

LYSOGENY IN STAPHYLOCOCCI

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ABSTRACT

BLAIR, JOHN E. (Hospital for Joint Diseases, New York, N. Y.) AND MIRIAM CARR. Lysogeny in staphylococci. *J. Bacteriol.* **82**:984-993. 1961.—Changes in the phage typing patterns of strains of staphylococci of the 80/81-52/52A/80/81 complex and of phage group III were produced by lysogenization with temperate phages derived from selected strains of *Staphylococcus aureus*. The phages used were of the serological groups A, B, and F. Certain changes of phage pattern were related to serologically specific prophage immunity; others were nonspecific, or resulted from the conversion of a strain from partial resistance to full sensitivity to certain typing phages. In addition to an alteration of their phage typing pattern, the lysogenization of certain strains by appropriate phages effected a reversal of their susceptibility to penicillin. The capacity to produce toxin was conferred upon certain nontoxigenic strains by lysogenization with a phage derived from a toxigenic strain.

It has been established that many coagulase-positive strains of *Staphylococcus aureus* are lysogenic. When the prophage harbored by a given strain is transferred to an appropriate host by lysogenization, the phage-recipient strain tends to acquire new attributes. Changes in the phage typing pattern have been shown by several investigators to follow the *in vitro* lysogenization of staphylococci by suitable phages. There is evidence that the phages harbored by lysogenic strains usually are active only upon other strains belonging to the same broad phage group (Wahl and Fouace, 1954; Rosenblum and Dowell, 1960).

Strains of type 80/81 are widely distributed and are encountered frequently in the course of routine phage typing of staphylococci. Often associated with them are strains which show the typing patterns 52/52A/80 or 52/52A/80/81. The similarity of the phage patterns suggests that such strains, and strains with patterns com-

prised of various combinations of these phages, are closely related. It is conceivable that some of the differences in the phage patterns of presumably related strains that are observed during typing are the result of lysogenization.

The relationships of strains of the 80/81-52/52A/80/81 complex have been demonstrated by Asheshov and Rippon (1959) and by Rountree (1959) and have been confirmed by Comtois (1960), Rosenblum and Jackson (1960), and Sakuri et al. (1961). Sakuri and his associates have presented evidence that subtypes exist among strains with the patterns 80/81, 52/52A/80, and 52/52A/80/81, and that one type can be converted to another by lysogenization.

The observations reported by Asheshov and Rippon (1959) and by Rountree (1959) are mutually confirmatory and indicate that certain changes in phage typing patterns which follow artificial lysogenization are the result of serologically specific prophage immunity; cross-immunity is responsible for some changes, for a serological group F phage was found to produce resistance to serological group A typing phages. The phage typing pattern of strains of type 80/81 was reported to be changed to 52/52A/80/81 after lysogenization by certain serological group A phages. The mechanism responsible for this apparent increase in susceptibility to the serological group B phages 52 and 52A has not been fully elucidated, although Rountree (1959) has suggested that the change may result from the selection of pre-existing, phage-sensitive mutants by the lysogenizing phage.

The studies on lysogenization in staphylococci mentioned above have been directed largely toward investigation of the relationships of strains of the 80/81-52/52A/80/81 complex and the possible significance of lysogenization in explanation of the differences in patterns that sometimes are observed in the routine typing of these strains. It is the purpose of this article to record observations in confirmation of these relationships, to

report changes in the phage typing pattern of group III strains after lysogenization, and to describe the transfer of certain other properties of staphylococci by lysogenization. These include the alteration of susceptibility to penicillin and the conversion of nontoxicogenic strains into strains capable of producing a potent toxin.

MATERIALS AND METHODS

Strains and phages used. Both field cultures and selected propagating strains for the standard typing phages were used. The majority of the field cultures were isolated in this laboratory; a few were received from other laboratories for phage typing. Cultures were typed with the 21 basic phages: 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 42D, 81, and 187, and with one additional phage, 83. Phage 81 and its propagating strain were received from E. T. Bynoe, Laboratory of Hygiene, National Department of Health and Welfare, Ottawa, Canada. Phage 83 was isolated by the authors, in this laboratory, and was originally designated as "phage VA4". All other typing phages and their propagating strains were received from the Staphylococcus Reference Laboratory, Central Public Health Laboratory, London, England, through the courtesy of R. E. O. Williams. A phage, 47C, which has been removed from the routine typing series, was employed in certain tests; it also was received from Dr. Williams.

