

THE GENETIC SIGNIFICANCE OF THE DISSOCIANTS OF STAPHYLOCOCCUS AUREUS¹

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In the study of bacterial dissociation variations in pigment formation and in colonial and biochemical characteristics have been noted many times. As early as 1897, Newman reported the recovery of white colonies, differing in acid and indol production, from a strain of *Staphylococcus aureus*. Mellon and Caldwell (1926), studying a white strain of *Staphylococcus*, concluded that it was a common ancestor of *Staphylococcus aureus* and *albus*. More recently Pinner and Voldrich (1932), in a detailed study of six strains of *Staphylococcus aureus*, reported the isolation of four white, one lemon yellow, and one rose variant. In the same year, the authors obtained 11 variants from a single strain of *Staphylococcus aureus*, and the cultural characteristics of four of these were reported.

The purpose of this work was to study the dissociation of different strains of *Staphylococcus aureus* and *albus* and to determine their genetic position and cultural stability.

EXPERIMENTAL

Two strains of *Staphylococcus albus* and four of *Staphylococcus aureus* were dissociated and the characteristics of their variants studied. All the strains, before dissociation was attempted, were plated repeatedly on agar plates to insure a pure culture. When purity of cultures had been established all strains and all variants obtained later from these strains were dissociated (Hoffstadt and Youmans, 1932) in lithium chloride broth, pH 7.8, and plain

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broth, pH 7.8. Daily transfers were made serially into these broth tubes for a period of sixty days and subcultures from each broth tube were made on agar plates at each transfer. Incubation was at 37°C. No variants were studied for cultural characteristics or further dissociation until purity of the culture was determined. Utmost care was taken to prevent contamination.

CULTURAL CHARACTERISTICS OF THE STRAINS

The two strains of *Staphylococcus albus* differed from each other in that strain No. 2 did not ferment glycerol, and differed from Bergey's (1930) species *Staphylococcus albus* in not fermenting mannitol; with the exception of the heavy white growth on agar slants they were like *Staphylococcus epidermidis* described by Bergey. Strains 5 and 6 of the orange Staphylococci had identical cultural characters, both fermenting mannitol, but not glycerol or salicin in which they differed from the American Type Culture Collection strain as described by Pinner (1932). Bergey's species of *Staphylococcus aureus* does not ferment mannitol. Strain 3 orange agreed more nearly than any form with the latter, but differed from the American Type Culture Collection strain in that it did not ferment glycerol. Strain 4 showed the greatest variation; it did not ferment lactose, levulose, salicin, galactose or glycerol (table 1).

DISSOCIATION OF THE TYPES STUDIED

No. 1 of the white staphylococci showed no dissociation at any time, while from No. 2, the G, the gonidial form, was recovered on the thirty-ninth transfer. This strain of G fermented glucose, maltose, levulose, and lactose only feebly. Variants were isolated from all of the orange forms. From No. 3, a rough yellow was obtained on the forty-third transfer in lithium chloride broth, and from the forty-second transfer in plain broth, but it did not remain stable. A smooth, white stable variant and a translucent stable form were isolated on the sixth transfer in lithium chloride broth and from it on the fifty-first transfer, a yellow form was obtained, but no G forms were found.

The dissociation in strain 4, as previously reported by us (1932)

TABLE 1
Cultural characteristics of undissociated strains of staphylococci

SPECIES	ALBUS 1	ALBUS 2	AUREUS 3	AUREUS 4	AUREUS 5	AUREUS 6
Source*	Acne abscess	Acne abscess	Mild abscess	Abscess with osteomyelitis	Osteomyelitis (fatal)	Severe abscess
Pigment.....	White	White	Orange	Orange	Orange	Orange
Virulence.....	-	-	-	+++	+++	+++
Agar slant.....	Smooth white	Smooth white	Smooth yellow	Smooth yellow	Smooth yellow	Smooth yellow
Maltose.....	+	+	+	+	+	+
Mannitol.....	-	-	-	-	-	-
Inulin.....	+	+	+	+	+	+
Sucrose.....	+	+	+	+	+	+
Glucose.....	+	+	+	+	+	+
Levulose.....	+	+	-	-	+	+
Lactose.....	+	+	+	-	+	+
Salicin.....	-	-	-	-	-	-
Galactose.....	+	+	+	-	+	+
Glycerol.....	+	-	-	-	-	-

* From purulent discharges with exception of strains 4 and 5 which were from blood cultures.

was again confirmed with the production of two stable, smooth, white variants (SW), a rough yellow (RY), a rough white (RW), a translucent (SR), and the G form. In addition, a yellow smooth form was recovered from the smooth white on the twentieth transfer. The fifth strain never produced rough colonies, but light yellow smooth colonies appeared on the thirty-seventh transfer and became stable white colonies on the fifty-third. The G colonies were recovered twice from the forty-ninth transfer on lithium chloride broth and on the fifty-fifth in plain broth. Both the G colonies showed feeble fermentative powers. A yellow colony was obtained from the white variant on the fifty-first transfer. The variants of strain 6 were three in number: RY obtained in plain broth on the twenty-sixth day, but which did not remain stable; a smooth white on the thirty-fourth day; and a G form on the thirty-eighth, the forty-first and forty-third day in lithium chloride broth and on the twenty-ninth day in plain broth. The G forms fermented actively maltose, mannitol, sucrose, levulose and glucose. A yellow variant was recovered from the smooth white variant on the sixth day.

