A STUĐY OF MIÇROBIC VARIATION IN A YELLOW PIGMENT-PRODUCING CÖCCUS

FRANCIS E. COLENG

Department of Bacteriology and Immunology, University of Minnesota, Minneapolis

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INTRODUCTION

A consideration of present views relative to the nature of microbic variation shows several differences of opinion. This study was undertaken in order to obtain further evidence in regard to the nature of variation, which for the past decade has become of interest not only to students of medical bacteriology and public health, but also to workers in the fields of agricultural and industrial bacteriology.

Microbic variation at the present time is a more or less universally accepted phenomenon. For an excellent and extensive review of the subject, including a complete survey of the earlier literature, the reader is referred to Hadley's work (1927). An extensive bibliography of the published works since Hadley's survey may be found in my original manuscript.

This work was undertaken for the purpose of studying the cultural, morphological, biochemical, and to a limited degree, the immunological phases of variation in a recently isolated yellow pigment-producing coccus. The study includes a consideration of the growth cycle of this coccus which showed a motile phase. The work is limited to a discussion of the methods of obtaining the variants, the character of the changes observed, the possibility of filterable forms and a growth cycle showing changes in the morphology of the organism.

1 Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the Graduate School of the University of Minnesota, June 18, 1934.

2 Library of the University of Minnesota, Minneapolis, Minnesota.
CHARACTERIZATION OF THE ORGANISM

The organism, a yellow pigment-producing coccus, was isolated from an apparently normal throat. When examined after 24 hours growth on an agar plate the colonies resembled Micrococcus citreus. However, after 48 hours the colonies became granular and in 4 to 5 days were definitely rough and showed mycelium (× 75) radiating from the periphery. Impression stains of the mycelium showed it to be made up of bundles or rows of coccus-like elements.

Morphology. The organisms were spherical, 0.8 to 1.0 µ in diameter, arranged in clusters (plate 2, fig. 1). Their morphology was studied when first isolated and again one and two years later. No marked changes were observed during that period. The organisms were Gram-positive and showed no irregular forms. Negative staining showed clear cut spherical forms in irregular clusters resembling staphylococci. The cocci were non-motile and did not form spores or capsules.

Agar plate. The streak plates were incubated at 37°C. for 24 hours and then placed in diffuse daylight at 22°C. for 24 hours. The colonies were circular, dome-shaped, 0.5 mm. in diameter, cadmium-yellow, and glistening with entire edges. The edges of the colonies became fimbriate in 4 days and later showed an abundant mycelium radiating from the periphery (fig. 2). The colonies were granular after 48 hours incubation and became definitely rough after 4 to 5 days.

Agar slant. Abundant, finely granular, cadmium-yellow; filiform, moist, entire edge (fimbriate after 4 days). The stab growth was moderate, beaded and extended to the bottom of the tube.

Nutrient broth. Moderate growth with uniform turbidity. The culture produced an abundant yellow flake-like pellicle which settled to the bottom of the tube forming a yellow viscid sediment which did not disintegrate on shaking.

Potato. Abundant, dry, yellow, spreading growth. The medium became slightly darkened.

Biochemical reactions. Acid but no gas was produced in glucose, sucrose, maltose, levulose and glycerol. Lactose, mannitol,
raffinose and salicin were not fermented. Litmus milk became acid in 3 days, but no further changes were noted after 14 days. Infundibuliform liquefaction was observed in gelatin after 7 days, becoming stratiform in 2 weeks. Indol was not formed and acetylmethylcarbinol was not produced. Moderate reduction of nitrates and marked diastatic action was shown. On amino-nitrogen assimilation medium, as used by Hucker (1924), the strain showed moderate growth and produced acid.

PURE CULTURE METHODS

The following methods were used for obtaining pure cultures of the organism: (1) serial plate method, (2) pour plate method, and (3) single cell isolation.

Twenty-four hour broth cultures were streaked on nutrient agar plates, pH 7.2. These were incubated at 37°C. and after 24 hours a well isolated colony was streaked on a second plate. After 24 hours incubation a well isolated colony from this second plate was streaked on a third plate. Colonies from this third plate were used in the experimental work.

All isolations were duplicated using pour plates. Some investigators believe that this method gives greater assurance that the colony arises from a single cell. The single cell method of Nirula (1928) was also used.

The serial plate method seems to be the best for isolating coccii in pure culture. It is reasonable to believe that a series of platings will eliminate filterable and similar forms by a process of dilution. In some cases plating was continued until all the colonies appeared uniform, and the microscopic examination of stained films from several colonies on each plate gave results that were identical.

