Richard Edward Mason, Jr. BIOCHEMICAL AND CULTURAL CHARACTERISTICS OF STAPHYLOCOCCUS AUREUS ASSOCIATED WITH LYSOGENIC CONVERSION TO LOSS OF BETA-HEMOLYSIN PRODUCTION. (Under the direction of Wendall E. Allen) Department of Biology, ECU, Greenville, N. C., February 1974.

The purpose of this investigation was: to determine the relationship between animal and human strains of Staphylococcus aureus to lysogenic conversion of the β-hemolysin and fibrinolysin characters; to determine if other physiological characteristics are affected by the phage conversion; and to study the mechanism of conversion. Strain 7-8, which is beta-hemolytic and non-fibrinolytic (β+K-) and of bovine origin, was lysogenically converted by several group F phages to β-K+. This is in accord with the work of Winkler et al. (1965). Serological group A phage 42E was found to convert strain 7-8 to β-K-. Production of coagulase, DNase, lipase, gelatinase, and mannitol fermentation were not correlated with lysogenic conversions to loss of β-hemolysin and gain in fibrinolysin production.

S. aureus strains of bovine origin are usually β-hemolytic, non-fibrinolytic, crystal-violet positive, and sensitive to group IV phages. Strains of human origin are usually non-β-hemolytic, fibrinolytic, crystal-violet negative, and insensitive to group IV phages. Beta-hemolytic and fibrinolytic strains can be lysogenically converted to loss of β-hemolysin production and gain in fibrinolysin production. Crystal-violet reactions were not affected by lysogenic conversion of β-hemolysin. Every lysogenically converted β- isolate was insensitive to group IV phage 42D. Further testing with additional group IV phages is necessary to determine if loss in sensitivity to group IV phages always accompanies

loss of β-hemolysin production.

Beta-hemolytic and non-fibrinolytic (β+K-) variants were regularly obtained from each of the 50 β+K+ appearing colonies of strain 7-8 (β+K-) isolated after treatment with nitrosoguanidine or ethyl methanesulfonate treated phage preparations. Since no stable β+K+ isolate was obtained from any of the β+K+ appearing colonies tested and since no β-K- colonies were found, the theory of Winkler et al. (1965) that conversion to β-K+ involves two separate loci on the phage chromosome was not confirmed by use of phage mutants.

Conversion of β-hemolysin by lysogenization of a serological group A phage has not previously been reported. Phage 42E conversions differed from the group F conversions since fibrinolysin was not affected. This indicates that conversion to β-K+ involves two separate loci on the phage chromosome.

BIOCHEMICAL AND CULTURAL CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* ASSOCIATED WITH LYSOGENIC CONVERSION TO LOSS OF BETA-HEMOLYSIN PRODUCTION

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Dedicated

to

My Wife, Mary

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INTRODUCTION

Many attempts have been made to correlate various biochemical and cultural characteristics to coagulase production in pathogenic strains of staphylococcus. DNase activity, yellow pigmentation, mannitol fermentation, and gelatinase activity have been correlated to coagulase production and have been considered cirteria for pathogenicity in staphylococci. Efforts to correlate coagulase activity with hemolysins, fibrinolysin, lipase, and lysozyme have been unsuccessful (Victor et al., 1969). Staphylococcal strains of bovine origin and human origin generally vary in some of these characteristics. Isolates of bovine origin are usually β-hemolytic, non-fibrinolytic, crystal-violet positive, and sensitive to phages of phage group IV. Isolates of human origin are generally non-β-hemolytic, fibrinolytic, crystal-violet negative, and insensitive to phages of phage group IV.

The production of β-hemolysin, α-hemolysin, δ-hemolysin, fibrinolysin, and lipase has been lysogenically converted in staphylococcal strains. Winkler et al. (1965) reported the double lysogenic conversion of S. aureus strains to loss of β-hemolysin and gain in fibrinolysin by lysogenization with serological group F bacteriophages.