Culture media. The culture media employed for all work with the phages were No. 162 Trypticase soy broth and No. 168 Trypticase soy agar, purchased from the Baltimore Biological Laboratory, Inc., Baltimore, Md.

Lysogenicity. Strains to be examined for their lysogenic capacity were grown in broth for 5 hr at 37 C. After centrifugation, the supernatants were spotted on suitable indicator strains spread on the surface of agar plates. The plates were incubated for 20 hr at 30 C.

Preparation of phages. The typing phages were propagated, titrated, and controlled by methods previously described (Blair and Carr, 1960a).

Appropriate indicator strains were used as propagating strains for the preparation of stocks of the phages derived from lysogenic strains. Propagation was initiated with the supernatant of a 5-hr broth culture of a lysogenic strain, and was carried out either on the surface of agar plates incubated for 20 hr at 30 C, or in broth

rotated during incubation at 37 C for 5 hr. Supernatants of the broth cultures or of broth washings from the agar plates were filtered through Seitz E. K. filter pads. The serological group of each phage was determined by methods described by Rountree (1949), using specific antisera prepared by the immunization of rabbits to phages of the serological groups A, B, F, and L; these sera were controlled with group-specific antisera received from R. E. O. Williams.

Lysogenization. Strains to be lysogenized were grown in broth at 37 C for 5 hr and spread with a sterile applicator on the surface of agar plates. About 0.1 ml of undiluted phage filtrate was deposited on the plate and spread over the seeded area with a loop, leaving a small portion as a control, and the plate was incubated for 20 hr at 30 C. The secondary growth within the area of confluent lysis was touched with a straight needle and streaked widely on an agar plate. After incubation overnight at 37 C, 10 well-isolated, non-eroded colonies were fished to agar, and subsequently subcultured to broth for determination of their phage patterns. This procedure eliminated any phage that might have been picked up with the secondary growth from the plate used for lysogenization. A strain was considered to have been lysogenized when: (i) it exhibited an altered phage pattern but was resistant to the lysogenizing phage, (ii) the supernatant of a broth culture lysed the parent strain, and (iii) it showed no evidence of contaminating phage when a whole broth culture was spread on agar.

For the purpose of this article, phages derived from lysogenic strains bear the number of the donor strain and are distinguished from the typing phages by the prefix "L", e.g., phage L2043 was carried by strain 2043. A lysogenized strain is identified by its number followed in parentheses by the number of the lysogenizing phage, e.g., 2003 (L2043).

Antibiotic susceptibility. All strains examined for their susceptibility to antibiotics were screened by the disc method, using low concentration antibiotic discs (Baltimore Biological Laboratory). The results of the disc tests were confirmed by the tube dilution method.

Penicillinase. To demonstrate the presence of penicillinase, pour plates were prepared containing 0.05 unit of penicillin/ml of agar and seeded with a suitable dilution of a known penicillin-sensitive strain. The indicator strain was

S. albus 151 (American Type Culture Collection), which had been shown by the tube dilution method to be inhibited by 0.05 unit/ml of penicillin. Sterile filter paper strips, 1 by 6 cm, were immersed in the supernatants of 24-hr broth cultures of the strains examined, drained free of excess fluid, and placed on the surface of the agar. Control strips immersed in sterile broth and in broth containing 10 units/ml of commercial penicillinase were included. The plates were incubated for 24 hr at 37 C. The presence of penicillin-inhibitor was indicated by growth of the indicator strain in a zone surrounding the paper strip.

Production and testing of toxin. For the production of toxin, cultures were grown in "soft" agar prepared by the addition of 0.5% agar and 1% proteose-peptone to Difco heart infusion broth (Difco Laboratories, Detroit, Mich.). The plates were seeded with 1 ml of a 20-hr broth culture of the test strain and were incubated at 37 C for 48 hr in an atmosphere containing 30% CO₂. The soft-agar cultures were strained through gauze and centrifuged, and the supernatants were filtered through Seitz E. K. filter pads.

