STUDIES IN MICROBIC HEREDITY

XI. THE GENETIC ORIGIN OF STAPHYLOCOCCUS ALBUS AND AUREUS FROM COMMON ANCESTRAL STRAINS

RALPH R. MELLON AND DOROTHY W. CALDWELL

From the Department of Laboratories, Highland Hospital, Rochester, New York

Received for publication October 31, 1926

PURPOSE OF THE STUDY

It is not the primary purpose of this paper to classify staphylococci but rather to obtain some clue as to the genetic origin of numerous types whose heterogeneity has helped to baffle those who have attempted to make satisfactory classifications. Incidentally, the reason for the mixed character of the group may become more apparent and the fundamental biological relationships of its individual members be somewhat clarified.

Although our approach to this subject has a serological basis, the genetic significance of the conclusions will have as their background Study III (Mellon, 1926) of this series in which serologic and cultural differences appear as the result of definite variability experiments. It has been shown in this study that from an organism possessed of two or more antigenic groups new individuals may be dissociated, having but one of these groups dominant. In short, the genetic origin of group and specific agglutinogens has been indicated, with and without change of cultural or morphologic character.

SOURCE OF MATERIAL

Although many of the cultures were obtained largely from diverse pathological conditions, the majority came from cases of *impetigo contagiosa bullosa* in whose epidemiology we were interested. Particularly, were we interested in isolating an epidemic type, but in this we were not successful (Mellon, 1925). A most

TABLE 1

Showing the agglutination reactions of the majority of the strains studied (40)

PIGMENT	SOURCE	NUMBER	MANNITOL	AUREUS—NO. 635				ALBUS—NO. 644				ALBUS—NO. 1 (GALL BLADDER)				NORMAL			NaCl SOLUTION	DATE
				1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:20	1:40	1:80		
White	Gall-bladder	1-21	−	2+	1+	1+	±	C−	C−	C−	C−	C	C	C	3+	1+	0	0	00	2/6
White	Gall stone	192-21	−	1+	0	0	0	C	C	C		0	0	0	0	1+	1+	0	00	2/6 and 6/12
White	Gall-bladder	470-22	−	2+	±	0	0	C	C−	C−	C−	C	C	C	2+	1+	0	0	00	2/13 and 9/24
White	Gall-bladder	891-22	−	1+	±	0	0	C	C	C	C	C	C	C	C	C	1+	0	00	2/18
White	Breast milk	644-23	−	0	0	0	0	C	C	C	C	C	C	C	C	0	0	0	00	1/18
White	Breast milk	646-23	−	0	0	0	0	3+	3+	3+	2+	C	C	C	C	1+	1+	0	00	2/15
White	Skin	657B-23	−	0	0	0	0	2+	2+	1+	0	C	C	1+	0	0	0	0	0 00	2/15 and 3/9
White	Breast milk	659-23	−	2+	2+	1+	0	3+	3+	3+	3+	3+	C	C	C−	3+	3+	1+	00	2/15
White	Breast milk	669-23	−	0	0	0	0	0	0	0	0	C	C	3+	1+	1+	0	0	.00	2/21
White	Nose culture	820-23	−	±	0	0	0	3+	3+	1+	1+	C	C	C	C	0	0	0	00	3/9 and 6/29
White	Urine culture	836-23	−	1+	0	0	0	C−	2+	2+	0					1+	0	0	00	3/9
White	Breast milk	136-24	−	1+	1+	1+	0	C	C	C	1+	C	2+	1+	0	2+	±	0	00	2/21
White	Mouth culture	164-24	−	1+	1+	±	0	C	C	C	C					1+	0	0	00	3/5 and 6/16
Orange	Gall-bladder	11.1-21	+	C	C	C	C−	1+	1+	±	0					1+	1+	0	00	1/18 and 5/22
Orange	Furuncle	180-23	+	C	C	C	C	1+	±	0	0					0	0	0	00	3/2
Orange	Osteomyelitis	404-23	+	C	C	C	C	2+	2+	0	0					0	0	0	00	3/9
Orange	Breast milk	630-23	+	C	C	C	C	0	0	0	0	1+	1+	0	0	1+	1+	1+	00	1/18