METHODS OF OBTAINING VARIANTS

In this investigation no methods were used to induce variation other than by aging the cultures in nutrient broth, antiserum broth and broth filtrates. The usual amount of peptone (Difco), salt and meat extract was used, and the broth was adjusted to pH 7.0 to 7.2. The antiserum broth consisted of nutrient broth
to which was added 10 per cent homologous antiserum. All the media were incubated at 22°C. and 37°C. before they were inoculated.

In aging cultures duplicate flasks were inoculated at each transfer. One flask was used for the microscopic and cultural examinations each week; the duplicate was not opened until the particular experiment was completed, or until a change in the appearance of the organisms was observed in the first flask culture. By this method the chances for contamination were reduced since no flask was opened and then used for further inoculations. Furthermore, the microscopic and cultural examinations of the duplicate culture provided a control as to the results obtained from the first culture. Several sterile uninoculated broth flasks were incubated with each experiment and served as a check on the media used.

Agar streak and poured plates were inoculated in duplicate. In addition, air contaminant control plates were used when inoculating agar plates. These control plates were uncovered during the process of streaking, or pouring, and any colonies that appeared were compared with the colonies appearing on the series plates. In a number of cases control plates and plate cultures were kept for several weeks without showing signs of contamination, even though the plate cultures had been opened for the purpose of obtaining material for subculture and microscopic examination. The inoculated plates were not discarded after the initial observation, 36 to 48 hours after inoculation, but were studied over a period of 3 to 4 weeks, since it was found that interesting changes in colony structure frequently occurred after prolonged growth on agar plates. In order further to eliminate the chances of contamination, the aged broth cultures were streaked on agar (solidified in a horizontal position) in 32 oz. medicine bottles.

**CHARACTERIZATION OF THE VARIANTS**

Three types of variant were obtained by aging the yellow pigment-producing coccus (96) in liquid media, namely—a smooth yellow variant (96S), a rough yellow variant (96-1) and a small
rough filterable form (96FV). Intermediate variants were also observed.

**Variant 96S.** This smooth yellow variant occurred regularly on agar plates inoculated from antiserum broth cultures that had been aged for 14 to 60 days at 22° or 37°C. The colonies were smooth, raised, cadmium-yellow and glistening with entire edges. They were larger than the original colony (96) and unlike this type did not form mycelium. Figure 3 illustrates two smooth and two original rough colonies. The variant produced a uniform turbidity in nutrient broth, surface growth in the form of a

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Biochemical reactions</th>
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<td></td>
<td>ORIGINAL STRAIN</td>
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<td>Glucose</td>
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<td>Lactose</td>
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<td>Sucrose</td>
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<td>Maltose</td>
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<td>Milk</td>
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<tr>
<td>Gelatin</td>
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<tr>
<td>Diastatic action</td>
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<td>Nitrate reduction</td>
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* Coagulated and reduced. Note: + in the case of the fermentable substances indicates acid production without gas; in the other cases it indicates a positive reaction.

ring, and a grey-white viscid sediment which readily disintegrated on shaking. When first isolated the variant consisted of a pure culture of short Gram-positive rods. After repeated transfers on agar slants during the course of a year, coccoid, bacillary and filamentous forms appeared at different periods of growth. Biochemically the variant was practically inactive, as shown in table 1. The variant was readily transferable on agar and as far as its colony structure was concerned remained stable for 2½ years. We observed the reversion of this smooth variant to the original rough form after aging in nutrient broth for 3 months.
Variant 96-1. This rough variant was obtained by aging the original rough strain in antiserum broth for 30 days at 22° or 37°C. The colony had a wrinkled "wall" around the periphery, a somewhat wrinkled and granular center, and a mass of mycelium extending some distance from the periphery (fig. 4). The mycelium of the variant was more compact and its outward growth from the periphery was more extensive than the mycelium of the original strain.

Variant 96FV. This variant occurred on agar streak plates inoculated with nutrient broth cultures of the original organism aged at 37°C for 21 days. The colonies appeared after the plates had been incubated for 7 days, and were visible only with the microscope. Under a Leitz ultropak (×110) the colonies had a rough "wooly" appearance and showed a mass of rhizoid mycelium extending into the medium (fig. 5). Stained films showed a mass of filaments, short and long rods, and irregular diphtheroid forms. Repeated attempts were made to subculture these colonies to nutrient agar, glucose agar and blood agar without success. The colonies were not transferable on solid media, but were transferable in nutrient broth where they produced uniform clouding with pellicle formation. These small colonies contained filterable elements and are probably identical with Hadley's (1931) G colonies. This variant will be further discussed below.