To determine the α -hemolysin titer, decreasing amounts of filtrate were dispensed in serological tubes and made up to 1 ml with physiological salt solution; an equal volume of 2% fresh, washed rabbit erythrocytes was added to each tube and the set, with a suitable erythrocyte control, was incubated in a water bath for 1 hr at 37 C. The hemolytic titer was recorded as the smallest volume of filtrate which produced complete lysis of the cells. To demonstrate lethal toxin, 0.5 ml of the undiluted filtrate was injected intravenously into white mice weighing from 16 to 20 g. Ten mice were used for each test for lethal toxin. *S. aureus* Wood 46, of known toxigenicity, was included as a control in the preparation and testing of each series of cultures examined.

RESULTS

Lysogenicity of strains. Strains showing the typing pattern 80/81 were found to be nonlysogenic. No evidence of lysis was observed when the supernatants of 5-hr broth cultures of 36 such strains were spotted on 48 indicator strains showing the patterns 80/81 or 52/52A/80/81. This confirms the reports by Asheshov and Rippon (1959) and by Rountree (1959) that

strains of type 80/81 do not harbor serological group B phage, and it agrees with the observation by Rosenblum and Jackson (1960) that none of 50 strains of type 80/81 was lysogenic. It may be noted, however, that Rountree (1959) demonstrated the carriage of a serological group F phage by three strains of type 80/81. The phage was active on only a single indicator strain, was propagated with difficulty, and could not be detected in three other strains of type 80/81. We were unable to demonstrate a serological group F phage in any of our strains of type 80/81, nor were Rosenblum and Jackson able to do so in the 50 strains which they examined.

In the present study, those strains of the 80/81-52/52A/80/81 complex that were found to be lysogenic showed the typing patterns 80, 81, 52/52A/80, or 52/52A/80/81. Of 37 strains showing one of these patterns, 15 (40.5%) were lysogenic. One strain was type 80 and two were type 81; 9 of 12 strains showing the pattern 52/52A/80 and 3 of 22 strains of type 52/52A/80/81 were lysogenic. The incidence of lysogenic strains in types 52/52A/80 and 52/52A/80/81 is comparable to the figures given for strains of those types by Asheshov and Rippon (1959) and by Rosenblum and Jackson (1960), respectively. In contrast, Rountree (1959) reported that 7 of 15 strains typing as 52/52A/80/81 were lysogenic.

In group III, 13 (86.6%) of 15 strains were found to be lysogenic when tested against 17 indicator strains of the same broad phage group.

TABLE 1. Changes in the phage typing patterns of strains of the 80/81-52/52A/80/81 complex

Strain	Phage pattern	Phage pattern after lysogenization	
		By phage L2026 (Serol. A)	By phage L2043 (Serol. B)
2003	80/81	52/52A/80	81
2005	80/81	52/52A/80	81
PS80	80/81	52/52A/80	81
PS81	80/81	52/52A/80	81
2015	52/52A/80/81	52/52A/80	81
2017	52/52A/80/81	52/52A/80	81
2044	52/52A/80/81	52/52A/80	81
2043	81	Nontypable	Not lysogenized

TABLE 2. *Lysogenization of strain 2043 by serological A phages*

Strain	Reaction* with phages at RTD					Reaction* with undiluted phages				
	47C	52	52A	80	81	47C	52	52A	80	81
2043	-	-	-	-	++	()	()	()	()	++
2043 (L2026)	-	-	-	-	-	-	()	()	()	()
2043 (L2026)(L2002)	-	-	-	-	-	-	++	++	++	++
2043 (L2026)(L2054)	-	-	-	-	-	-	++	++	++	++

* Symbols used: ++ = strong lysis; () = inhibition; and - = no lysis.

Included among the lysogenic strains were all of the propagating strains for the nine group III typing phages used in this study. This incidence of lysogenic strains corresponds closely to the observation by Rosenblum and Dowell (1960) that a total of 28 (90%) of 31 strains showing group III patterns was lysogenic.