The purpose of this investigation was: to determine if S. aureus strain 7-8 of bovine origin could be lysogenically converted to loss of β-hemolysin production and gain in fibrinolysin production by serological group F phage; to determine if β-hemolysin production could be affected by lysogenization with phage of serological groups A and B; to determine if other biochemical and physiological characteristics are simultaneously

affected along with lysogenic conversion to loss of β-hemolysin production; and to study the mechnaism of conversion. The results of this investigation may provide a better understanding of viral influence on staphylococcal characteristics and a better understanding of the nature of variation between S. aureus strains of human and bovine origin.

LITERATURE REVIEW

Characterization of Pathogenic Staphylococci of Human and Animal Origin

The most widely used method for distinguishing pathogenic staphylococci from closely related saprophytic species is the coagulase test (Victor, Lachica, Weiss, and Deibel, 1969). Many efforts have been made to correlate coagulase activity to other physiological characteristics. Yellow pigmentation is considered analagous to coagulase production in S. aureus strains of bovine or human origin (Cohen, 1972). Victor et al. (1969) reported that 95% of all coagulase-positive strains they tested produced DNase. Allen and Fellowes (1940), and Evans (1948) considered mannitol fermentation a criterion of pathogenicity, however, according to Cohen (1972) this may not be the case with strains of animal origin. Marks (1952) considered α-hemolysin production an indicator of virulence of S. aureus strains of human origin. It has been suggested that nearly all coagulase-positive S. aureus strains produce gelatinase and that strains of animal origin may be more active in that respect than strains of human origin (Cohen, 1972).

Variation in Physiological Characteristics

According to Victor et al. (1969) investigations relating the production of hemolysins, fibrinolysin, lipase or lysozyme to coagulase production failed to demonstrate a high correlation of any of these characteristics. Several workers reported that coagulase-positive strains of staphylococci of animal origin usually produce β-hemolysin and do not produce fibrinolysin, while coagulase-positive strains of

human origin usually produce fibrinolysin and do not produce β-hemolysin (Cohen, 1972). Meyer (1967) used crystal-violet reactions to distinguish S. aureus strains of bovine origin from strains of human origin. Of 2471 cultures of human origin, 84.9% were crystal-violet negative, while 81.2% of 618 bovine isolates were crystal-violet positive.

These studies indicate pathogenic S. aureus strains of bovine and human origin are likely to be coagulase positive, DNase positive, yellow pigmented and gelatinase positive. In general, human strains are also likely to be crystal-violet negative, fibrinolytic, non-β-hemolytic, mannitol fermenters and sensitive to phages other than those of phage group IV. Bovine isolates are likely to be crystal-violet positive, non-fibrinolytic, β-hemolytic, and sensitive to phages of phage group IV (Cohen, 1972).

Basis for the Differences in Physiological Characteristics

Omenn and Friedman (1970) reported the use of nitrosoguanidine (NTG) or ethyl methanesulfonate (EMS) for mutation of S. aureus strains to simultaneous loss of nuclease, coagulase, and β-hemolysin. Of 40 nuclease-negative strains, 39 were also coagulase negative and non-β-hemolytic. Recovery of all three properties after treatment with EMS was observed in 10 revertants from two mutant strains. Forsgren, Nordström, Philipson, and Sjöquist (1971) found that 34 of 60 S. aureus protein A-deficient mutants also lacked nuclease, coagulase, α-hemolysin, fibrinolysin, mannitol utilization, and the phage-type pattern. Mutants with combinations of these properties were also isolated. Simultaneous revertants for all or the combinations of these properties could be

induced or occurred spontaneously. The results of Omenn and Friedman (1970) and Forsgren et al. (1971) suggest that synthesis or release (or both) of a number of extracellular products of S. aureus is controlled by a common regulatory mechanism. The possibilities of a hypothetical plasmid carrying genes for all of these products or the deletion of these genes from a chromosomal location is ruled out since total revertants were isolated. Harmon and Baldwin (1964) reported isolation of a mutant that failed to produce detectable amounts of α-hemolysin, fibrinolysin, lipase, gelatinase, and casinase, but did produce nuclease, coagulase, and β-hemolysin. Omenn and Friedman (1970) suggested these results may indicate that two or more sets of genes for these extracellular enzymes are under co-ordinate but separate control. According to Zabriskie (1966), lysogenic conversion may also play an important role in mediation of production of bacterial toxins and other cellular products.

