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Transformation and Culture Phase Dissociation of Staphylococcus (*Micrococcus pyogenes*) (23494)

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A distinctive pattern of dissociation evolved from studies on *Gaffkya tetragena*. A specific immunologic white colony type gave rise to yellow, pink, pink-yellow and brown types, each with its mucoid, smooth and rough phases, and in addition a translucent form (1,2,3). The variants emerged best in aging cultures in broth or on agar and seemed to appear by chance. Because of a close relationship between *G. tetragena* and *Staphylococcus,* tests were made with similar methods to see if a parallel dissociative pattern pertained.

Methods. Cocci from a single yellow colony of *Staphylococcus PCI 209 P* (U.S. Food & Drug Admin.) served as the inoculum of 100 ml nutrient broth in flasks. After incubation at room temperature for 3 to 7 months, subcultures were made by streaking or as 5 or 6 point colonies on nutrient agar 7-10 mm thick in plates sealed with a strip of parafilm to delay drying. The plates were similarly aged and continually inspected for visible evidence of colonial changes.

Results. Point colonies made from a 54-day flask culture were of the smooth(S) yellow phase 10 mm wide after 20 days. At first they were of the usual yellow butter color and consistence, but later faded until some were yellow in the center shading into white; others turned cream-color or white. On further aging a sharply defined, indented, colorless, translucent rim appeared around some; others were studded or rimmed or both with minute yellow and white daughter colonies. The white ones usually were yellowish by transmitted light. Subcultures from the yellow, white and translucent areas, and from the yellow and white nubbins, with exceptions to be described, grew as the original S-yellow form as if youth and crowding had delayed the formation of pigment. Shades from nearly white, cream, buff, pale yellow (citreus), yellow (aureus), to deep orange (auranticus) appeared in many streaked cultures and point colonies for no discernible reason. Subculture usually reproduced the original yellow tint. Growth at 10°C or 37°C made little difference in color change. One set of cream-colored point colonies 90 days old and 22 mm broad were studded and edged with pale daughter colonies. These in turn were surrounded by a translucent band with another rim of minute colonies (Fig. 1).

Cocci from the S-yellow colonies were coagulase-positive, fermented mannitol, liquefied gelatin and hemolized blood.

Origin of the Mucoid-Yellow Phase. Subcultures from the pale areas of a 58-day S-yellow colony grew as thick, glistening, pale yellow colonies, confluent where crowded (Fig. 2) in contrast with the usual discrete S-