Changes in phage pattern following lysogenization of strains of the 80/81-52/52A/80/81 complex. Four strains of type 80/81, three of type 52/52A/80/81, and one of type 81 were lysogenized by the serological group A phages L13, L2002, L2026, and L2054, and by the serological B phages L2043 and L2050. The results obtained with the serological A phage L2026 and the serological B phage L2043 are typical of those produced by the several other lysogenizing phages and are illustrated in Table 1. Strain 2026, the carrier of a serological A phage, shows the typing pattern 52/52A/80 and is resistant to phage 81. Strain 2043 carries a serological B phage, is type 81, and is not lysed by the B phages 52, 52A, and 80.

When strains showing the pattern 52/52A/80/81 were lysogenized by the serological A phage L2026, the resulting pattern was characterized by a loss of sensitivity to phage 81; the pattern, in fact, was identical with that of the phage donor strain. Strains of type 80/81 also were made resistant to phage 81; in addition, they acquired sensitivity to phages 52 and 52A to give the pattern 52/52A/80. It was noted by Rippon, cited and confirmed by Rountree (1959), that the transfer of certain serological A phages to strains of type 80/81 may result in a gain in sensitivity to the serological B phages 52 and 52A. It has been pointed out by Asheshov and Rippon (1959) and by Rountree (1959) that strains which type as 80/81 at the routine test dilution (RTD) in reality are not completely resistant to the

typing phages 52 and 52A. Rountree (1959) reported, confirmed by us, that when strains of type 80/81 are exposed to undiluted phages 52 and 52A and to the serological A phage, 47C, they exhibit reactions of "inhibition" by those phages. She has suggested that the apparent gain in sensitivity to phages 52 and 52A following lysogenization by a serological A phage represents a change from partial resistance to full sensitivity rather than from complete resistance to sensitivity. Such strains become resistant to phage 47C.

When lysogenized by the serological B phage L2043, seven strains with the patterns 80/81 or 52/52A/80/81 were converted to type 81 and thus assumed the typing pattern of the phage donor strain. Strain 2043 could not, of course, be lysogenized by the phage which it carried.

Lysogenization by an appropriate phage may convert a typable strain into one which is nontypable at RTD. Strain 2043 is type 81 and is inhibited by undiluted phages 52, 52A, and 80. When lysogenized by the serological A phage L2026, the resulting strain 2043 (L2026) was not lysed by the typing phages at RTD (Table 2). However, it retained some degree of susceptibility to these phages, for it showed inhibition reactions when the phages were applied undiluted. All clones of 2043 (L2026) were coagulase-positive. An attempt was made to convert the nontypable 2043 (L2026) to full sensitivity to the serological B phages by lysogenization with the serological A phages L2002 and L2054. Although it remained refractory to typing at RTD, it would appear that the susceptibility of strain 2043 (L2026) to the phages was increased to some degree because, instead of inhibition, all colonies exhibited strong lysis in the pattern 52/52A/80/81 when they were exposed to those phages undiluted.

Changes in phage pattern following lysogeniza-

tion of strains of group III. Six strains which exhibited phage-typing patterns in group III were lysogenized by phages derived from four group III strains. The lysogenizing phages were LPS47, LPS53, LPS83, all derived from the propagating strains for standard typing phages, and L1738, which was carried by a strain isolated from an infected wound. The typing phages should not be confused with the lysogenizing phages carried by their propagating strains. The typing phage 47 is serological group A, and its propagating strain yielded the serological A phage designated as LPS47; the typing phage 53 is serological B, and its propagating strain carried a serological A phage, LPS53; the typing phage 83 also is serological B, and a serological F phage, LPS83, was isolated from its propagating strain. Phage L1738 belongs to the serological group A.

When six group III strains were lysogenized by two serological A phages, LPS47 and LPS53, a variety of changes was observed in the resulting phage patterns (Table 3). The variants produced with strain 157 showed identical patterns which differed distinctly from that of the parent strain. On the other hand, both lysogenizing phages tended to produce variants of the five other strains with patterns which differed from each other and from that of the parent strain. In general, the changes were such that, if the patterns had been encountered in the course of routine typing, the parent strains and their variants would be regarded as unrelated. Although strains 2056, 2058, and 2059 exhibited identical typing patterns, they were quite un-

related epidemiologically, both as to their source and the time of their isolation. In spite of their identical patterns, neither phage consistently produced identical variants from the three strains. The patterns of strains 2058 (LPS47) and 2059 (LPS47) were the same, but they differed by one reaction from that of strain 2056 (LPS47). Strains 2058 (LPS53) and 2059 (LPS53) showed patterns which differed by only a single reaction, but phage LPS53 did not lysogenize strain 2056.