FILTERABILITY EXPERIMENTS

In this investigation a search was made for filterable forms of the yellow pigment-producing coccus, or its variants. Cultures aged in nutrient broth and in antiserum broth for 3 to 6 months were filtered through Berkefeld filters V, N and W, and through Seitz filters. The first 15 cc. of filtrate were collected in large test tubes (inside the filter flasks) containing 15 cc. of nutrient broth. The filtrates were incubated at 37°C. for 24 hours. Only 2 filtrates out of 48 were contaminated. In the filtrates in which filterable forms occurred, clouding developed after 36 hours.

A modified Hauduroy (1929) technique was used to demonstrate the presence of filterable forms. Two loopfuls of the broth filtrate were spread over the surface of nutrient agar plates.
Three plates and a bottle flask were inoculated with each filtrate. These plates and flasks and uninoculated control agar plates were incubated at 37°C. for 48 hours and then at 22°C. for 10 days. Two loopfuls of sterile broth were spread over the agar surfaces of the plates and flasks. Without flaming the loop, the broth washings were transferred to a second series of plates and flasks and incubated in the same manner as the first set. If growth did not appear on the first series of transfers a second series of washings was made from the first set of cultures. Only two series of washings were made, since it appeared that the chances for contamination increased with each series. In the successful cases growth usually appeared on the plates inoculated with the first filtrate washings. In every case where growth was detected it was checked with the bottle flask cultures and with the control plates. The original broth filtrates also were examined microscopically to detect the presence of growth.

CHARACTERIZATION OF THE VARIANTS OBTAINED BY FILTRATION

Variant 96F. Variant 96FV was inoculated into 50 cc. of nutrient broth, incubated for 10 days at 37°C. and filtered through a Berkefeld N candle. After 36 hours growth at 37°C. the filtrate gave rise to a pure culture of Gram-positive branching filaments. After cultivation on nutrient agar for 4 weeks these filaments gradually changed into short rods and finally a coccus form, morphologically and culturally similar to the original strain. The change from the coccus to the filterable form and to the original coccus as well as the R to S variation are diagrammatically illustrated in plate 1.

Streak plates made from the filtrate of 96FV after aging for 3 weeks at 37°C. showed a pure culture of small cadmium-yellow colonies (96F). These colonies grew more slowly than those of the original organism (96), were smaller and of a more uniform size. This variant had the same biochemical reactions as the original organism with the exception of its behavior in milk, in which it showed acid formation, coagulation and reduction, whereas the original strain produced only acid. The growth on agar slants, on potato medium and in nutrient broth also re-
semblled strain 96, with the exception that in broth the variant produced a ring surface growth.

**Variant 96FW.** This white variant appeared on agar plates inoculated from a broth filtrate of variant 96FV which had been aged at 22°C. for 3 months. The streak plates showed small yellow colonies which resembled the original strain (96). Some of these small yellow colonies, after 7 days growth at 22°C., gave rise to white daughter colonies and white sectors. Subcultures from these areas on agar remained stable for over 2 years. This white variant differed from strain 96 by fermenting lactose and mannitol.

**Variant 96M.** Streak plates from the broth filtrate of 96FV showed small yellow colonies. After incubation for 2 weeks at 22°C., in diffuse daylight, the colonies showed a slight dark coloration when observed through the bottom of the plates. This central area gradually became larger and after 3 weeks colonies with well developed maroon centers were seen. At the end of 4 weeks the maroon color extended to within a few millimeters of the periphery (plate 2, fig. 6). Gram-stained slides from these colonies showed no characteristic bacterial forms with the exception of a few coccoidal "shell" forms (2.0 X 0.8 µ). The staining was uneven and showed both Gram-positive and Gram-negative properties. This maroon-colored variant was transferable in broth, but thus far we have not been able to obtain the same colony form by direct transfers to agar. The maroon color was not produced in liquid media.

In addition to the filtration experiments using variant 96FV, the original coccus was aged in antiserum broth and filtered through Berkefeld and Seitz filters. After incubation at 37°C. for 10 days small pin-point colonies developed on the plates and flasks inoculated with the first filtrate washings (Hauduroy technique). Gram-stained slides from these colonies showed irregular stained forms, filaments, short and long rods, diphtheroids and a mass of faintly staining cocci. These coccus forms looked like a "shell" which had lost the material which made up the cell substance. These forms, like those obtained from the filtrate of 96FV, were not transferable on solid media. Aged
nutrient broth and antiserum broth cultures of variant 96S and 96F were also filtered. No growth was obtained on any of the plates or flasks.