Orange	Impetigo	636-23	+	3+	C	C	C	1+	1+	1+	±	0	0	0	0	±	0	0	00	2/21
Orange	Breast milk	639-23	+	C	C	C	C	0	0	0	0	0	0	0	0	0	0	0	00	1/28
Orange	Eye exudate	657A-23	+	C	C	C	C	0	0	0	0	0	0	0	0	0	0	0	00	2/6
Orange	Eye exudate	658-23	+	C	C	C	C	1+	1+	±	0	0	0	0	0	0	0	0	00	2/6
Orange	Impetigo	664-23	+	C	C	C	1+	1+	1+	0	0		0	0	0	0	0	0	00	2/18
Orange	Impetigo	725-23	+	C	C	C	C	2+	2+	1+	0	1+	1+	0	0	2+	±	0	00	3/2
Orange	Osteomyelitis	856-23	+	3+	C	C	C	3+	2+	2+	0					2+	2+	0	00	3/2 and 6/16
Orange	Breast abscess	26-24	+	C	C	C	C	1+	1+	0	0					±	0	0	00	3/2
Orange	Impetigo	32-24	+	C	C	C	C	2+	2+	1+	0	2+	2+	0	0	2+	0	0	00	3/2
White	Gall-bladder drainage	39-24	+	C	C	C	C	0	0	0	0	0	0	0	0	0	0	0	00	2/21
Orange	Empyema	45-24	+	C	C	C	C	1+	1+	1+	0	2+	2+	1+	0	1+	1+	0	00	1/28
Orange	Impetigo	96-24	+	C	C	C	3+	2+	0	0						1+	±	0	00	3/2
White	Abscess	108-24	−	C	C	C	C	2+	1+	0	0					C	3+	1+	00	6/26
Orange	Impetigo	132-24	+	3+	C	C	C	2+	1+	±	0					1+	0	0	00	3/5
Orange	Conjunctivitis	153-24	+	C	C	C	2+	0	0	0	0					0	0	0	00	3/5
Orange	"Stitch" abscess	162-24	+	C	C	C	C	0	0	0	0	0	0	0	0	0	0	0	00	3/2
Orange	Abscess	169-24	+	C−	C−	C−	C−	1+	1+	1+	1+					0	0	0	00	6/26
Orange	Impetigo	173-24	+	C	C	C	C	1+	1+	1+	0					0	0	0	00	3/5
Orange	Impetigo	175-24	+	C	C	C	C	1+	±	±	0					0	0	0	00	3/5
Orange	Impetigo	213-24	+	2+	C	C	0	0	0	0	0	0	0	0	0	0	0	0	00	3/2
Orange	Impetigo	214-24	+	2+	C	C	C	0	0	0	0	0	0	0	0	0	0	0	00	3/2
Orange	Impetigo	215-24	+	2+	C	C	C	1+	±	±	0	0	0	0	0	0	0	0	00	3/2
Orange	Impetigo	217-24	+	C	C	C	C−	0	0	0	0	0	0	0	0	0	0	0	00	3/2

interesting anomalous group came from pathological conditions in the gall-bladder, the flora of which has been subjected to special study. The remainder were obtained from miscellaneous lesions. The total of the series was 58 strains, which in a preliminary way had been tested on mannitol broth with the idea of determining whether this character could be correlated with pigment formation. If so, it would form a ready method of recognizing aureus strains temporarily non-pigmented (see table 1).

A COMMON ANCESTOR STRAIN FOR AUREUS AND ALBUS STRAINS

It was our good fortune to encounter a strain made up apparently of at least three distinct antigenic groups. One of these groups was serologically albus; the others serologically aureus. The strain itself, no. 644, was never pigmented while under our observation. The cardinal value of this strain for our purpose is illustrated by the observations of Study III of this series (1926), which shows that strains having specific agglutinogens may be dissociated from a parent strain containing both group and specific agglutinogens. The presumption is strong, therefore, that the same thing holds for the staphylococci. Moreover, the common ancestor strain offers a plausible explanation for the diversity among these cocci which has caused certain workers to abandon efforts at satisfactory classification. The bearing of this proposition will become clearer in light of the serologic data.

