminant (Fig. 4). To our surprise, the morphology of satellite colonies was altered. In addition to colonies of unaltered Gram negative organisms, the majority of satellites consisted of Gram positive bacteria with variable morphology. Some consisted of very small coccobacillary forms (Fig. 8), some of larger rods corresponding in size to *Salmonella*, and some of large long rods, sometimes growing as segmented filaments, which continued to grow as cocci (Fig. 5). Other colonies contained long chains of large round cocci (Fig. 6) of regular size or very small cocci sometimes without chain formation. Also, groups of very large round cocci were present (Fig. 7). These various Gram positive organisms in the satellites could not have been produced by contamination from the central colony of the contaminant, the cocci of which were regular-sized organisms corresponding to micrococci. The satellites were not in contact with the contaminant and outside the satellites only colonies of the Gram negative bacterium were present.

TABLE 1. DEVELOPMENT OF GRAM POSITIVE STREPTOCOCCI IN CULTURES OF 1GN

March, 1968	Growth of a single small bacillary colony on a BAP culture of <i>Mycoplasma laidlawii</i> . Transplant of this single colony yielded a few colonies of pleomorphic Gram negative rods (1GN).
April 2	Single colonies of 1GN transferred to BAP. Growth of a few colonies of Gram negative rods.
April 6	Single colonies streaked on the uninoculated area of the same BAP.
April 10	Satellite colonies seen around an "air coccus" contaminant. The satellites contain Gram positive streptococci of variable morphology.
April 13	1GN transplanted from an area of the April 6 culture not exposed to the air coccus. The transplant is incubated with and without exposure to the air coccus.
April 16	Growth of satellite colonies containing Gram positive streptococci in the area surrounding the air coccus colonies. No Gram positive organisms seen in 1GN not exposed to the air coccus.

On April 13, one-half of a blood agar plate was inoculated from the April 6 transplant of the Gram negative bacterium not exposed to the influence of the air coccus, and a streak of the air coccus was made across the inoculated area. The other half of the plate was inoculated in a similar way from the large satellite colonies. On April 16, Gram preparations were made from the cultures, using imprints made by agar fixation.

In the area inoculated with the unchanged Gram negative organisms far from the growth of the air coccus, only small colonies of the Gram negative rods were present. The colonies are illustrated at low and high magnification (Figs. 1, 2, 3). In the same inoculation, near the air coccus, Gram positive organisms grew out of the same type as indicated in the April 6 transplant. In the transplant made from the satellite colonies near the air coccus on the April 6 plate, Gram positive and Gram negative colonies developed that also showed satellite growth near the streak made with the air coccus.

From the cultures made on April 6 and 13, 20 well-isolated colonies were transferred to blood agar plate. They were picked from three and ten-day old cultures selecting large, flat, slightly spreading colonies, situated as near as possible to the air coccus. Unaltered Gram negative organisms developed in ten transplants. In the others, Gram positive colonies developed alone or together with Gram negative colonies. The prevalence of Gram negative cultures resulted from the scarcity of well-isolated colonies in proximity to the air coccus. In transfer of a large bacterial colony from the April 6 plate only mycoplasma developed, and in two other transplants mycoplasma was mixed with the bacterial colonies. The gram positive organisms in the descendants of single colonies were of the same type as in the satellite growth of the 3rd and 4th transfers. The small coccobacillary forms and large cocci in chains were prevalent and often both were present in the descendants of single colonies.

The greatest variety was seen in the descendants of one single colony, designated as N, isolated from the April 6 transplant. No growth was apparent in the transplant of colony N after 24 hours' incubation. After 2 days' incubation, one small colony developed and was surrounded by very small satellite colonies. After 72 hours several such small colonies appeared, surrounded by tiny satellite colonies. Stained agar preparations and impressions for Gram staining were made from the culture, and also transplants and smears for staining separately from the central colony and the satellites. An excised block of agar with the central colony and the satellites was also streaked out on BAP.

The central colonies consisted of thin, small rods and coccobacillary forms which stained Gram negative. In the transplants made from the central colonies small Gram negative and Gram positive coccobacillary forms grew.

The satellites were produced by long chains of small or large cocci, some Gram negative and some Gram positive. The centers of satellite colonies consisted of a few large bodies and large cocci (Figs. 13-15). Outside the satellite growth a few large bodies were visible on the medium similar to those in the center of the satellites.

The growth developing in the smear of the agar block containing a central colony and satellite is of interest. Examined after one day of incubation, the smear from the central colony could be recognized as masses of large, refractile and darkly stained large bodies, most of them decolorized in Gram staining (Figs. 11, 12). Adjoining this area were growing colonies of streptococci of the same type as the satellite colonies. Some of the small colonies consisted only of large bodies as in Fig. 13. Farther away on the surface of the agar, as in the first transplant of colony N, large bodies were present, singly or in small groups, with occasional growth of a streptococcal chain from them.