Differences in Phage Susceptability Patterns

In addition to testing for biochemical and cultural characteristics, many public health and hospital laboratories throughout the world have adopted phage typing for the further characterization of coagulase-positive staphylococci (Blair and Carr, 1960; and Blair and Williams, 1961). According to Cohen (1972) the recommended typing set contains 22 phages divided into four groups. In group I were phages 29, 52, 52A, 79, and 80; in group II were 3A, 3C, 55, and 71; in group III were 6, 42E, 47, 53, 54, 75, 77, 83A, 84, and 85; in group IV was 42D. In addition, some laboratories use other phages in routine phage typing.

According to Blair and Carr (1960) groups I, II, and III are broad groups, each comprising phages that exhibit some degree of specific action against staphylococci belonging to serologic groups 1, 2, and 3 respectively.

Staphylococcal phages have been serologically grouped by Rountree (1949) and Rippon (1956). According to Blair and Williams (1961) the recommended typing set contains phages of serological groups A, B, F, and L. Seto, Kaesberg, and Wilson (1956) reported differences in morphology of serological group A and B phages. Bradley and Kay (1960) studied the morphology of staphylococcal bacteriophages. Bradley (1963) divided staphylococcal phages into two morphological groups. Phages of serological groups B, F, and L were morphologically similar but distinct from serological group A phages. Dowell and Rosenblum (1962) reported a difference in caesium chloride buoyant densities of phages of serological groups A and B. According to Rosenblum and Tyrone (1964) staphylococcal phages of the same serological grouping are homogeneous in buoyant density and morphology. Group A phages have distinct oval heads with relatively long tails ending in a terminal knob. Phages of serological groups B, F, and L have round heads, usually smaller than group A phages, and a relatively short tail ending in a terminal knob. Bradley (1963) further subdivided the group B, F, and L phages (having round heads) into two subgroups based upon the length of the tail: (1) A short-tailed group consisting of the B and L phages, and (2) A long-tailed group consisting of the F phages. Rosenblum and Tyrone (1964) suggested the variation in length of the tails of these phages was not pronounced.

S. aureus strains of human origin are usually lysed by some phages of phage groups I, II, and III and may not be lysed by phage 42D or other phages of group IV. Bovine isolates are usually lysed by phage 42D or additional phages of group IV and may not be lysed by phages of groups I, II, and III (Cohen, 1972).

Lysogeny

Bacterial cultures isolated from nature were shown to be a primary source of phages (Adams, 1959). Bacteria latently infected with bacteriophage are called lysogenic and have two dominant characteristics: (1) they carry the potentiality to produce and release phages as a stable heritable trait; (2) they are immune to lysis by the lysogenizing phage (Hayes, 1968).

Detection of lysogeny usually depends upon the chance isolation of a sensitive indicator strain on which the phage will form plaques. For example, the K-12 strain of Escherichia coli was realized to be lysogenic for phage as a result of the accidental discovery that cultures of the strain produced plaques when plated with a mutant of strain K-12 that had lost the phage (cured) and had therefore become sensitive to it (Lederberg, 1951). The results of Rountree (1949), Gorrill and Gray (1956), and Blair and Carr (1961) suggested that most strains of S. aureus carry temperate bacteriophages. Lysogeny is so widespread among the staphylococci that it should be considered normal, rather than exceptional (Hayes, 1968).

Prophage-Bacterium Relationship

Lysogenic bacteria do not contain or harbor infectious phage

particles as no phages are released when these bacteria are disrupted (Hayes, 1968). Lwoff and Gutmann (1950) confirmed an earlier hypothesis that lysogeny represented a hereditary potentiality of the bacteria to generate phages. They used a micromanipulator to separate individual lysogenic cells in micro-drops for many generations. Their findings were: (1) many generations can pass without release of a single phage particle; (2) the appearance of phages in the micro-drop was correlated with the rapid disappearance of a bacterium; and (3) the mean number of phage particles liberated by lysis of each bacterium was high.