EXPERIMENTAL

Technique. Immune sera were prepared by immunization of rabbits with killed cultures of strains nos. 644, 635 and 1. Nos. 644 and 1 were non-pigmented strains not fermenting mannitol (*S. epidermidis* according to Bergey). The strains were purified beyond question before inoculation. The first dose consisted of 0.5 cc. of the organisms suspended to a turbidity of one billion per cubic centimeter. Five doses, gradually increasing up to 1 cc. of a two billion suspension, were given at three- to four-day intervals. One week after the fifth inocu-

lation the animals were bled from the heart. The day following the bleeding a sixth dose was given and after another week the animal was bled again.

Antigens were prepared after the method of Hooker. Four-day-old agar cultures were used and the growth washed off with 0.1 per cent formaldehyde in 0.85 per cent NaCl solution. The suspensions were allowed to stand from three to four days, washed, suspended in fresh formolized salt solution, filtered and brought to a turbidity of two billion per cubic centimeter.

Dilutions of serum of 1:25, 1:50, 1:100 and 1:1200 were used in 0.5-cc. amounts. An equal amount of antigen was added to each dilution tube, doubling the final dilution in each tube. No attempt was made to secure a serum of very high titer, nor, to determine the titer of a serum except for the homologous antigen. For the homologous antigen the titer was at least 1:800. Controls in normal serum in final dilutions of 1:20, 1:40 and 1:80 were used, as well as salt solution controls. The earlier set-ups were incubated for two or three hours at 37°C. and then placed in the icebox over night. The results were so unsatisfactory that the tests had to be repeated and for all later work the tubes were incubated at 55°C. for sixteen hours. Second readings were made 24 hours later.

For the absorption of agglutinins the following procedure was used. A twenty-four-hour culture of the organism was emulsified in a small volume of 0.85 per cent NaCl solution, heated to 56°C. for twenty minutes, then centrifuged at high speed until clear. To the sediment thus obtained a 1:10 dilution of the serum was added. The suspension of bacteria in diluted serum was incubated at 37° for two hours and held in the ice box over night, centrifuged until clear, the serum pipetted off and added to another heavy sediment of organisms precipitated as before and the absorption repeated, after which agglutination tests were made.

SEROLOGIC DATA

From table 2 it is clear that strain no. 644, although growing white and not fermenting mannitol, contains a pigmented sub-

group which is reciprocal serologically with the antigen of no. 635. The reactions here are precisely those of group and specific agglutinins between any two strains, strain no. 635 being a minor agglutinogen in the complex antigen of no. 644. We do not expect no. 644 antigen to agglutinate noticeably in the serum of no. 635 nor to absorb antibodies from it, while we do expect no. 635 antigen to absorb its own antibodies from serum no. 644 but to leave untouched the group antibodies of no. 644. This is exactly what happened.

TABLE 2

Agglutination reactions of strain no. 635

ANTIGEN	NO. 635 (AUREUS)									NO. 644 (WHITE)				NO. 1 (WHITE)				NORMAL SERUM				NaCl
	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:20	1:40	1:80	1:160	
635	C	C	C	C						C	C	C	C	C–	C–	C–	C–	1+	1+	1+	O	–
*	C	C	C	C	C	C	C	C	C													
644	O	O	O	O						C	C	C	C									

	NO. 635A635†				NO. 635A644				NO. 644A644				NO. 644A635				NaCl
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	
635	2+	1+	1+	1+	C	C	C	C	1+	O	O	O	O	O	O	O	00
644									O	O	O	O	C	C	C	C	00

* Means the second or overnight reading.

† Means that no. 635 serum is absorbed (A) by no. 635 antigen.

Strains nos. 597, 666 and 41 behaved as did no. 635 when run against the three antisera. They all ferment mannitol and are pigmented. Pigmented strain no. 645 represents a subgroup of no. 635 inasmuch as it only removed part of the agglutinin from serum no. 635 on absorption.

Strain no. 744 did not ferment mannitol and was non-pigmented, yet it agglutinated no. 635 serum strongly. Its reaction with serum no. 644 and no. 1 was not complete. It is entirely conceivable that such a strain was dissociated from a no. 644 type with its fermentation of mannitol temporarily in

abeyance. Certain of our experimentally dissociated strains have exhibited just such characters.