GROWTH CYCLE—MORPHOLOGICAL CYCLE AND MOTILE PHASE

The original culture (96) was kept through many test tube generations and was frequently tested culturally and microscopically. After being subcultured for about 2 years on agar slants the strain spontaneously changed from cocci to rods and filaments, a change apparently associated only with the morphology. In order to determine whether this change was a part of an orderly and natural growth cycle 30- and 60-hour growth studies were made.

For the 30-hour growth study agar slants, inoculated with the coccus form, were incubated at 37°C. and examined every 3 hours in Gram-stained slides, negative stained slides and hanging drop preparations. Congo-red negative staining, according to the method of Benians (1916), was especially useful for identifying the changes in form that occurred during the morphological cycle of this organism.

During the 30-hour growth period the organism showed a complete change from cocci to rods, to filaments, to rods, to coccolidial forms to the original coccus form,—a morphological cycle completed in 30 hours. The organism changed from the coccus to a short rod in about 6 hours. Observations after 9 hours growth showed branching filaments which gradually broke up into long rods after 12 hours growth. The rods gradually became shorter and after 27 hours had changed into small coccolidial forms. These forms changed into the typical coccus, arranged in irregular clusters, in 30 hours.

For the 60-hour growth study, agar slants were inoculated with the filamentous form, which had spontaneously developed after subculturing the original coccus on agar slants for about 2 years. Examinations made every 3 hours, according to the method used in the 30-hour study, showed that the filaments gradually changed into short rods in about 27 hours. After 39 hours growth only very short rods and coccolidial forms were observed. At the end
of 60 hours the short rods and coccoidal forms had changed to cocci. This morphological change from the filamentous to the coccus form is illustrated in plate 3. It is interesting to note that it required 60 hours for the organism to change from the filaments to the cocci while it required only 30 hours to complete the cycle from cocci to filaments, to cocci.

**Motile phase.** During the growth study motility was observed in some of the hanging drop preparations. Further observations in nutrient broth cultures of the original organism (96) and the smooth variant (96S) showed that the short rods and coccoidal forms were actively motile. The filamentous forms showed no true motility. It was observed that motility was present only during the 24- to 48-hour growth phase, with the greatest number of motile cells and greatest activity being shown after 36 hours growth at 22°C.

In order to confirm the motility observed in the hanging drop preparations the organisms were grown in a semi-solid medium similar to that used by Jordan, Caldwell and Reiter (1934). Semi-solid agar plates were inoculated with a 10-day agar culture of the original organism (96), a 48-hour culture of the same organism which showed a short rod to coccoidal form and a 48-hour agar culture of variant 96S. The plates were incubated at 22°C. for 3 days. The semi-solid agar inoculated with the coccus form showed all non-motile colonies, the plates inoculated with the coccoidal form showed 9 motile and 15 non-motile colonies, and those inoculated with the smooth variant showed 8 motile and 2 non-motile colonies. Six semi-solid medium plates were inoculated with each strain and the results were consistent in each case.

To study further the motility of these organisms flagella-stained slides were made of the coccus, short rod form and the filamentous form of the original rough organism (96) and the short rod form of the smooth variant (96S). The flagella stain described by Safford and Fleisher (1931) and modified by Jordan, Caldwell and Reiter (1934) was used.

The flagella-stained slides confirmed previous observations on hanging drop preparations and on semi-solid medium. No flagella were observed on preparations made from the coccus form
of 96 or any of the filamentous forms while flagella were found on the short rod or coccoidal forms (10 per cent). The majority (95 per cent) of the cells of the smooth variant, which consisted entirely of coccoidal forms, showed flagella (plate 2, fig. 7). Slides made from young cultures, less than 21 hours old, or cultures over 5 days old, showed no flagella. Throughout these experiments the smooth variant showed a greater number of motile cells than the original rough organism. Colquhoun and Kirkpatrick (1932) stated that many organisms developed motility on semi-solid medium while they are non-motile when grown in the ordinary fluid or solid media. Our slides were made from agar slants and not from colonies on semi-solid medium. We are therefore certain that the motility here observed cannot be explained by growth in semi-solid medium. The flagella-stained slides verify the fact that this organism has a motile stage, the coccoidal or short rod form.