The colony N was transplanted after 10 days' incubation. Whether this colony consisted originally of the Gram negative rod (1GN) or of Gram positive organisms is not known. It contained very few viable organisms which produced colonies of the Gram negative bacterium only after long incubation. Small groups of well-stained large bodies similar to those which developed in the transplant made from the Gram negative central colonies were scattered over the inoculated area. The microscopic preparations indicated that these large bodies grew into streptococci and produced satellites under the influence of the developing Gram negative colonies. The small Gram negative rods in the central colonies had the tendency to transform into coccobacillary forms or to grow into the special type of large body in transplant. Twelve isolated colonies were picked from the first and second transfers of colony N. Only Gram positive cocci were recovered from them in transplant and no Gram negative filamentous rods.

The descendants of the 20 single colonies isolated from the transplants of April 6 and April 13 and of 12 colonies descended from colony N were maintained and studied for about six months. The descendants of 10 colonies remained in the original Gram negative form of 1GN. Upon exposure to the influence of the air cocci which were first used, no change in morphology was observed. One of us (W. Pachas) tested the culture of 1GN with many air coccus colonies found as contaminants. Upon exposure to one freshly isolated contaminant, streptococci again developed in colonies of 1GN as secondary growth (Fig. 10). Another contaminant induced slight growth of similar colonies. These observations indicate not only that the growth as streptococci in the colonies of 1GN was variable but the influence of coccus strains to induce such growth also varied.

The morphology of the Gram positive organisms in the first transplant from the April 6 and April 13 cultures varied in the same way as in the original plates near the air coccus. Transitions between the different types of Gram positive organisms were more often seen in the transplants than in the two original plates. The small coccobacillus form, partly Gram positive, partly decolorized, was prevalent in most (Figs. 16, 17). After many transfers only these small organisms and large pleomorphic streptococci, often in long chains, were present. Transition between these two forms was suggested in this case also. In several cultures at the edge of the inoculated area, the colonies of the small coccobacilli grew to larger size, and short chains or groups of the large Gram positive cocci developed at their periphery. This is illustrated in photographs 19 and 20.

The small organisms produced *alpha* hemolysis on BAP. Slowly they grew to large colonies under which secondary colonies developed. These contained small bacillary forms embedded in the agar. Secondary colonies often develop under large bacterial colonies of various species. Some of these cultures grew well without change in morphology with about 200 units of penicillin per ml. in the media. They grew well in plain or serum broth as long chains of small coccal forms (Fig. 18). Transferred from broth to agar, they returned to the elongated coccobacillary form, producing only a few short chains. In the colonies and in the chains large fusiform or diphtheroid-like organisms or large cocci were occasionally present, indicating that the tendency to produce such forms was not lost. In some cultures the organisms were strongly Gram positive, but usually they were partly decolorized.

The colonies of the large streptococci grew slowly and remained small. They did not produce hemolysis. They grew in long chains in broth and on agar, and usually they were strongly Gram positive. The proximity of the culture of the small coccobacillary organisms produced satellite growth of the large streptococci.

In many tests without osmotic protection or with 0.5 M NaCl or sucrose, no large bodies and no L forms were produced by penicillin from 1GN or from any of the Gram positive organisms recovered from its cultures.

Observations with Gram negative filamentous bacteria isolated from human throats

These organisms were isolated in three different periods from throat cultures obtained from human patients in late April and early May 1968, in August 1968 and in March of the following year. Several colonies were transferred from these plates to BAP and only those cultures were studied which, after one or two days' incubation did not contain Gram positive cocci. The results with these cultures are not as clear-cut as those obtained with

1GN, but Gram positive cocci similar to those obtained from the culture of 1GN developed in several cultures. These organisms are easily available, and their study may give decisive evidence concerning the origin of Gram positive cocci developing in their cultures.

The organisms isolated from the throat plates were Gram negative and they decolorized easily. The morphology was very variable in every strain. The colonies after 24 hours' incubation usually consisted of quite large or smaller rods or segmented filaments (Figs. 21, 22). After longer incubation the organisms usually became very pleomorphic with swellings developing on the rods and filaments and they produced small-sized round forms, large bodies and granules (Figs. 23, 26). All these forms took strong staining and were mechanically resistant as in 1GN and in the altered L form of *Proteus*.⁹ Autolysis of the cultures was prevalent in all strains and the development of secondary growth of pleomorphic organisms. It is of interest for the present study that in most strains, after several transplants, a few colonies or the whole culture transformed into small, uniform granules 0.5-1.0 μ in size. The granules continued to grow in this form (Figs. 24, 25). In some cultures the granules were mixed with short, slender rods, but usually the granules multiplied without admixture of rods. The granules were Gram negative and did not grow into the medium.