In contrast to the somewhat random changes in phage pattern effected by these two serological A phages are the results which followed lysogenization of the same strains by another serological A phage, L1738, and by a serological F phage, LPS83 (Table 4). Except for strain 157, the patterns of each pair of lysogenized strains were identical and differed from that of the parent strain by at least two reactions. Patterns of the lysogenized strains of 157 were closely similar, although not identical, but they were distinctly different from that of the parent strain. The patterns resulting from lysogenization by these phages were quite unlike those produced by lysogenization of the same strains by phages LPS47 and LPS53. The notable feature of lysogenization by phages L1738 and LPS83 is the consistency with which both produced resistance to the typing phages 54 and 75. The development of resistance to "blocks" of phages, often rather closely related genetically, after lysogenization was observed by Lowbury and Hood (1953). The transfer of resistance to a block of genetically related phages occurred, of course,

TABLE 3. Changes in phage typing patterns of strains of group III following lysogenization by serological A phages

Strain	Original phage pattern	Lysogenized by phage	Phage pattern after lysogenization	Loss
PS6	6/7/47/53/54/75/77/83	LPS47	47/53/75/83	6, 7, 54, 77
		LPS53	53/75/77/83	6, 7, 47, 54
PS54	7/47/53/54/75/77/83	LPS47	7/47/53/54/75/77/83	—
		LPS53	7/53/75/77/83	47, 54
157	6/7/42E/47/54/75/83	LPS47	6/7/54/75	42E, 47, 83
		LPS53	6/7/54/75	42E, 47, 83
2056	47/53/54/75/77/83	LPS47	47/53/54/83	75, 77
		LPS53	47/53/54/75/77/83	—
2058	47/53/54/75/77/83	LPS47	47/53/54/75/83	77
		LPS53	53/75/77	47, 54, 83
2059	47/53/54/75/77/83	LPS47	47/53/54/75/83	77
		LPS53	53/54/75/77	47, 83

TABLE 4. *Changes in phage typing patterns of strains of group III following lysogenization by a serological A phage and a serological F phage*

Strain	Original phage pattern	Lysogenized by phage	Phage pattern after lysogenization	Loss
PS6	6/7/47/53/54/75/77/83	LPS83	6/47/53/77/83	7, 54, 75
		L1738	6/47/53/77/83	7, 54, 75
PS54	7/47/53/54/75/77/83	LPS83	7/47/53/77/83	54, 75
		L1738	7/47/53/77/83	54, 75
157	6/7/42E/47/54/75/83	LPS83	6/7/47/83	42E, 54, 75
		L1738	6/47/83	7, 42E, 54, 75
2056	47/53/54/75/77/83	LPS83	47/53/77/83	54, 75
		L1738	47/53/77/83	54, 75
2058	47/53/54/75/77/83	LPS83	47/53/77/83	54, 75
		L1738	47/53/77/83	54, 75
2059	47/53/54/75/77/83	LPS83	47/53/77/83	54, 75
		L1738	47/53/77/83	54, 75

when strains of type 52/52A/80/81 were lysogenized by the serological B phage L2043, for the related typing phages 52, 52A, and 80 are phenotypic modifications of a single phage type.

The typing phages 54 and 75 are of serological group A; they are not related genetically but their lytic spectra show that they have a rather similar host range. Strains 1738 and PS83 show closely similar typing patterns, both of which are characterized by resistance to the typing phages 54 and 75. It might be supposed therefore that their carried phages would confer immunity to phages 54 and 75, as is illustrated in Table 4. While this does not represent immunity to a group of genetically related phages, it would appear that an appropriate lysogenizing phage may confer resistance to those serologically related typing phages which have a closely similar host range. The phages L1738 and LPS83 are serologically different and exhibit distinctly different lytic spectra which have in common only strong lysis of PS54. The transfer of resistance to the serological A phages 54 and 75 by a serological F phage represents an example of cross-immunity between phages of unrelated antigenic structure, such as was reported in the 80/81-52/52A/80/81 complex by Asheshov and Rippon (1959) and by Rountree (1959).