**SERUM AGGLUTINATION REACTIONS**

Serum agglutination experiments were carried out with the original rough organism (96) and its smooth variant (96S) in order to furnish additional evidence on the relationship between R and S forms. A study was also made of the serological relationship between these R and S forms and *Micrococcus aureus*.

Antiserum was prepared from the R and S stains. Rabbits were immunized by injecting, intravenously, 1 cc. of a saline suspension of organisms grown on agar at 37°C. for 24 hours. The rabbits were given 7 injections at 5 day intervals. They were bled from the heart 5 days after the last injection and the serum was preserved with 0.3 per cent trikresol. In the agglutination tests the antigens were prepared by suspending the growth from a 24-hour slant in 0.25 per cent saline and adjusting the density to 3 on McFarland's nephelometer. In the test, 0.5 cc. of the antigen was added to 0.5 cc. of the diluted serum and incubated in a water bath at 56°C. for 4 hours and at 22°C. for 18 hours. The results of the agglutination experiments are shown in table 2. The original rough antiserum agglutinated the homologous organism in a dilution of 1:640, but did not agglutinate the
smooth variant or *Micrococcus aureus*. The smooth antiserum agglutinated the smooth strain in a dilution of 1:1280, but did not agglutinate the original rough strain or *Micrococcus aureus*. All of the control tubes were negative. There was no antigenic relationship between the original organism (96) and the smooth variant (96S), and between these strains and *Micrococcus aureus*.

**TABLE 2**

*Agglutination reactions*

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<td>1:2560</td>
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<td>Control</td>
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**DISCUSSION**

The present studies show that a coccus goes through a progressive morphological change from cocci to short rods to long rods to filaments and back again to long rods to short rods to coccoidal forms and to cocci. Novak and Henrici (1933) reported similar morphological changes in a yellow staphylococcus isolated from a filtrate of a broth culture of an actinomycete. The colony structure and cultural characteristics of their organism are essentially similar to the coccus described in this paper.

Other workers have reported changes in bacterial forms which simulate a morphological cycle. Wittern (1933) working with "*Microbacterium* mesentericus" found that rods and filaments divided into coccus-like fragments which developed into the normal form in liquid medium. Cunningham (1930–1931) observed that *Bacillus saccharobutyricus* (von Klecki) may produce coccoid,
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rod, dwarf growth and fungoid forms corresponding to the types of Löhnis and Smith. Henrici (1928) observed a series of morphological changes in various bacterial species to which he applied the term "cytomorphosis."

Mellon's (1919) study on a fuso-spirillary organism and his several other studies on microbic heredity display a similarity to our observations. He showed that the fuso-spirillary organism could produce various forms which showed transformations from one form to another; transformations between staphylococci and branching filaments and between diphtheroid forms and branching filaments. In 1927 he presented bacteriologic evidence for an in vivo life cycle that involved bacillary coccus transformations. The report shows a relationship between pigmented staphylococci and the long thread and bacillary forms that apparently dissociated in vivo. In another study Mellon and Jost (1926) showed that Bacillus alkaligenes can exist as a diplococcus and then be transformed into pure line bacillary and filamentous forms, variants which bred true and were serologically reciprocal.

We found no reference in the literature to a growth cycle transformation between various phases which differ not only in morphology, but also physiologically. The coccoidal and short bacillary forms showed motility and flagella formation, a characteristic not possessed by either the filaments or the true cocci.

In our work we present a pleomorphic cycle which forecasts the direction into which the culture can dissociate. Two of the forms, a filamentous and a short rod (S form) were stabilized and later reverted to the original coccus form. Mellon and Jost (1926) have shown that the colon-alkaligenes group may exist in a stabilized actinomycotic stage. Our growth cycle meets the requirements of Mellon's pleomorphic principle, "Each characteristic pleomorphic phase of a microorganism is capable of being stabilized as such, the culture thereafter being capable of multiplying into what were formerly pleomorphic forms." Mellon (1926) pointed out that the various phases of the pleomorphic cycle represent the ontogeny of the strain; when the phases are

* Personal communication.
stabilized through mutation they collectively represent the phylogeny of the strain. Winslow (1935) stated, "If a unicellular organism shows a definite series of morphological and physiological alterations in response to certain changes in environment which are likely to occur with reasonable frequency in its natural life we may call it a 'life cycle' if we wish. . .".