From each of the ten plates inoculated from throat specimens in the Spring of 1968, several colonies were isolated that were suspected of being *H. hemolyticus* (H.H.). Of 13 isolates selected for further study, three proved to be mixed with streptococci after 24 hours' incubation and were discarded. In six strains, the development of Gram positive cocci resembling streptococci was observed in some experiments. Five strains designated as follows were studied intensively: 39, 89-1, 89-2, 89-3, and 89-4. In strain 89-4 the development of Gram positive cocci has not been seen. Gram positive cocci were seen to develop in two of the remaining five strains. One of these became contaminated with diphtheroids; in the other cocci were seen only in the second transplant and not later.

Young colonies of strain 39 usually consisted of medium-sized regular bacilli (Fig. 12) which after two days' incubation became pleomorphic (Fig. 26). In older cultures with advancing autolysis, masses of medium-sized round forms and large bodies were produced (Figs. 27, 28). All these variable forms were Gram negative and exposure to a freshly isolated coccus, which induced strong satellite growth in a strain of *H. influenzae*, did not influence their growth and Gram staining. The third transplant of the culture 17 days after isolation autolyzed after 24 hours of incubation, and only a few large bodies and small very pleomorphic secondary colonies consisting of a few large bodies and small granules remained intact. Transplants from

this plate and all their later descendants consisted of small granules similar to those seen in some transplants of almost all H.H. strains studied, but the granules were more or less Gram positive. The Gram staining was more strongly positive in colonies growing in proximity to the air coccus. The colonies and the organisms in them were similar to the preponderant organisms among the descendants of 1GN, and no further change in the culture, and no growth of Gram negative filaments were seen during four months of observation. The autolysis and alteration of the culture of strain 39 occurred without using penicillin or interfering with the development of the culture in any other way.

From the throat culture marked 89, four H.H. colonies (89-1, 2, 3, 4)

TABLE 2. TRANSPLANTS FROM STRAIN 89, ISOLATE 1 (89-1)

<i>Days of incubation</i>	<i>Exposure to air coccus</i>	<i>BAP</i>	<i>BAP with oleic albumin complex</i>	<i>Plain agar</i>
1	without	no cocci	Gr. pos. cocci from one bacillary colony	Slight growth of Gr. neg. rods; many tiny colonies of Gr. pos. cocci. Periphery de-colored
	with	Gr. pos. cocci from one bacillary colony	Gr. pos. cocci in several bacillary colonies	Better growth of Gr. neg. rods; Gr. pos. coccus colonies with less decolorization
3	without	Bacillus colonies autolyzed; Gr. pos. cocci from one colony	Bacillary colonies not autolyzed; no cocci	Bacillary colonies autolyzed, many with Gr. pos. cocci
	with	Secondary growth in autolyzed bacillary colonies; many have some Gr. pos. cocci.	Bacillary colonies autolyzed; many contain Gr. pos. cocci	Bacillary colonies autolyzed; many contain Gr. pos. cocci and a few large streptococcus colonies
6	without	Autolyzed colonies; no Gr. pos. cocci.	Few Gr. pos. cocci mixed in with autolyzed colonies	Colonies all autolyzed; a few mixed with Gr. pos. cocci
	with	Autolyzed colonies; no Gr. pos. cocci.	Few Gr. pos. cocci mixed in with autolyzed colonies	Like without air coccus; in several autolyzed colonies, large pleomorphic cocci in chains

The Gram negative organisms developed in all transplants after 24 hours of incubation.