Reference to table 1 shows clearly that practically all of the pigmented strains agglutinated in no. 635 antiserum but practically not at all in no. 644 serum. Since a pigmented agglutinogen exists in this non-pigmented no. 644 as a minor agglutinogen (no. 635) the no. 635 agglutinogen must be distinct from the more common *Staphylococcus aureus*, which does not agglutinate to any extent in no. 644 antiserum. Therefore it is reasonable to suppose that our pigmented, mannitol fermenting *Staphylococcus aureus* has been dissociated from a non-pigmented non-mannitol fermenting wild type, differing from no. 644 only in the kinds of pigmented agglutinogens that it contains.

Since the former are more virulent it is conceivable that the adaptation to the host was the result of such a dissociation, as we have shown experimentally with the alkaligenes-colon group (Study III—Mellon, 1926). Viewed in this way strain 744 can be looked on as an intermediate or perhaps a transition form which, by virtue of its failure to ferment mannitol and to yield pigment, is not yet the fully developed aureus indicated by its serological characters. There is no good reason for trying to make a separate group of such *apparently* anomalous strains, nor yet for saying that they do not fall into the group typified by strain no. 635.

THE GALL-BLADDER COCCI

Of rather special interest from the standpoint of the possible influence of environment in the transformations indicated is the similarity of the pigmented strains isolated from the gall-bladder wall or bile. This group, nos. 763, 3, 143 and 761, are lemon yellow, but mannitol negative, and by their agglutination with no. 644 serum show their relation to the aureus component of this coccus.

Table 3 clearly shows that the reaction of pigmented strain no. 761 with the serum no. 644 is with its aureus group, with which it forms a subgroup, since absorption of the no. 635 aureus serum removes the agglutinins specific for no. 761 and leaves

those of no. 635. The agglutination of no. 761 with the no. 635 serum is then with minor agglutinins produced by the latter.

Strains no. 143 and 3 in their reactions indicate that strain

TABLE 3

ANTIGEN	NO. 635				NO. 644				NO. 1				NORMAL			NaCl
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:20	1:40	1:80	
761	C C	3+ C	3+ C	C C	C C–	C C	C C	C C	C–	C–	C–	C–	0	0	0	00

	NO. 635A635				NO. 644A644				NO. 635A761				NO. 644A761			
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400
761	1–	0	0	0	C	C	3+	1+	0	0	0	0	0	0	0	0
635	0	0	0	0					C	C	C	C				
644					0	0	0	0					C	C	C	C

TABLE 4

ANTIGEN	NO. 635				NO. 644				NO. 1				NORMAL			NaCl
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:20	1:40	1:80	
3	1+	1+	0	0	C	C	C	C	C	C	C	C	2+	1+	0	00

TABLE 5

ANTIGEN	NO. 635				NO. 644				NO. 1				NORMAL			NaCl
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:20	1:40	1:80	
143	C C	C 2+	C 0	2+ 0	C C	C C	C C	C C–	2+	C	C	C	C	C	0	00

	NO. 635A635				NO. 635A644				NO. 644A644				NO. 644A635			
	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400	1:50	1:100	1:200	1:400
143	0	0	0	0	C	C	C	C	0	0	0	0	C	C	C	1+
635	0	0	0	0	C	C	C	C	0	0	0	0	0	0	0	0

no. 644 has another yellow component rather distinct from no. 635. The agglutination of these strains in no. 635 antiserum is slight or questionable, and when the no. 644 antiserum is ab-

sorbed by the no. 635 aureus antigen the antibody corresponding to it is removed but not that corresponding to no. 143. Strain no. 763 is much like no. 143.