Mellon (1927) found that bacteria may reproduce by various methods besides transverse fission. He reported that transitions of a major type, such as between filaments and cocci and *vice versa* were brought about through the medium of reproductive forms, either filterable microgonidia or macrogonidia. Some of our observations indicate that the coccus reproduced by other methods than transverse fission. We observed bud-like structures on some of the filaments, on negative-stained slides, and deeply staining bodies in the diphtheroid forms of the G colony which resembled gonidia.

The various rough and smooth colonies were distinct types and reproducible, true to form, many times under varying conditions. Arkwright (1921) found that the differences between the various colony types were often indefinite. Two of our variants were of an unusual type, one with a pronounced "wall" structure around the periphery, the other a maroon-colored colony. Fleck and Elster (1932) in studying the variation of streptococci also obtained a rough colony with a "wall" around the periphery and a rust-colored variant.

The elements contained in the G colony were filterable and gave rise to filamentous forms which developed into the original coccus. Our organism passed through an R, S and G form similar to the cyclic change described by Hadley (1931) in his studies on the life history of the Shiga dysentery bacillus.

In evaluating any work of this nature it is necessary to show, within reasonable limits, that the observations made cannot be explained by contamination. The results reported here are reproducible, and the majority of forms were obtained from various sources. It may seem that some of our observed variations appear to be tinted with an extreme view of pleomorphism, but in spite of this, all the variants represent a phase in the life cycle.
of the original organism. We therefore feel that the common argument of contamination cannot be invoked to explain these findings.

SUMMARY

Microbic variation was studied in a recently isolated strain of an unidentified yellow pigment-producing coccus. Variants were produced by growing the organism in nutrient broth, 10 per cent antiserum broth and in broth filtrates. After aging the cultures in nutrient broth and antiserum broth three different variants were observed; a smooth yellow variant, a rough yellow variant and a filterable form. Several intermediate forms were also obtained.

Variants were obtained from the broth filtrates of the filterable form. After incubation for 36 hours a filamentous form was obtained which developed into the original coccus after prolonged growth on agar. A white variant and a maroon-colored variant were also obtained from aged broth filtrates of the filterable form. No antigenic relationship was found between this yellow pigment-producing coccus and Micrococcus aureus, or between the the smooth variant and Micrococcus aureus. No cross agglutination occurred between the rough type and the smooth variant.

The original strain after cultivation on nutrient agar for two years spontaneously changed from a coccus to a filamentous form. Growth studies showed that this filamentous form was part of a morphological cycle which was completed in 30 hours. During this cycle the cocci changed to rods and filaments which finally reverted to cocci. The filamentous form required 60 hours to go through the various forms from filaments to cocci.

During this morphological cycle motility was shown in the short rod or coccoidal phase. This motility was observed in hanging drop preparations, by the presence of motile colonies in semi-solid medium and by flagella stained slides.

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Safford, C. E., and Fleisher, M. S. 1931 Stain Technology, 6, 43.
PLATE 1
MICROBIC VARIATION

(Francis E. Colien: Microbic variation in pigment-producing coccus)
FIG. 1. Unidentified yellow pigment-producing coccus, original rough, from twenty-four hour agar slant, stained by Gram's method. Gram-positive. \( \times 1000 \).

FIG. 2. Original rough colony of the yellow pigment-producing coccus, 6 days growth on nutrient agar. Note the mycelium extending from the periphery. Transmitted light. \( \times 75 \).

FIG. 3. Two smooth colonies of variant 96S, and two original rough colonies, 10 days growth on nutrient agar. Transmitted light. \( \times 50 \).

FIG. 4. Rough variant colony 96-1 obtained by aging the original strain in 10 per cent antiserum broth, 14 days growth on nutrient agar. \( \times 16 \).

FIG. 5. A small colony, filterable form, 7 days growth on nutrient agar. Leitz ultropak microscope. \( \times 110 \).

FIG. 6. Variant colony 96M, maroon center, 4 weeks growth on nutrient agar. \( \times 16 \).

FIG. 7. Safford and Fleisher flagella stain of the short rod or coccoidal form of the yellow pigment-producing coccus from a twenty-four hour agar slant. \( \times 1800 \).
(Francis E. Colien: Microbic variation in pigment-producing coccuss)
PLATE 3

MORPHOLOGICAL VARIATION OF A YELLOW PIGMENT-PRODUCING COCCUS. THE TRANSFORMATION OF FILAMENTS TO COCCI

Each figure represents three hours in the growth cycle; 3, 6, 9, up to 60 hours at 37°C. Negative stained preparations (Congo red) from nutrient agar. X 1000.
(Francis E. Colien: Microbic variation in pigment-producing coccus)