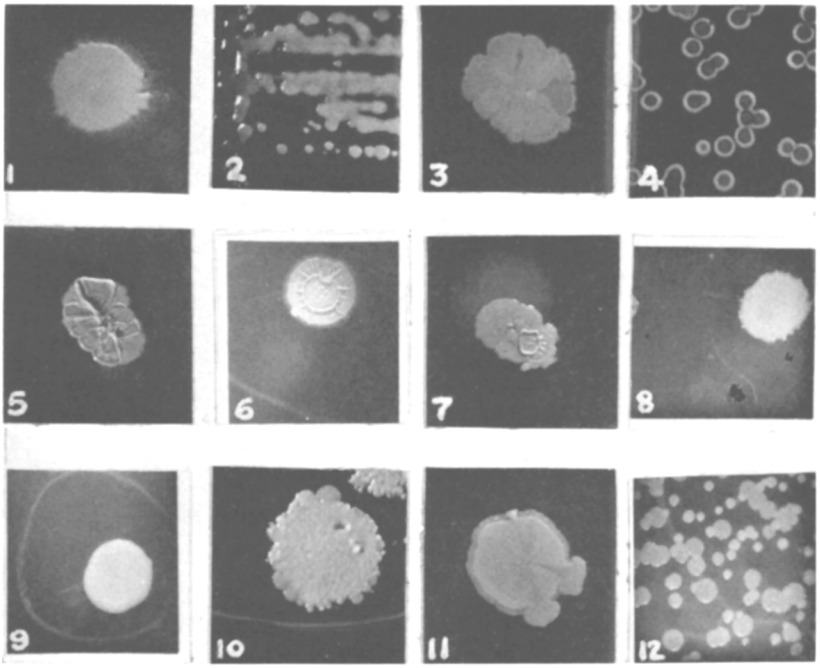

FIG. 1. S-yellow colony 90 days old studded and edged with daughter colonies surrounded by a translucent band with another rim of barely visible colonies.
FIG. 2. Confluent M-yellow colonies.
FIG. 3. M-yellow colony with sectors.
FIG. 4. Rimmed M-yellow colonies.
FIG. 5. R-yellow colony.
FIG. 6. R-yellow colony with a white daughter in the translucent rim.
FIG. 7. R-yellow colony with M-yellow sector in the middle and a white one to the right.
FIG. 8. S-white colony studded with yellow and white daughters.
FIG. 9. S-white colony.
FIG. 10. S-white colony, 2 mo. old studded with white papillas and with mucoid-white outgrowths.
FIG. 11. M-white colony with M-white and S-white outgrowths.
FIG. 12. M-white colonies and smaller S-white ones.
All colonies are in natural size.

yellow ones. Touched with a wire, they were tough or glairy and pulled like mucin. Further transfer on streaked plates bred true. Point colonies usually reverted to the S form or formed M colonies (Fig. 3), and a few grew as rough yellow colonies. Senile flask cultures of S-yellow usually changed to M-yellow. On occasion, colonies seemed to autolyze in the center but had a thick mucoid-yellow rim (Fig. 4). Subcultures from both areas bred true.

Cocci of the M colonies were smaller than the parent ones. They caused mucinous growth in broth, were unclustered, appeared at times to have capsules and often were enmeshed in a stringy pink-stained matrix, possibly of capsular substance. No swelling or clumping occurred when immersed in homologous antiserum. They were coagulase-positive, fermented mannitol, liquefied gelatin and were hemolytic.

Origin of the Rough-Yellow Phase. A 97-day flask culture of S-yellow cocci streaked on agar at first grew as homogenous cream-colored colonies. After a month, a few colonies were larger and less opaque. Both kinds on transfer grew as viscid, confluent M-yellow ones. On a point colony plate, cocci from the small colonies reproduced S-yellow ones, but those from the large ones grew into 10 mm, flat, yellow, lusterless, deeply crinkled and concentrically or radially ridged colonies, obviously the hitherto undescribed R-yellow phase (Fig. 5 and 6). When fished with a

wire, large, dry, crumbly portions lifted away. Each colony was surrounded later by a translucent fringe. As mentioned in the preceding section, M-yellow colonies at times gave rise to R-yellow ones.

In one month-old colony, a white daughter colony emerged at the tip of a ridge (Fig. 6). Subcultures of the R-yellow base gave M-yellow colonies. Subcultures from the white outgrowth at times reverted to mucoid-yellow colonies, but at others, formed different slightly ridged pale yellow colonies with a white rim. One colony in addition developed a large paler yellow sector and a white sector both of which were ridged (Fig. 7). Transfers from the paler yellow sector gave M-yellow colonies. From the white sector 2 kinds of white colonies grew, one as S-white, the other as a new form of high-domed glossy mucoid white.

Another point R-yellow colony was pale, slightly ridged and surrounded by a whitish rim in which a deep yellow sector grew after 3 weeks. Transfer from the pale yellow center gave typical R-yellow colonies on one occasion and M-white on another; the deep yellow rim sector was a reversion to the S-yellow phase.

Subcultures of R-yellow cocci on streaked plates usually grew as the M-phase; point colonies usually grew as R-yellow, but often gave rise to M or S yellows and to a mucoid white. Some point colonies and occasionally crowded ones less rough than the extremely rough ones may have represented intermediate forms. Evidently the R-yellow phase is less stable than the M or S.

R-yellow cocci were small, clustered but with no evidence of capsules or ropy growth and had biologic characteristics in common with the M and S ones.