According to the "insertion hypothesis", presented by Hayes (1968), the phage genome (prophage) becomes integrated into the bacterial chromosome by some kind of recombination event so that a continuous structure is formed. Thus the prophage should be considered an episome that, according to Jacob and Wollman (1961), exists in alternation between a stable state of attachment to the chromosome and a state of autonomy.

Induction

Lwoff and Gutmann (1950) reported the proportion of bacteria liberating phages varied from experiment to experiment. They suggested phage production might be induced by external factors. Lwoff, Siminovitch, and Kjelgaard (1950) showed exposure of lysogenic Bacillus megaterium to UV-irradiation could result in induction of lysis of an entire culture. Gorrill and Gray (1956) used UV-irradiation for induction of lysogenic S. aureus cultures. Many other effective inducing agents have been found, e.g. X-rays, γ-rays, nitrogen mustards, hydrogen peroxides, organic peroxides, 6-azauracil, fluorodeoxyuridine, mitomycin-C and

thymine deprivation (Hayes, 1968).

There is reason to believe the phage genome is prevented from expressing its function by a cytoplasmic immunity substance or "repressor" that becomes inactivated upon UV-irradiation (Hayes, 1968). According to Lwoff et al. (1950) induction of lysis occurred only in rich growth medium. This suggested the response to inducing agents may be effected by the physiological condition of the host (Adams, 1959). Alternately, Gorrill and Gray (1956) suggested inducability was mainly a property of the prophage.

Curing

A bacterium that irreversably loses its episomal or plasmidal deoxyribonucleic acid (DNA) is said to be cured of that DNA. Barber (1949) observed that S. aureus strains were spontaneously and irreversably cured of penicillinase plasmids at a low frequency. This frequency increased after treatment at elevated temperatures (May, Houghton, and Perret, 1964) and after exposure to compounds such as acridine dyes (Harmon and Baldwin, 1964; and Hashimoto, Kono, and Mitsuhashi, 1964), and ethidium bromide (Bouanchaud, Scavizzi, and Chabbert, 1969). Sonstein and Baldwin (1972) reported 96.1 to 100% curing rates with two strains of S. aureus after treatment with the anionic surface-active agent sodium dodecyl sulfate.

Several additional methods were reported to cure lysogenic strains of S. aureus of their prophage. Duval-Iflah (1972) used: (1) exposure to high doses of UV-irradiation; (2) growth in the presence of acriflavine; and (3) repeated subculturing in the presence of 0.02 M sodium citrate. Jan, Van Der Vijver, Van Es-Boon, and Michel (1972) reported

curing of lysogenic S. aureus by 30 min. exposure to 1 μg/ml mitomycin-C while in the presence of specific antiphage sera. Cohen and Sweeney (1973) used UV-irradiation in presence of 0.1 M disodium ethylenediaminetetra-acetate (EDTA) and 0.15 M NaCl.

Lysogenic Conversion in Staphylococcus

Lysogenization is known to produce various changes upon the host bacterium, e.g. changes in phage sensitivity patterns, acquision of properties, and suppression of properties (Zabriskie, 1966).

Many workers (Asheshov and Rippon, 1959; Rountree, 1959; and Blair and Carr, 1961) have demonstrated changes in phage sensitivity patterns occurring upon lysogenization. According to Duval-Iflah (1972) three patterns of change may occur: (1) induction of specific prophage immunity, characteristic of lysogeny; (2) induction of broader, less specific phage immunity; and (3) increase in susceptibility to certain phage (See: Lowbury and Hood, 1953; Rountree, 1959; Asheshov and Rippon, 1959; Comtois, 1960; Rountree and Asheshov, 1961; and Rosendal and Bülow, 1965).

Several authors reported lysogenic conversion to gain of various properties by staphylococci. Blair and Carr (1961) reported a gain in penicillinase production in 4 strains of S. aureus by lysogenization with a serological group B phage and, in another case, a gain in α-hemolysin production by 3 group A phages and 1 group F phage. Both types of conversions were accompanied by changes in phage sensitivity. Clecner and Sonea (1966) reported lysogenic conversion to gain of δ-hemolysin production. Jan et al. (1972) reported lysogenic conversion to P-V leucocidin production by a serological group A bacteriophage.