Here is a group of strains, some of which represent the dissociation of an additional pigmented group from that contained by the "common ancestor" white coccus. This group is distinct from the pigmented agglutinogen of no. 635. As was the

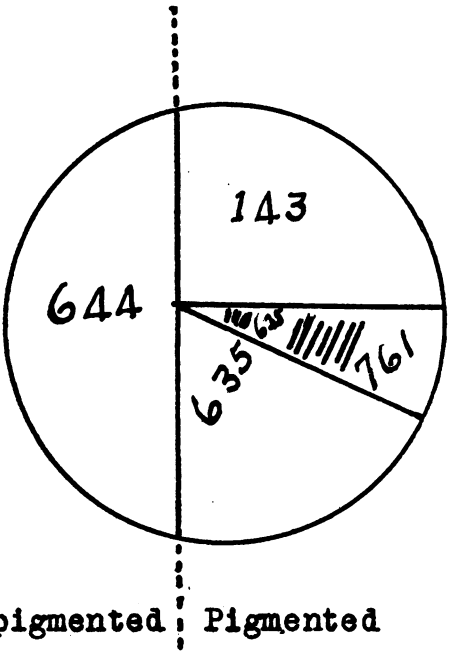

FIG. 1. The left hand sector represents the agglutinogen corresponding to *Staphylococcus albus* no. 644. Sector no. 635 represents a pigmented agglutinogen which contains at least one subgroup as shown by no. 761. Sector no. 143 represents a pigmented agglutinogen distinct from nos. 635 or 761.

case with no. 744 we have an incomplete strain of the type of no. 635, but the approach to it is a closer one because the latter are pigmented a light or lemon yellow even though they do not ferment mannitol. As is known, too, their virulence is apt to be intermediate.

So although, as has been shown in table 1, many strains show

a correlation of pigment production and mannitol fermentation, others have not reached the type—so to speak—in either one or both particulars, so that the implications of the term *Staphylococcus aureus* and *S. albus* may be quite different with individual cocci unless they have been subjected to detailed study. What would be called the anomalous character of such strains, as regards fermentation, may be correlated with their origin from the bile since all pigmented strains isolated from this source were similar.

Diagramatically then, the common ancestor strain may be viewed as an agglutinogenic composite having at least the groups depicted in figure 1, and perhaps more, had we encountered the strains to bring them out.

On the basis of these considerations it becomes clear how dissociated strains within the animal body may not necessarily be related serologically and yet have come from the same source in an hereditary sense. On the other hand, they may play quite different rôles in the host, depending of course on their relative virulence.

OTHER SEROLOGICALLY DISTINCT STRAINS

While the majority of the aureus strains studied showed serologic relation with aureus strain no. 635, a few did not. Moreover, they showed no agglutination in the antiserum of no. 644, indicating that they were serologically distinct from the pigmented antigenic components of this strain. Thus we have encountered at least three distinct groups of aureus, to say nothing of subgroups. The strains distinct from no. 635 are nos. 628, 716, and 770-O. In addition to being pigmented these also ferment mannitol.

Similarly strains nos. 1030, 650 and 770-W, which are not pigmented and mannitol negative, do not react with the antiserum of strain no. 644. Strain no. 770-W came from the breast milk of the same case as no. 770-O. Not only are they quite distinct from the strains above considered but they are not related serologically to each other, as was shown by preparing antisera for each and doing cross agglutinations and

TABLE 6

Showing the agglutination results with the "white" variants of the pigmented cocci

ANTIGEN	PIGMENT	MANNITOL	NO. 635						NO. 644						NO. 635A635						NO. 644A635						NORMAL			NaCl	DATES
			1:50	1:100	1:200	1:400	1:800	1:1600	1:3200	1:50	1:100	1:200	1:400	1:800	1:50	1:100	1:200	1:400	1:800	1:50	1:100	1:200	1:400	1:20	1:40	1:80		
635	Orange	+	C	C	C	C	C−	C−	0	C	C	C	C	0	1−	0	0	0	0	0	0	0	0	1−	0	0	00	5/8
635A	White	+	C	C	C	C	C	C−		C	C	C	0	0	1+	1−	1−	0	0					C	1+	0	00	
597	Orange	+	C	C	C	1−	0	0		C	C	C−	0	0	0	0	0	0	0	0	0	0	0	0	0	0	00	
597A	White	+	C	C	C	C	C	1+		C	C	C	C	1+	0	0	0	0	0	0	0	0	0	0	0	0	00	
666	Orange	+	C	C	C	C	2+			C	C	C	0	0	0	0	0	0	0	0	0	0	0	1−	0	0	00	
666A	White	+	C	C	C	C	1+			C	C	C	1+											C	1+	0	00	
635	Orange	+	C	C	C	C	3+	1+	0																		00	5/12
635A	White	+	C	C	C	C	C−	1+	0																		00	
597	Orange	+	C	C	C	2+	1+	0																			00	
597A	White	+	C	C	C	C	C−	2+	1+																		00	

absorptions. It was thought at first that no. 770-W might be the non-pigmented form of no. 770-O.