Origin of a Smooth-White Type. As stated previously, the original senile S-yellow point colonies had yellow and white daughter ones (Fig. 8). Subcultures of both usually evolved into the S-yellow phase, but on several occasions those from the white daughters produced mostly yellow and a few white colonies. From a 7-month S-yellow flask culture, a streaked plate revealed almost all M-yellow colonies and a row of about 20 white ones. Point colonies from both sources grew more slowly than the yellow and formed smaller, white, porcelain-like, flat colonies 10 to 12 mm wide after a month or two (Fig. 9). They were regarded as the S-white phase. Subcultures were stable. Old colonies studded with white papillas had the deceptive appearance of roughness and had outgrowths of mucoid white (Fig. 10).

The cocci were usually much larger than those previously mentioned, had no capsule and many were in diploform and tetrads. Both the cocci and the colonies were morphologically indistinguishable from those of S-white *G. tetragena.* They were weakly hemolytic, coagulase-negative and did not ferment mannitol nor liquefy gelatin.

Origin of the Mucoid-White Phase. Subcultures from a 3-month flask seeded with S-white cocci grew as colonies different from the parent. On one occasion a mucoid white sector appeared in an M-yellow colony. Papillas on a 2-month S-white point colony (Fig. 10) and a sector of an R-yellow one (Fig. 7) also differed. The colonies therefrom were glistening, pearly-white and less opaque; later they flattened, became drier and developed sectors, areas and outgrowths of opaque white or of even more translucency (Fig. 11). Young colonies, touched with a wire were viscid and drew out in filaments; older ones, though drier, were more fluid than the S-white ones. They were confluent when crowded resembling those of Fig. 2. Numbers of small, dense, white, domed colonies appeared among the more translucent ones (Fig. 12) indicating reversion to S-white. Thus far, neither the M-white nor the S-white reverted to the yellow type and, as with *G. tetragena,* no R-white form emerged.

Cocci of the M-white phase were clustered, often in tetrads, seemed to have less prominent capsules than the M-yellow, often were enmeshed in pink stained material, were larger than the yellow ones. Growth in broth was slimy. The biologic reactions were those of the S-white.

Translucent forms. Subcultures of a white daughter colony from a giant S-yellow one grew into crowded yellow colonies after 2

days and scattered about were minute, flat, thin, barely visible, discreet and confluent, colorless, translucent ones. On only a few other occasions did similar translucent colonies appear among S-yellow ones. They were suspected as being the anticipated translucent form or the so-called G form. Some of them were immature yellow ones delayed in development by crowding because subcultures usually gave rise to yellow colonies. Subcultures on agar or in broth at times bred true, grew slowly or not at all and some died. Point colonies seldom exceeded 2 or 3 mm in width and daughter colonies formed before growth ceased. On no occasion did evidence of reversion to any other form appear as was often the case with *G. tetragena*. For that reason the question of contamination was not excluded. The cocci were small, many in tetrads and clumps, pleomorphic and polychromatic similar to those of the G or translucent form described for other bacteria. They were coagulase-negative, did not liquefy gelation, ferment mannitol nor hemolyze blood.

Small Colony Variants. These forms described by others seldom were seen. Small ones often were small because they were crowded. A few small yellow and white colonies occasionally remained small on subculture but usually grew into large ones when transferred. Growth in penicillin-broth did not induce their appearance better than aging of cultures.

Comment. Previous investigations of the dissociative pattern of *Staphylococcus* did not reveal the classic M, S, R changes as established for *G. tetragena*(3), *Pneumococcus*(4) or *Streptococcus*(5). Pinner and Voldrich described what now appears to be type transformation of the yellow (aureus) into the lemon-yellow (citreus), white (albus), and pink (roseus) forms, but saw no R forms and M forms were not mentioned(6). The citreus forms were stable, but the albus reverted to the aureus after growth in homologous antiserum, and the roseus to the albus after inoculation into animals. They regarded color transformation as analogous to phase variation of other bacteria. Color-type transformation was similar to that I later observed in *G. tetragena.*

Bigger(7) described a scheme difficult to reconcile with the usual pattern. A number of aureus and albus variants were classified as smooth with varying degrees of viscidity and as rough viscid forms. Some sticky ones which pulled out like glue probably were mucoid forms. Photographs of his 3-day-old rough colonies bear no resemblance to the R form here described.