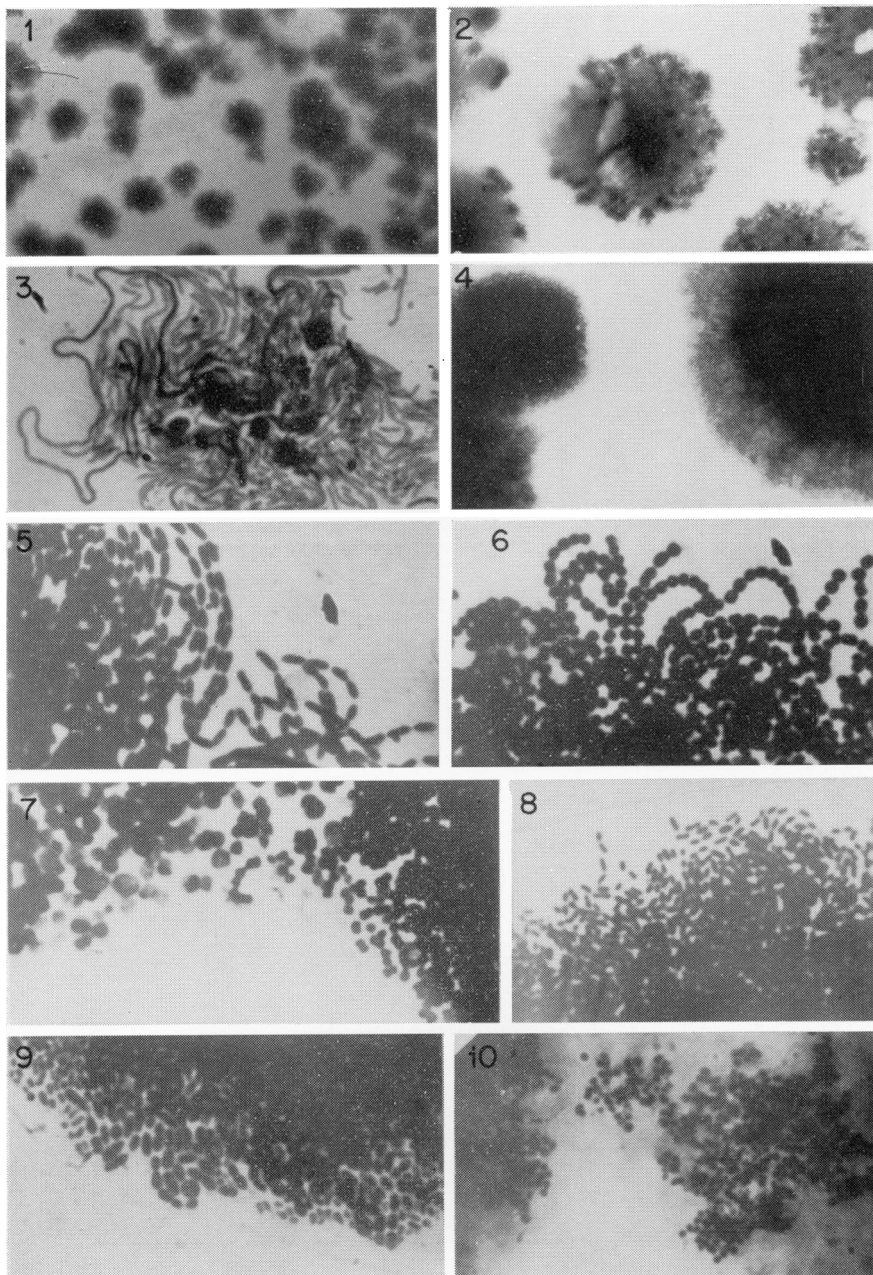


PLATE I

FIG. 1. Small colonies of 1GN on BAP from an area far from the air coccus after three days of incubation. $\times 250$.

FIG. 2. Autolyzed colony of 1GN with darkly stained tiny secondary colonies of Gram negative rods. $\times 250$

FIG. 3. Young colony of 1GN with high magnification. $\times 2250$

FIG. 4. Three-day-old satellite colonies in culture of 1GN in the area near to the air coccus. $\times 250$

FIGS. 5, 6, 7, 8, 9. The periphery of Gram positive satellite colonies shown in Fig. 4 with high magnification. $\times 2250$

FIG. 10. Two small Gram positive secondary colonies in 1GN several months after its isolation in proximity of an air coccus. $\times 2250$