In this connection it should be noted that the dissociation of non-pigmented strains from pigmented ones is by no means infrequent. Our no. 635 strain dissociated white colonies spontaneously on more than one occasion when the strain had been carefully purified. However, the greater number of these white colonies regain their orange color later, but the individual colonies vary greatly in this respect.

Inasmuch as strain no. 635 was used for developing a pure "yellow" antiserum it was desirable to be sure that the white colonies that it occasionally dissociated were not different antigenically. In fact they were always identical serologically as is clearly shown in table 6 by the agglutinations and absorptions. Moreover the "white" colonies always fermented mannitol.

RESULTS OF MANNITOL FERMENTATION

Despite the discrepancies in the reports as to the reliability of mannitol fermentation as a basis of differentiation, all of the cultures included in this series were tested in mannitol broth. Sugar free veal infusion broth, fermented with *B. coli*, and adjusted to a pH of 6.8 was used, plus bromothymol blue indicator after the method of Baker (1922). One per cent mannitol was added after sterilization. Cultures were incubated for seven days and readings made at one, three, five and seven days, respectively. As a rule, acid forming cultures had decolorized the green of the indicator to a yellow at the end of the third day. All acid forming cultures were further tested with methyl red. Readings were made after the method of Clark (1920) and no electrometric readings were taken.

All of the orange pigmented cultures fermented mannitol with the formation of acid but not of gas, reaching a pH of 6.0 to 5.4 at the end of seven days. All of the non-pigmented cultures, with one exception, *failed* to form either acid or gas in mannitol broth. Only one non-pigmented staphylococcus of this series fermented mannitol. Four yellowish cocci from gall-bladder material failed to ferment mannitol.

The results of the fermentation tests are summarized in table 7.

In view of the fact that our results with mannitol fermentation agree with those of Hine (1922) and are at variance with those of Winslow (1908), of Walker and Adkinson (1917) and of Julianelle, tests were repeated on a few of the cultures with the media of Julianelle (1922) and of Winslow, consisting of a 1 per cent pepton broth to which 1 per cent mannitol was added. The results are shown in table 8.

TABLE 7

Showing the results of the mannitol fermentation tests

COLOR	MANNITOL POSITIVE	MANNITOL NEGATIVE	TOTAL
Orange	33	0	33
White	1	20	21
Yellow	0	4	4
Total	34	24	58

TABLE 8

Showing the results in pH of further mannitol fermentation tests after seven days incubation

STRAIN	INFUSION BROTH	PEPTON BROTH
644	6.8	6.8
646	6.8	6.8
657-B	6.8	6.8
659	6.8	6.8
734	6.4	5.2
630	5.6	5.2
635	6.0	5.4
657	6.0	5.4

It is therefore apparent from table 7 that with the strains under consideration mannitol fermentation was in the main correlated with pigment formation. Upon the addition of mannitol, either sugar free infusion broth or pepton broth provided a suitable medium for differentiation.

For routine diagnostic work staphylococci that produced an orange pigment were reported as *Staphylococcus aureus* and non-pigmented cultures as *Staphylococcus "albus."* The aureus

cultures sometimes showed good color in twenty-four hours, but in many cases several days passed before pigmentation was obvious. Upon plating, orange colonies and white colonies were sometimes obtained from a pigmented culture. Some of the white colonies so obtained remained colorless during a long series of subcultures but later became pigmented. Some of the chromogenic cultures lost and regained chromogenesis during the period that they were under observation. This is in accord with the observations of other workers who have found chromogenesis to be a variable function, easily influenced by environmental conditions.