All the forms, except one, which showed dissociation were pigment producers and all gave rise to white stable strains. Three, strains 3, 4, and 6, dissociated into the G strain. Strain 4 produced the largest number of variants, and strain 3 only one variant, a feebly fermenting G. A stable rough form was recovered only from strain 4.

CHANGES IN THE CULTURAL CHARACTERISTICS OF THE WHITE VARIANTS OF ORANGE STAPHYLOCOCCI

Eight stable white variants were studied for cultural characteristics: two were isolated from culture 3, four from culture 4 and one each from cultures 5 and 6. They fell into two groups, those which fermented mannitol and those which did not (table 2). The former could again be subdivided into those which were glycerol-positive and those which were glycerol-negative. In the group which fermented both glycerol and mannitol, 3a and 4a were alike; however, 3a fermented both mannitol and levulose which the source, strain 3, did not. White 6a differed from the

TABLE 2
Cultural characteristics of white variants of orange staphylococci studied

	3a	3b	4SW	4SW ₂	4SR	4RW	5a	6a
Virulence.....	-	-	-	-	-	-	+++	-
Agar slant.....	Opaque	Translucent	Opaque	Translucent	Opaque	Opaque	Opaque	Opaque
Source.....	3	3	4	4	4	4	5	6
Maltose.....	+	+	+	+	-	+	+	+
Mannitol.....	+	-	+	-	-	+	+	+
Inulin.....	-	-	-	-	-	-	-	-
Sucrose.....	+	+	+	+	+	+	+	+
Glucose.....	+	+	+	+	+	+	+	+
Levulose.....	+	-	+	+	+	+	+	+
Lactose.....	+	+	-	-	-	-	-	-
Salicin.....	-	-	-	-	-	-	-	-
Galactose.....	+	-	-	-	+	+	+	+
Glycerol.....	+	-	-	-	-	-	-	+
Changes from original yellow culture	Mannitol + Levulose + Glycerol +	Galactose -	Levulose +	Mannitol - Lactose +	Maltose - Mannitol - Levulose + Lactose +	Levulose + Lactose + Galactose +	Pigment formation only	Glycerol +

biochemical reactions of its parent culture only in its fermentative action on glycerol. Of those which did ferment glycerol and mannitol, 4 RW and 5a were alike while the third, 4 SW differed only in not fermenting lactose. It is interesting to note that 5a was the only white strain which did not lose its virulence after dissociation and that it differed from the parent culture only in pigment production; 4 SW differed from the original in the addition of levulose fermenting power and 4 RW in acid formation in lactose and galactose.

Only one form, 4 SW₂ agrees in fermentation reactions with any form described by Bergey, *Staphylococcus epidermidis*, but it was not in the least similar to it colonially. Not a single strain showed the fermentation reactions described by Bergey for *Staphylococcus albus* or *pharyngis*.

CULTURAL CHARACTERISTICS OF THE ORANGE VARIANTS

Five orange variants were obtained, one from strain 4 directly, a rough yellow, and one each from white strains 3b, 4 SW, 5a, and 6a. 5b and 6b were similar to each other, except that 5a fermented galactose, while all of the others differed in one or more fermentations. All differed from the white variant from which they were obtained, and from the original source culture (table 3).

SUMMARY AND DISCUSSION

All of the orange strains of staphylococci dissociated to some degree. Only one white strain showed any change, producing a feebly fermenting G form. White variants were recovered from all orange strains and G cultures from all but strain No. 3. Apparently the biochemical activity and the frequency of the appearance of the G form depend on the stability of the parent culture.

The progressive fermentative powers of the variants of strain No. 4, the most actively dissociating organism, are shown in table 4. The original cultural characteristics of this strain differed from all cultures of yellow and orange staphylococci described in that it did not ferment lactose. Of the first two variants isolated in its dissociation, 4 SW lost its pigment and like its parent did not