Stability of lysogenized strains. The lysogenized strains were maintained on agar slants at room temperature, with occasional subculture, and their phage typing patterns were determined at intervals of a few months for periods up to 15 months. During the time of observation none

was found to revert to the pattern of the parent strain or to show other alterations of pattern.

Transfer of susceptibility to penicillin. In addition to the alterations of phage typing pattern described above, it was found that the lysogenization of certain strains also conferred a change in their susceptibility to penicillin. The susceptibility of phage-donor, phage-recipient, and lysogenized strains to 10 antibiotics was determined by the disc method, using low concentration antibiotic discs (Baltimore Biological Laboratory). The antibiotics employed were: penicillin, oxytetracycline, tetracycline, erythromycin, novobiocin, matromycin, oleandomycin, chloramphenicol, kanamycin, and dihydrostreptomycin. Twenty-one phage-donor and phage-recipient strains and 32 lysogenized strains were examined. A definite alteration of susceptibility to penicillin accompanying a change in the phage pattern was observed in certain strains. No reversal of susceptibility to the nine other antibiotics was observed.

The sensitivity of all lysogenized strains which exhibited altered susceptibility to penicillin by the disc method, together with that of the corresponding phage-donor and phage-recipient strains, was confirmed by the tube dilution method. In addition, the capacity of these strains to produce penicillinase was determined. Table 5 illustrates the reversal of susceptibility to penicillin, both from sensitivity to resistance and from resistance to sensitivity, and a corresponding change in the capacity to produce penicillinase in three group III strains and in one strain each of type 80/81 and type 52/52A/80/81.

TABLE 5. *Reversal of susceptibility to penicillin by lysogenization*

Strain	Phage pattern	Penicillin sensitivity		Production of penicillinase†
		Disc*	Tube dilution (inhibiting concentration in units/ml)	
PS6 Recipient	6/7/47/53/54/75/77/83	S	0.09	—
PS53 Donor	53/54/75/77/83	R	1.5	+
PS6(LPS53)	53/75/77/83	R	3.1	+
2005 Recipient	80/81	S	0.09	—
2026 Donor	52/52A/80	R	1.5	+
2005(L2026)	52/52A/80	R	50.0	+
2044 Recipient	52/52A/80/81	R	50.0	+
2054 Donor	52A/80	S	0.04	—
2044(L2054)	52/52A/80	S	0.04	—
2059 Recipient	47/53/54/75/77/83	R	50.0	+
PS83 Donor	6/47/53/77/83	S	0.04	—
2059(LPS83)	47/53/77/83	S	0.09	—
PS54 Recipient	7/47/53/54/75/77/83	R	1.5	+
PS54(LPS83)	7/47/53/77/83	S	0.04	—

* Discs contained 2 units of penicillin. R = resistant; S = sensitive.

† Production of penicillinase indicated by growth of indicator strain (+).

TABLE 6. *Transfer of toxigenic capacity by lysogenization*

Strain	Phage pattern	α -Hemolysin titer*	Lethal toxin†
2043 donor	81	0.03	+
2003 recipient	80/81	0.2	—
2003 (L2043)	81	0.01	+
2015 recipient	52/52A/80/81	0.2	—
2015 (L2043)	81	0.2	—
2017 recipient	52/52A/80/81	0.2	—
2017 (L2043)	81	0.02	+

* Volume of culture filtrate producing complete lysis of 1% rabbit erythrocytes in 1 hr at 37 C.

† Symbols used: + = death of all of 10 mice within 60 min after intravenous injection of 0.5 ml of culture filtrate; — = survival of all of 10 mice for 7 days after intravenous injection of 0.1 ml of culture filtrate.