A few of the gall-bladder cocci were yellowish in color and seemed to be of the citreus type. The majority of the cultures however, were definitely either orange or colorless. Practically all of the staphylococci studied showed definite hemolysis when planted on blood agar.

LITERATURE REVIEW AND DISCUSSION

The earliest attempts at classification appear to have been based largely upon differences in pigmentation. Kolle and Otto in 1902 first differentiated pathogenic from saprophytic forms by means of agglutination tests. Gordon (1905), after extensive work on the classification of the streptococci, undertook the classification of the staphylococci. His contributions in 1905 and 1906 deal with studies on several hundred staphylococci, "mainly white forms," and using liquefaction of gelatin, coagulation of milk and acidification of mannitol and glycerol as means for separation into four types.

Dudgeon (1908) found slightly more active fermentative powers associated with the production of orange pigment but considered the white and orange cocci varieties of a single species. The orange forms attacked mannitol, glycerol and raffinose actively. The Winslows, also in 1008, divided the cocci into two main groups, one essentially parasitic and active in fermentative powers (orange and white) and the other (yellow and red) saprophytes and with more restricted fermentative powers.

They further divided them on the basis of gelatin liquefaction associated with pigment production and considered the orange and the white forms as constituting different genera. Kligler (1913) substantiated the Winslows' basis of separation by means of the rate of liquefaction of gelatin.

Walker and Adkinson (1917), working with sera of rather low titer (C/250), found that aureus immune serum would agglutinate with aureus but not with albus strains, and that albus immune serum would agglutinate with albus but not with aureus strains. They used Hiss serum water for fermentation tests and found that while aureus always fermented mannitol, ten strains of albus were manitol-negative while five were mannitol-positive. Winslow, Rothberg and Parsons (1920) find that staphylococci from pathogenic sources tend to ferment more strongly then saprophytic, but concluded that there is no justification for a "generic distinction between the orange and the white staphylococci." They place mannitol among the carbohydrates "attacked so rarely as to be of no serious diagnostic value."

Julianelle (1922) has done intensive work on the serological and biochemical relations of 25 staphylococci, including four saprophytic forms, and divides them into three main groups and two subgroups on the basis of agglutination and absorption tests. But "these groups do not conform strictly with differences in pigment formation and other biological characters (Bergey)." Julianelle finds that mannitol is always fermented by his staphylococci, in fact finds no difference in fermentative faculties between his parasitic and saprophytic forms.

Hine (1922) classifies 81 pathogenic staphylococci by serological means into two main groups, the pyogenes and the epidermidis, and finds that in general the pyogenes group are pigmented and ferment mannitol while the epidermidis group in general are non-pigmented and do not ferment mannitol. By absorption tests he found further subgroups within his two main groups.

In Bergey's "Manual of Determinative Bacteriology" (1923) the staphylococci are classified first on the basis of pigment formation, orange pigmented being called *Staphylococcus aureus*,

lemon yellow cocci *Staphylococcus citreus*, and the white forms *Staphylococcus epidermidis*, *albus*, etc. Aureus, citreus and albus are fermenters of mannitol while epidermidis is not. Bergey does not indicate the serological relationship of the various strains. Culture no. 39 would be *Staphylococcus albus* according to Bergey but serologically it is identical with *Staphylococcus aureus* and we have so classified it, regarding it as a non-pigmented aureus. *Staphylococcus citreus*, according to the "Manual," is a lemon yellow mannitol fermenter. Our lemon yellow cultures are all mannitol-negative and serologically belong to our type aureus, or are of a subgroup, or they may be a distinct antigenic aureus. Moreover these aureus groupings are components of the common ancestor strain no. 644.

Our results with mannitol fermentation are particularly interesting in view of the divergent reports of other bacteriologists. Winslow and Rothberg report that the staphylococci are generally mannitol-negative; Julianelle reports them invariably mannitol-positive; Walker and Adkinson found that while pigmentation and agglutination were correlated and aureus always fermented mannitol, *Staphylococcus albus* (Epidermidis?) was mannitol-positive in a few cases and mannitol-negative in others. Hine on the other hand found pigmentation, mannitol fermentation and serological affinities to be closely correlated and most of our strains happen to confirm this correlation in a general way.