Staphylococcus aureus of all strains tested by Hoffstadt and Youmans gave rise to white and G colonial forms(8). They described 8 kinds called rough, but some were translucent bluish-white, and photographs again do not resemble the R colonies described here. These investigators and Swingle(9) observed small colony variants (G form), and others(10,11) obtained them by growth in broth containing penicillin. They usually reverted to the parent form on subculture. Similar small colonies appeared occasionally in my experiments but not more often after exposure to penicillin than after aging.

The studies of these investigators and the ones presented here indicate that dissociation of *Staphylococcus* like that of other bacteria is more complicated than the simple, visibly or serologically detectable type and type-color transformation and respective M, S, R phase variation. Thus far the simplified scheme appears as:

Mucoid yellow	Mucoid white
Smooth yellow	Smooth white
Rough yellow	

It is the counterpart of the scheme depicted for other Gram-positive pathogenic cocci, namely, *G. tetragena, Streptococcus,* and *Pneumococcus.* For the latter it is:

Type I	Type II	Type III	
Mucoid	Mucoid	Mucoid	etc.
Smooth	Smooth	Smooth	
Rough	Rough	Rough	

Conversion of pneumococci of one type into another was induced by growth of M-pneumococci or of R-pneumococci derived from any type in animals or in broth with dead M cocci containing deoxyribonucleate of the donor cocci(12,13). Type transmutation of *G. tetragena* and of *Staphylococcus* was spon-

taneous. Strains established as aureus, albus and roseus probably are variant forms of a common ancestor.

Granting an analogy of dissociative patterns, one may predict an R-white phase, a pink type as Pinner found, and perhaps others for *Staphylococcus*. Effort to educe them is under way. It would be of importance to know the significance of the mucinous substance of the M phase, whether the yellow and white staphylococci are type specific by serologic or 'phage methods and if both forms convert to different respective serologic yellow and white types.

Staphylococcus and *G. tetragena* seem to be much more closely related than generally thought. The resemblance in colonial appearance, and in coccal size, shape, staining and arrangement probably often leads to bacteriologic diagnostic error. It is impossible to differentiate the respective S-yellow or S-white forms of either on that basis. The biologic differences as listed(14) are minor and may vary even among identical strains; and they differ from some reported here. Officially, *St. albus* liquefies gelatin and ferments mannitol; my white forms did not. *G. tetragena* is said not to liquefy gelatin nor ferment mannitol, in agreement with my tests(1), but the myth of pathogenicity for mice is perpetuated. In my hands, growth of staphylococci and of tetragenas was inhibited by similar amounts of penicillin, the M cocci of both made a mucinous substance and the yellow forms grew more luxuriantly and resisted heat better than the white forms.

Summary. A partial pattern of dissociation into color-type and phase variation emerged after aging a strain of *Staphylococcus*. From the smooth yellow (aureus), a mucoid yellow and a rough yellow colony form were derived, and in addition a smooth white type (albus) and its mucoid white phase. Thus far the dissociative pattern is analogous with that of *G. tetragena* and *Pneumococcus* as regards type and phase variation and interconvertibility. It may apply to *Staphylococcus* in general.

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Increased Sensitivity to Estrogen in Uterine Hyperplasia in DBA x CE and Reciprocal Hybrid Mice.* (23495)

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At 6 to 7 months of age, virgin female DBA x CE and reciprocal hybrid mice spontaneously develop a progressively severe endocrine imbalance involving the ovaries, adrenal cortex and pituitary(1,2). One of the more prominent morphological features of this syndrome is marked enlargement of the uterus

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