As examples, the phage-donor strain 2054 was inhibited by 0.04 unit of penicillin and produced no penicillinase; the phage-recipient strain, 2044, required over 50 units of penicillin for inhibition and produced penicillinase, but after lysogenization by phage L2054 it produced no detectable penicillinase and its titer of inhibition by peni-

cillin dropped to 0.04 unit. When strain 2005, inhibited by 0.09 unit of penicillin and penicillinase-negative, was lysogenized by phage L2026, derived from a penicillinase-producing strain which was inhibited by 1.5 units, it was converted to a penicillinase-producing strain which was inhibited by 50 units of penicillin. It may be noted that the reversal of susceptibility to penicillin did not accompany an alteration of phage pattern in every instance. For example, phage LPS83 conferred sensitivity on two resistant strains but had no such effect on two other resistant strains, although their phage patterns were altered by lysogenization.

Four attempts were made to demonstrate penicillin-sensitive mutants in the resistant strain 2044 by the replica plating method of Lederberg and Lederberg (1952). Of a total of 1158 colonies replicated onto agar containing 2 units/ml of penicillin, only one (0.086%) was found to be sensitive; it was inhibited by 0.04 unit of penicillin and produced no penicillinase. In contrast, the incidence of changes in susceptibility to penicillin shown by the lysogenized strains was greater than could be explained by the presence of occasional mutants; when a reversal of susceptibility to penicillin occurred, it

was exhibited by all clones of the lysogenized strains examined.

Transfer of toxigenic capacity. Strain 2043 produces an exotoxin which lyses rabbit erythrocytes and is rapidly lethal for white mice when injected intravenously. Phage L2043, derived from this strain, was used to lysogenize three nontoxigenic strains, two of which acquired the capacity to produce lethal toxin.

Extensive experience in this laboratory has shown that the α -hemolysin titer rather closely parallels toxigenicity; a filtrate which completely lyses rabbit erythrocytes in a volume of 0.03 ml or less is lethal for mice within a period of from 2 min to about 4 hr. While nontoxigenic strains often produce zones of hemolysis on rabbit blood agar, their culture filtrates usually produce partial to complete hemolysis of 1% rabbit erythrocytes only in titers of 0.1 to 0.5 ml.

Table 6 illustrates the effect of lysogenization by phage L2043 on the toxigenic capacity of three nontoxigenic strains. The phage donor strain 2043 exhibited an α -hemolysin titer of 0.03 ml, and was lethal for mice in from 9 min to 3 hr. All three nontoxigenic strains showed α -hemolysin titers of 0.2 ml, and all mice injected intravenously with their culture filtrates survived for 7 days. Strain 2003 (L2043) showed an appropriate increase of α -hemolysin titer, and its culture filtrate was lethal for all of 10 mice within 2 min. Strain 2017 (L2043) showed a comparable titer of α -hemolysin, and its culture filtrate was lethal for all of 10 mice within a period of 10 to 60 min. All mice that died exhibited the characteristic symptoms of rapid respiration, paralysis of the hind legs, incontinence, and terminal convulsions. Transfer of the toxigenic capacity was accompanied by alteration of the phage pattern. Conversely, phages derived from two nontoxigenic strains did not reverse the toxigenic capacity of two toxigenic strains, although they conferred an alteration of phage pattern upon those strains.

The capacity to produce toxin was not conferred upon strain 2015, although lysogenization by phage L2043 effected a change in the phage pattern (Table 1); the α -hemolysin titer was unaltered and all mice survived intravenous injection of the culture filtrate for 7 days. The same strain did not acquire the capacity to produce toxin when lysogenized with phage L2054, also derived from a strain of known toxigenicity.

DISCUSSION

From the observations reported here, which confirm the studies by others on lysogenization of strains of the 80/81-52/52A/80/81 complex, it appears reasonable to conclude that those strains which show phage typing patterns composed of various combinations of these phages are closely related. It does not appear, however, that either type 80/81 or 52/52A/80/81 can be regarded with assurance as the prototype from which the several variants have been derived. Theoretically, either pattern could arise from the other. Strains of both types have existed for many years (Rountree, 1959; Blair and Carr, 1960b). In this laboratory, among 43 strains isolated between 1927 and 1947 which eventually were found to belong to the 80/81-52/52A/80/81 complex, 29 showed the patterns 52/52A/80 or 52/52A/80/81. When they were originally typed before phages 80 and 81 were available, many of them had shown the patterns 52, 52A, or 52/52A; one strain of type 80/81 was isolated in this laboratory in 1935 (Blair and Carr, 1960b).