Although pigment formation and mannitol fermentation taken together served to allocate in a general way the majority of the strains with which we worked, with another series of strains these characters might not be so successful. Indeed as we indicate they are by no means final criteria. Then, too, a mannitol-negative, non-pigmented strain may not be an epidermidis strain in the sense of being serologically distinct from the aureus strain as nos. 644 and 1 show clearly.

Although as we have already remarked, our primary object was not to set up a classification of staphylococci, the probable lines of descent indicated by the common ancestor strain possessed of component groups—both as to antigenic constitution

and pigment formation—help us to see the characters of the group in somewhat better perspective. The results seem to indicate that on a broad biologic basis it is not feasible to consider but one character in classification, however desirable it may be on practical grounds. The characters must be taken as a whole and with flexibility of limits, else inevitably we shall have a varying number of unclassifiable strains.

CONCLUSIONS

1. We have described a non-pigmented strain of staphylococcus which we regard as a probable common ancestor of distinct strains of *Staphylococcus aureus* and *S. albus*.

2. The aureus strains, which cross agglutinate and absorb from the antiserum of the non-pigmented common ancestor strain, represent a dissociation of a strain possessed of "specific," "pigmented" agglutinogen, potentially present in the parent non-pigmented strain.

3. This pure parent strain contains as its antigenic components at least two distinct "aureus" agglutinogens and at least one "albus" agglutinogen.

4. Explanation is offered for the genetic origin of many of the "atypical" strains, i.e., those where correlation of such characters as pigment production and mannitol fermentation fail.

5. The heterogeneity resulting from this situation renders classification especially difficult, if the resulting system is supposed to make for ready identification on the basis of a single set of characters.

6. Of the 58 strains studied relatively few of the apparently pure "aureus" agglutinogens were closely related to these groups present in the common ancestor strain. They may have originated from other similar ancestral strains with multiple agglutinogens. On the other hand, the majority of the albus strains were similar to the "white" agglutinogen of the "parent" strain.

7. It seems probable that this represents one of several wild or aboriginal saprophytic strains that become dissociated into

the more parasitic albus and aureus strains. This dissociation may be a mechanism of adaptation when a strain becomes pathogenic.

REFERENCES

BAKER 1922 Jour. Bacteriol., **7**, 301.

BERGEY, DAVID H. 1923 Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore.

CLARK, W. MANSFIELD Determination of Hydrogen Ions. The Williams & Wilkins Co., Baltimore.

DUDGEON 1908 Jour. Pathol and Bacteriol., **12**, 242. (Cited after Winslow.)

GORDON 1905 Supp. 33rd Ann. Report Local Govt. Bd. containing Report of Medical Officer for 1903–1904, 388. (Cited after Winslow.)

GORDON 1906 Ibid., 1904–1905, 387. (Cited after Winslow.)

HINE 1922 Lancet, **203**, 1380.

JULIANELLE 1922 Jour. Infect. Dis., **31**, 256.

KLIGER 1913 Jour. Infect. Dis., **12**, 432.

KOLLE AND OTTO 1902 Ztschr. Hyg. u. Infekt., **41**, 369. (Cited after Winslow.)

MELLON, RALPH R., CALDWELL, DOROTHY W., AND WINANS, W. W. 1925 The mother's milk as a source of infection in epidemic pemphigus neonatorum (impetigo contagiosa bullosa). Amer. Jour. Med. Sci., **169**, 419.

MELLON, RALPH R., AND CALDWELL, DOROTHY W. 1925 Further observations on the epidemiology of pemphigus neonatorum. Amer. Jour. Med. Sci., **169**, 736.

MELLON, RALPH R., AND JOST, ELIZABETH 1926 Studies in microbic heredity. III. The hereditary origin of group and specific agglutinogens among the colon-alkaligenes organisms. Jour. Immunol., **11**, 139.

WALKER AND ADKINSON 1917 Jour. Med. Res., **35**, 373.

WINSLOW 1908 The Systematic Relationships of the Coccaceae. John Wiley & Sons, N. Y.

WINSLOW, ROTHBERG AND PARSONS 1920 Jour. Bacteriol., **5**, 145.