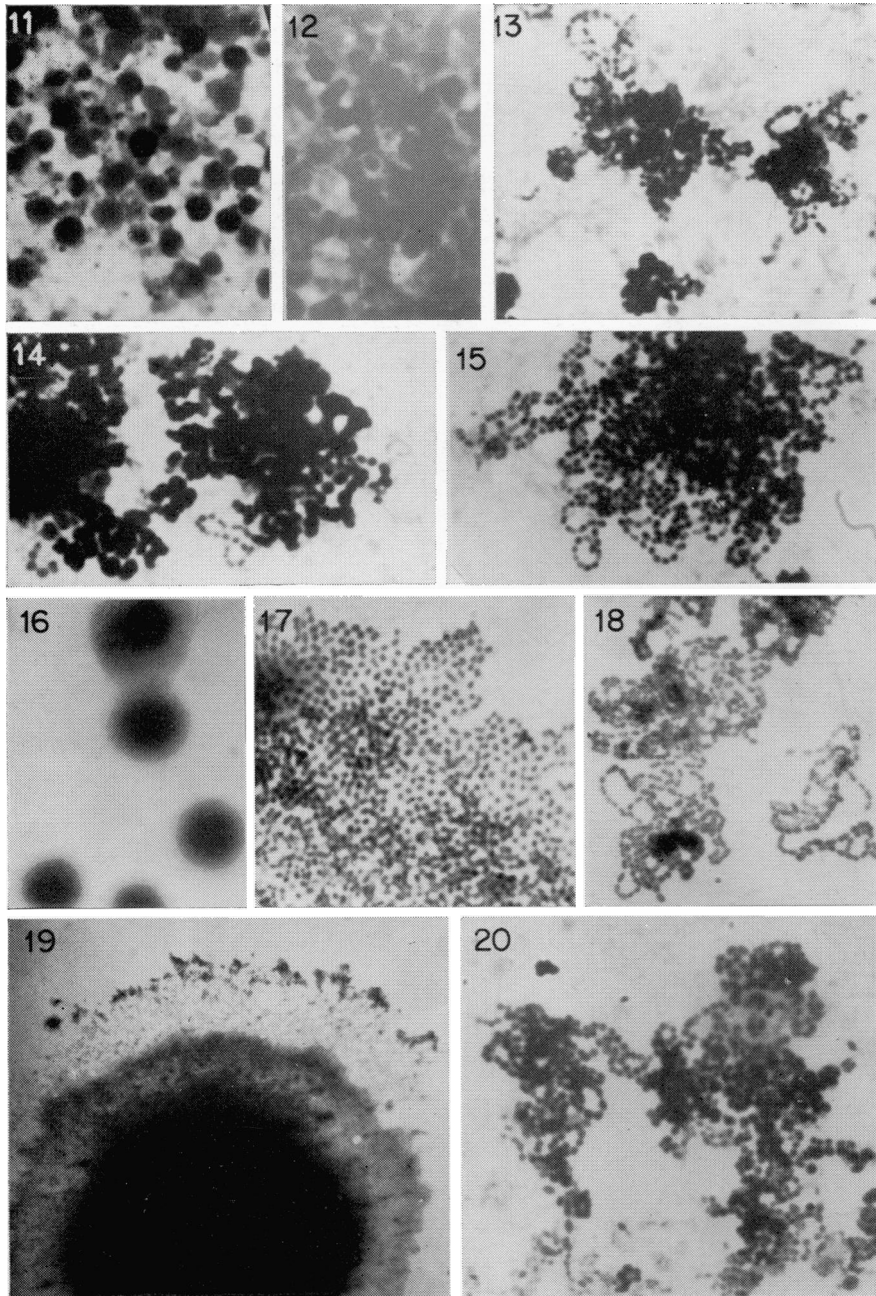


PLATE II

FIGS. 11-15. Descendants of colony N of 1GN.

FIGS. 11, 12. Large bodies after 24 hours of incubation developing from the center colony of satellite growth in the transplant of colony N. $\times 2250$

FIGS. 13, 14, 15. Satellite colonies in the transplant of colony N and in the consecutive transplant. $\times 2250$

FIGS. 16-20. The predominating type of Gram positive cocci and of their colonies after several transplants.

FIG. 16. Colonies on BAP after one day of incubation. $\times 250$

FIG. 17. The periphery of a colony with high magnification. $\times 2250$

FIG. 18. Chain formation in broth culture. $\times 2250$

FIGS. 19-20. A large colony at the edge of inoculated area.

FIG. 19. Low magnification indicates development of chains and groups of long cocci at the periphery of the colony. $\times 250$

FIG. 20. With high power the morphology of the large cocci is visible. $\times 2250$