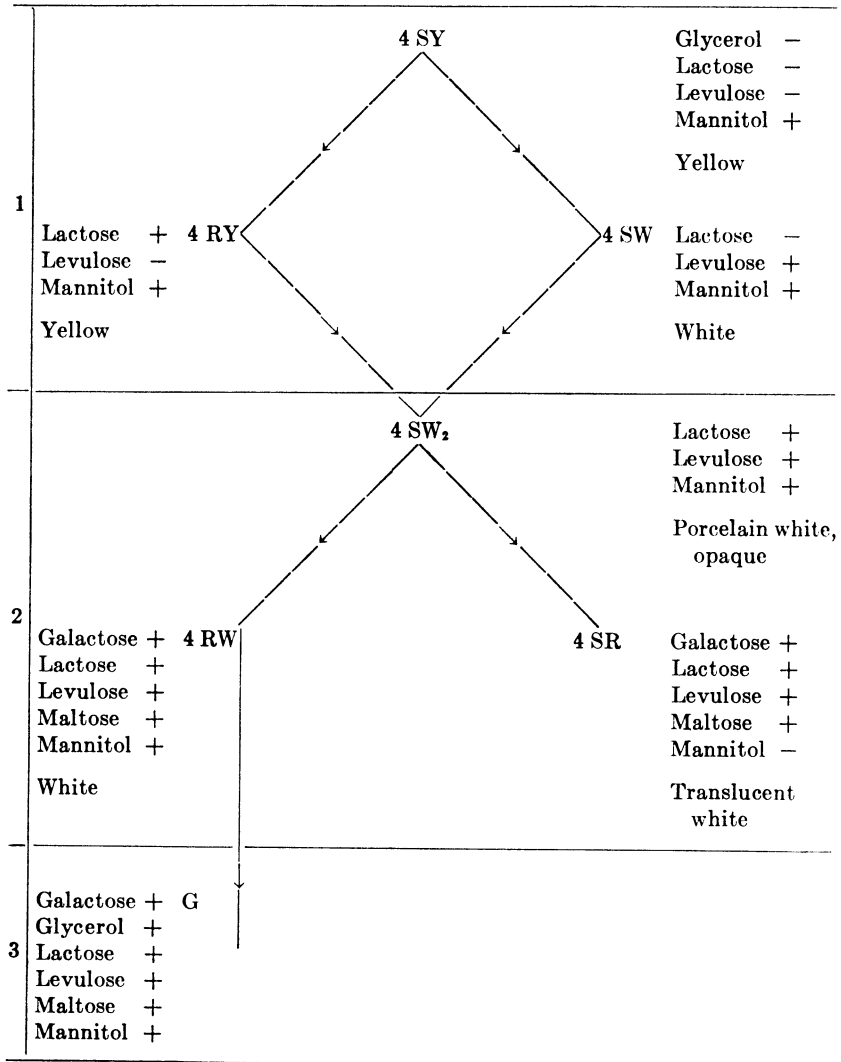
TABLE 3
Cultural characteristics of yellow variants

	3c	4a	4RY	5b	6b
Virulence.....	-	-	-	-	-
Source.....	3a	4SW ₂	4	5a	6a
Maltose.....	+	+	+	+	+
Mannitol.....	+	+	+	+	+
Inulin.....	-	-	-	-	-
Sucrose.....	+	+	+	+	+
Glucose.....	+	+	+	+	+
Levulose.....	+	-	-	+	+
Lactose.....	+	+	+	+	+
Salicin.....	-	-	-	-	-
Galactose.....	+	-	-	-	-
Glycerol.....	+	-	-	-	-
Changes from source form.....	Levulose +	Mannitol + Levulose + Galactose -	Lactose +	Galactose -	Galactose - Glycerol -
Changes from original yellow form.....	Mannitol + Levulose + Salicin + Glycerol +	Lactose +	Lactose +	Pigment galactose -	Galactose -

ferment lactose, but did ferment levulose, the other 4 RY, rough yellow, retained its pigment and fermented lactose, but not levulose. The next variant 4 SW₂, white, fermented both

TABLE 4

Distinguishing cultural characteristics of variants of strain 4 orange staphylococcus



lactose and levulose and although it did not ferment mannitol, produced 4 RW, rough white, which fermented mannitol, and 4 SR, translucent, white, which did not ferment mannitol. Both of these also fermented galactose and the G form, the last dissociant, fermented glycerol in addition. The loss of virulence and pigment production accompanied the change in fermentative activity. This was confirmed in all orange strains studied by Pinner and in all strains used in this study with the exception of strain 5 which did not lose its virulence and did not change its cultural characteristics when the pigment was lost.

It is not an unknown phenomenon in biological evolution for a biological variant to become a stable species. The evolution of the strain 4 variants with increasing fermentative powers has been divided into three sections (table 4). The first includes the establishment of lactose and levulose fermentation; the second includes the addition of galactose, and the third the addition of glycerol. Examining the cultural characteristics of the orange strains described by us, numbers 5 and 6, those possessing the greatest virulence, fall in section 1. In cultural characteristics the American Type Culture Collection strain falls low in this division and *Staphylococcus aureus* as described by Bergey falls at its juncture with section 2. The white variants fall within range 2 and 3, while *Staphylococcus epidermidis* as described by Bergey is nearest in cultural characteristics to the blind branch 4 SR₂ in section 2, and *Staphylococcus albus* and *pharyngis* in range 3.

Might not the 4 RY be the parent of the *Staphylococcus aureus* and 4 RW of *Staphylococcus albus*? Roughness is a relative term when applied to colonial form for many rough colonies may have a smooth appearance on ordinary media; hence a well established smooth-rough form may never appear colonially rough. This may account for Pinner's statement that he obtained no rough forms. We realize that the number of strains used is small, and in order to clear up the actual number of species within the staphylococcus group many more should be studied. However, if we are going to continue to use cultural characteristics in the classification of bacteria, the place of established dissociants, variants

and cultures which have the power of dissociation will have to be established.

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