Strains of type 52/52A/80/81 have been reported in recent years to emerge late in the course of prolonged hospital outbreaks in which the original inciting agent was a strain of type 80/81. It has been suggested that they were derived from type 80/81 (Asheshov and Rippon, 1959). The evidence cited earlier in this article suggests that this is quite possible and that the strains may have arisen either through the converting agency of a lysogenizing phage or by spontaneous mutation. It is less clear whether the pattern 80/81 may be derived from 52/52A/80/81. If this were so, type 80/81 should carry a serologic B phage which confers immunity to the typing phages 52 and 52A; serologic B prophage has not been demonstrated in strains of type 80/81. However, Sakuri and his associates (1961) have presented some preliminary evidence suggestive of the conversion of strains of type 52/52A/80/81 to type 80/81 by lysogenization with a phage derived from a type 80 strain. The lysogenized strains showed the inhibition by undiluted phages 52 and 52A that is characteristic of the classical type 80/81, but they differed from 80/81 by carrying a phage that was lysogenic for strains of types 52/52A/80 and 52/52A/80/81. Conversion to the pattern 80/81 was not always complete. The serological grouping of the phages was not described.

It is not known whether, or how frequently, lysogenization may take place in vivo. If it does occur, it could account for some of the differences in patterns that are shown by presumably related strains in the course of routine typing. It might also be responsible for the emergence of variants with identical or closely similar patterns from strains that have no epidemiological relationship. The hypothesis that in vivo lysogenization is responsible for some variations in phage pattern would be strengthened if it were possible to show that either of two strains present in a mixed infection in a single individual was lysogenic for the other. A few attempts by the authors to demonstrate such a relationship have been unsuccessful, but instances have been cited by Asheshov and Rippon (1959), Rountree (1959), and Sakuri and associates (1961) which suggest the possibility that lysogenization may take place under natural conditions. This is not to imply that all differences in the phage typing patterns of related strains can be attributed to natural lysogenization; this probably is not the case. However, the possibility that in vivo lysogenization may occur re-emphasizes the need to interpret carefully the patterns shown by a set of cultures that have been isolated from epidemiologically related sources. This interpretation is best accomplished by the close cooperation of the bacteriologist and the epidemiologist.

The reversal of susceptibility to streptomycin and novobiocin by staphylococcal phage has been reported by others. A change from sensitivity to resistance to streptomycin by lysogenization of a sensitive strain with a phage from a resistant strain was described by Cavallo and Terranova (1955); no alteration of sensitivity to penicillin was observed, although the phage donor strain was also resistant to that antibiotic. The transfer of resistance to streptomycin and novobiocin by the typing phage 53 was reported by Morse (1959). On the other hand, Comtois (1960) reported that a change in phage pattern following lysogenization of strains of type 80/81 was not accompanied by a change in their antibiotic susceptibility when the lysogenized strains were tested by the disc method against 20 antibiotics.

It is known that penicillin-sensitive, penicillinase-negative variants may emerge in cultures of resistant, penicillinase-producing strains (Barber, 1949). However, it would appear that the reversal of susceptibility to penicillin de-

scribed here was not due to the random appearance of variants but, rather, that it is a manifestation of the effect of the transferred prophage upon the capacity of a strain to produce the enzyme penicillinase. This is supported by the fact that the lysogenized strains acquired or lost their capacity to produce penicillinase in direct parallel with the presence or absence of that capacity in the phage donor strain.

Our observations indicate that the ability to produce toxin may be conferred upon certain nontoxigenic strains by lysogenization with a phage carried by a known toxigenic strain. It remains to be determined whether toxin production by *S. aureus* is controlled genetically by phage, as is the case with *Corynebacterium diphtheriae* (Barksdale, Garmise, and Rivera, 1961). Investigations on the relationship between phage and toxin production by staphylococci are now under way.

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