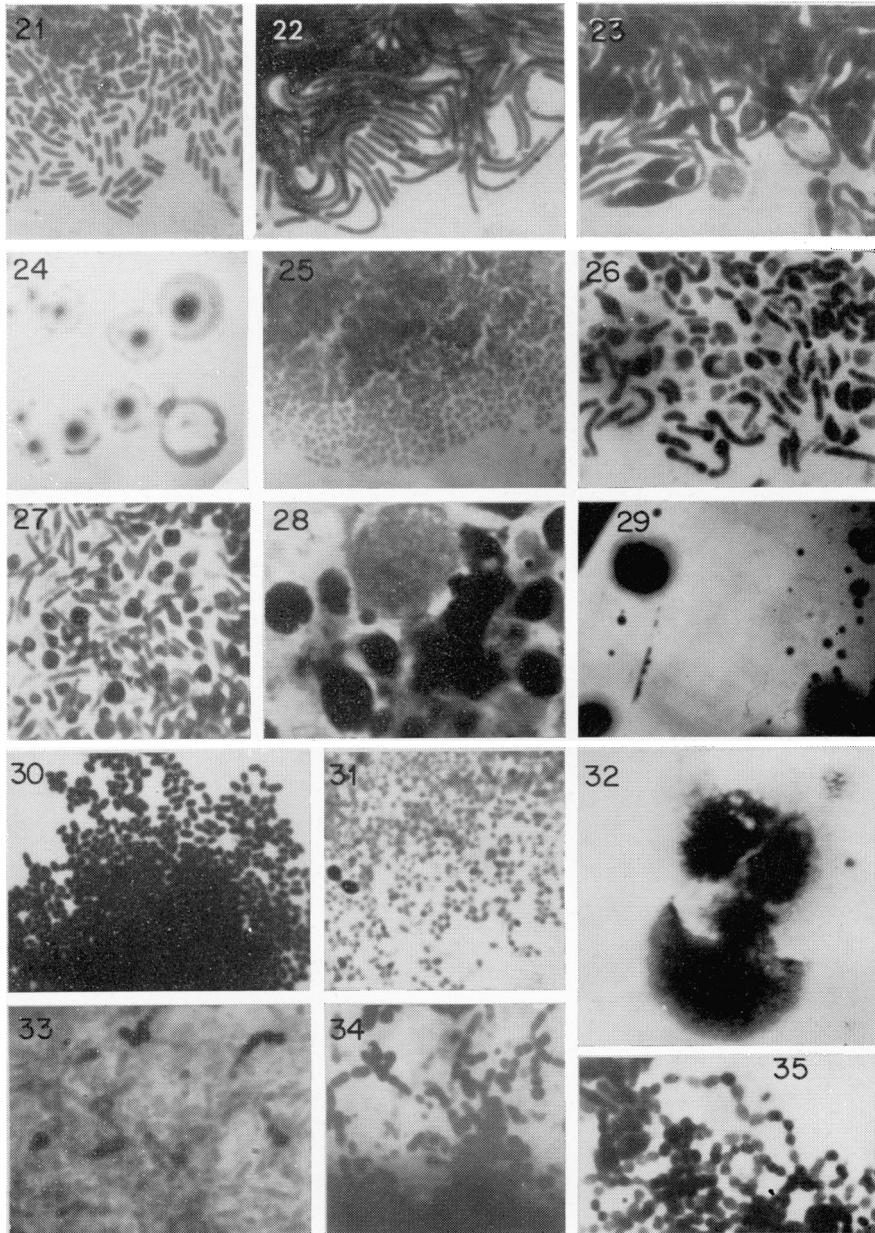


PLATE III

FIGS. 21, 22. The usual form of H.H. on BAP after 24 hours of incubation. $\times 2250$

FIG. 23. Edge of a colony of strain 89 after two days of incubation. $\times 2250$

FIGS. 24, 25. Growth of H.H. in forms of very small Gram negative coccoid organisms. In Fig. 24 continuation of growth in such forms on the periphery of an autolyzed colony with low power ($\times 85$); in Fig. 25 the small organisms with high power ($\times 2250$)

FIGS. 26, 27. Pleomorphic Gram negative organisms in cultures of H.H. strain 39. $\times 2250$

FIG. 28. Groups of large bodies in autolyzed culture of strain 39 from which Gram positive coccoid forms developed. $\times 2250$

FIG. 29. Colonies of small Gram positive and negative coccoid organisms derived from strain 39. $\times 85$

FIGS. 30, 31. Strongly Gram positive and mixed Gram positive and Gram negative organisms in colonies illustrated in Fig. 29. In Fig. 31 few large Gram positive organisms are mixed with the culture.

FIG. 32. Development of five colonies of Gram positive cocci in an autolyzed large colony of H.H. strain 89 and such growth starting also from the periphery of the colony. The outline of the autolyzed colony is marked with ink. $\times 130$

FIGS. 33, 34, 35. Gram positive cocci in autolyzed colonies of H.H. strain 89. In Fig. 33 the pleomorphic cocci are almost entirely autolyzed.

were isolated on BAP. After two days' incubation only Gram negative rods were seen in the cultures. Transplants were made from the two-day old cultures of isolate 89-1 on 3 plates: BAP, and BAP and plain agar containing oleic albumin complex. Part of the cultures was exposed to the air coccus. The growth developing on the plates is presented in Table 2. Gram negative rods developed in all transplants. After one day's incubation Gram positive coccoid forms were not seen on BAP without exposure to the air coccus; exposed to the influence of an air coccus, Gram positive cocci developed in one colony of 89-1. On BAP with oleic albumin complex the growth of Gram positive coccoid forms was observed in one H.H. colony without the influence of the air coccus; with the air coccus, they were observed in several H.H. colonies. After 3 days' incubation Gram positive coccoid forms were mixed in with many of the H.H. colonies on all three plates. The coccoid forms were not seen after six days on the BAP without the oleic albumin complex; with the oleic albumin complex in the BAP, they did not develop into colonies or form chains. On the plain agar tiny colonies of Gram positive coccoid forms developed after one day's incubation among the colonies of the Gram negative organisms. When exposed to the air coccus, a few of the Gram positive colonies grew to larger size after three days. After six days, both the Gram negative and the Gram positive organisms were autolyzed, but in a few colonies exposed to the air coccus, chains of large Gram positive cocci remained.

The four cultures obtained from single colonies of throat plate 89 were transferred after eight days of incubation to BAP and plain agar, both containing oleic albumin complex and incubated both with and without exposure to the air coccus. The cultures were examined after 1, 2 and 5 days. Gram negative rods developed in all transplants. No Gram positive cocci were seen in any of the cultures of 89-1 and 89-4, either on BAP or on plain agar. In culture 89-2, no cocci were seen on BAP. On plain agar exposed to the air coccus, after two days, Gram positive cocci started to grow at the periphery of a few large colonies of the Gram negative rods. In culture 89-3 on BAP, Gram positive cocci were seen in the autolyzed bacillary colonies only after five days' incubation and exposure to the air coccus. On plain agar, after two days, a few colonies of Gram positive cocci were present, both with and without exposure to the air coccus. After five days no cocci were seen without exposure to the air coccus. With the air coccus a few colonies of Gram positive cocci were present and cocci were also mixed with autolyzed colonies. The colonies of cocci were similar to those seen in strain 39.

In the following experiments descendants of strain 89-1 were studied. They were transferred from the plain agar culture of the first experiment

in which Gram positive cocci were well developed. Most cultures obtained from single colonies of the bacillus remained free from cocci. In those in which cocci developed, they were seen on BAP after 24 hours' incubation. We did not succeed in obtaining a culture like 1GN which was apparently free from cocci and which produced cocci only under the influence of the air coccus.

The Gram positive coccoid organisms in descendants of strain 89 were in most cases similar to those obtained from strain 39. When chains of large cocci developed, they were pleomorphic and subject to autolysis (Figs. 33-35). They were different in morphology and Gram staining from the streptococci usually seen in throat cultures. In successive transplants of cultures containing cocci, we have not seen the development of streptococci of the usual appearance. It is remarkable that with a few exceptions the Gram positive cocci were seen to grow from the periphery of the bacillary colonies or to develop inside the autolyzed colonies. In Fig. 32 a large autolyzed bacterial colony is visible with five small and large coccus colonies developing within it, and one large coccus colony growing from the periphery.

In two series of experiments, one started in August, and the other in March, single colonies were isolated from nine throat cultures. Five strains were not contaminated with streptococci and were studied for periods of three to five weeks. Development of Gram positive cocci was not seen in them, but in all a few colonies or the whole culture were transformed to small Gram negative coccoid forms, and remained in this form in successive transplants. Whether the difference between the first and later experiments indicates the fluctuation of the properties of the cultures at different times of the year or whether it is due to some unnoticed differences in the media or in the whole procedure of the experiment is undecided.

DISCUSSION

The studies described in this paper grew out of an unexpected observation. We make no claims that this and subsequent observations are sufficient to prove the derivation of the cocci from the Gram negative rods. Our impression is that there is no reason to believe that such a transformation is impossible and observations suggesting it deserve careful study. Some of the significant developments in bacteriology such as the recognition of phages, L forms, sexuality, and the production of penicillin resulted from accidental observations. The main difficulty in continuing the studies described is to find appropriate strains. They may be found if attention is called to them; the filamentous Gram negative bacilli in human and animal throats may possibly provide such strains.

Important morphological variations in bacteria seem less improbable at present than some years ago. Variations in biochemistry, in genetic makeup and in structure, as well as the transmission of strain characteristics between bacteria are well known. The authors are less reluctant than most bacteriologists to consider the possibility of morphological transformations. They have seen such transformation in connection with L forms.⁹ The transformation of a Gram negative pleomorphic filamentous organism to Gram positive streptococci was suggested in consecutive blood cultures in a case of severe septicemia observed some years ago.⁸

It is not possible to review and evaluate the literature of this subject here. It seems to be well established by recent studies that the morphology and growth requirements of bacteria may differ considerably from the usual properties of the species immediately after their cultivation from pathological processes. Reference is made only to two careful studies. Studying the aphthous lesions of the mouth, Barile⁹ obtained cultures which immediately after isolation consisted of various Gram positive and negative organisms, including Gram negative large, round forms and filaments. After several transplants, the cultures were identified as *Streptococcus sanguis*. The initial cultures which Charache¹⁰ isolated from Whipple's disease presented similar pleomorphism. The methods used in these studies to determine the morphology of the pleomorphic cultures did not give as clear information as the method used in the cases studied by us. The study of the morphology of bacteria in pathological lesions and in initial cultures obtained from them with appropriate methods may give much new information on the morphological variability of bacteria.

In the case of 1GN it is well supported by the observations that the Gram positive cocci developed from organisms which were present in the culture of the bacillus. Contamination on the plate would not explain their growth as satellites on two different plates or the variable morphology of the Gram positive organism. They were morphologically different from the air coccus and, while they were being studied, cocci similar to the air coccus were not seen to develop from them. The observations with H.H. are not as clear, but the development of cocci in several cases exclusively inside the autolyzed bacillary colonies and the characteristics of the cocci similar in different strains of the rods and in different experiments seem to exclude contamination on the plates.

Development of the cocci from organisms present in cultures of the Gram negative bacilli does not in itself prove the derivation of the cocci from the bacilli. Hidden sources of error may always be present. In the case of 1GN it is apparent that the cocci which grew out of it were not present in the culture in such a form. The H.H. and possibly also 1GN, originated from

the mucous membranes. As an example, it is conceivable that streptococci were present on the mucous membranes in an altered Gram negative form similar to those observed in some bacteria during the reproduction of full bacterial structure from L forms.⁹ A small admixture of such organisms and their consequent proliferation in connection with the rods would not be detected by the methods employed and, under appropriate conditions, they could regain full structure. If transformation of bacteria to cocci occurs, it is more likely that definite proof of it will be obtained by biochemical and genetic studies and not by morphological studies alone. However, several features of the observations described suggest that such transformations occurred, and although they cannot be regarded as decisive, the observations deserve further studies.

The 1GN and the strains of H.H. studied were in a stage of great variability and the cultures autolyzed within a few days. In contrast to various autolytic strains, L forms were not produced either spontaneously or with penicillin. The small and large round bodies and granules were similar to those seen in altered L cultures as transitional forms during reconstruction of full bacterial structure.⁹ Cultures consisting entirely of small coccoid forms and multiplying in such forms were a regular part of the morphological variability of H.H. strains. These cultures differed from the prevalent Gram positive cultures obtained from 1GN and H.H. apparently only by being Gram negative. It is possible that the variability of structure extends also to the bacterial structures responsible for Gram staining. The morphology of the Gram positive colonies of 1GN was very variable immediately after their production. It was less variable in successive transplants, but it continued to conform to streptococci. The coccal forms obtained from H.H. originally had variable morphology, although to a smaller degree, and in several experiments were autolyzed together with the Gram negative bacilli. Broth cultures of the descendants of H.H. were not studied, but the agar cultures were similar to the descendants of 1GN. The coccal forms both in 1GN and H.H. were variable in Gram staining in the same colony and the Gram staining was stronger in cultures exposed to the influence of the air coccus.

Most suggestive for the derivation of the Gram positive cocci from the Gram negative rods are the initial variability of the cocci in 1GN, the similarity of the preponderant coccoid forms in the descendants of all strains and their difference from streptococci usually seen in the human throat. It is not likely that accidental contamination could produce such uniform results in several experiments made during a period of two years.

It may be disturbing to some readers that the most significant observations concerning the development of cocci from 1GN could be made only

for a short period after the isolation of the culture. Bacteria exhibit not infrequently a similar change of properties following isolation. They are often pleomorphic, produce large bodies, and some also produce L forms spontaneously. This variability disappears in most cases after a few transplants or in a few days or weeks. The study of bacteria immediately after isolation from their natural habitat, and in an environment as similar as possible to their natural habitat may be necessary for the observation of morphological forms and processes which are not apparent in our cultures maintained on artificial media.

The influence which a certain bacterium exerts on the growth of the same organism or on an organism of a different species is of importance. In the case of *H. influenzae*, several bacteria may provide a necessary ingredient for growth. Transduction by DNA and by phages is a more complicated process. Bacillus Y²¹ makes possible or greatly enhances the reversion to bacterial forms in L cultures of *H. influenzae* and in some L forms of *Proteus* and streptococcus. It has already been mentioned that both the influence of the bacteria and the susceptibility for such influences may vary in different developmental stages of the cultures. The study of these processes is of great interest. They may play an important role in the reproduction and continuation of the observations described in this paper. The satellite growth, not only around the air cocci, but of the streptococcus around the Gram negative bacilli and of the pleomorphic large streptococcus around the small streptococci is remarkable. It indicates that something which was necessary for growth of the large streptococci or greatly helped it was absent in the media and could be provided by two different strains that were descendants of the same colony from which the large streptococci also originated.

No attempt was made to study the influence of the media, although it was apparent that with H.H. the development of cocci was favored when plain nutrient agar was used. The autolysis of the central part of the colonies of these bacteria, while growth progressed in the periphery, indicates that the availability of nutrients and the diffusion of metabolic products are important factors for growth, and for the preservation of the organisms. The study of growth requirements and biochemical variation of various growth forms may be necessary to clarify the mechanism underlying the alteration of morphology.

SUMMARY

In a culture of a filamentous pleomorphic Gram negative rod, large colonies consisting of pleomorphic streptococci developed as satellites around a contaminant colony of "air" cocci. In an experiment made a few days later,

Gram positive streptococci developed again in the culture of the Gram negative rods as satellites around the culture of the air coccus. Later experiments with this coccus yielded negative results but when numerous air coccus contaminants were tested, in two instances a few streptococcus colonies developed in the culture of the Gram negative bacterium. Without exposure to an air coccus, the development of Gram positive cocci has not been seen in it. The development of Gram positive cocci has also been seen in several strains of filamentous Gram negative bacteria isolated from the human throat. All these Gram negative rods were very variable and pleomorphic, and they autolyzed within a short time. The Gram positive cocci isolated from them were similar in many respects to each other and different in appearance from the usual streptococci of the human throat. Although these observations are not sufficient to prove the derivation of streptococci from the Gram negative bacteria, they suggest it, and they deserve further study.

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