Several authors have reported lysogenic conversion to the loss of various properties. Lysogenic conversion to loss of "Tween"-splitting enzyme activity was reported by Rosendal and Bülow (1965) and Rosendal, Bülow, and Jessen (1964). Duval-Iflah (1972) reported lysogenic conversion to loss of lipase production. Winkler, Waart, and Grootsen (1965) showed that lysogenic conversion to loss of β-hemolysin production by group F phages was always associated with a gain in fibrinolysin production. Only phage of serological group F have been reported to effect conversions concerning β-hemolysin production.

Phage Mutations Affecting Lysogenic Conversions in S. aureus

Various mutagenic agents have been used to increase the frequency of phage mutants. Benzer and Freese (1958) reported phage mutations were produced by ultraviolet light, nitrogen mustards, streptomycin, and proflavine. Benzer and Freese (1958) used the DNA base analogue 5-bromouracil, which is incorporated into the DNA of phages in place of thymine, as a phage mutagen. Novick (1967) used ethyl methanesulfonate (EMS) and nitrosoguanidine (NTG) to obtain staphylococcal-phage mutants.

Winkler et al. (1965) suggested that the correlation between lysogenic conversion by serological group F phages to loss of β-hemolysin and gain in fibrinolysin production might result from activity on two independent loci on the phage genome, or, the double conversion might result from action on a single regulator gene having a repressive effect on β-hemolysin production and a derepressive effect on fibrinolysin production. Winkler et al. (1965) used UV-irradiation in an attempt to obtain phage mutants that could convert one of these properties independent

of the other. One phage mutant appeared to convert a β-hemolytic, non-fibrinolytic strain of S. aureus to gain in fibrinolysin production without affecting β-hemolysin production. This phage mutant established an unstable lysogenic relationship with the host that easily reverted to the non-lysogenic state, remaining β-hemolytic but becoming non-fibrinolytic. Winkler et al. (1965) suggest this is the result of activity on two independent loci on the phage chromosome.

MATERIALS AND METHODS

Cultures

The culture of Staphylococcus aureus strain 7-8 used throughout this investigation was isolated from the teat of a cow with mastitis. S. aureus strains 879, 57, 491, 509, and lysogenic strains 879(491), 879(756), 879(269), 57(L80), and 879(509), were obtained from Dr. K. C. Winkler, State University, Utrecht, Netherlands. All lysogenic strains are indicated by the S. aureus strain number followed by the infecting phage strain number in parenthesis. All bacteriophages and S. aureus propagating strains (PS) of the International Phage Typing Series were purchased from The Sylvana Company, Milburn, New Jersey.

All cultures were maintained at 4 C on Trypticase Soy Agar, TSA, Baltimore Biological Laboratories (BBL), Baltimore, Maryland, containing 0.5% (w/v) Yeast Extract (BBL) and 400 μg/ml $CaCl_2$. In this report, all media containing $CaCl_2$ (CA) or Yeast Extract (YE) in the concentrations specified above will be indicated by the abbreviation of the added ingredient following the appropriate abbreviation for the medium, e.g. the medium just described will be called TSA-YE-CA. Incubation temperature was 37 C unless otherwise specified.

Cultural Characteristics

Beta-Hemolysin

Production of β-hemolysin was determined by growth of colonies on sheep blood agar plates. Sheep blood agar was prepared by adding 3% (v/v) whole sheep blood (BBL) to sterile molten TSA at 45 C. Eight strains

could be tested on a single petri plate of the blood agar by making uniform 2 cm long streaks of each culture with an inoculating loop and incubating the dishes overnight at 37 C followed by one hour at 4 C. Beta-hemolytic strains showed the typical hot-cold lysis on sheep blood agar medium.

Fibrinolysin

Fibrinolysin test agar, FTA, was prepared by a modification of the method of Christie and Wilson (1941). A 250-ml flask containing 88 ml Nutrient Agar, Difco Laboratories (Difco), Detroit, Michigan, and 0.5% (w/v) Bacto-Peptone (Difco) was melted and cooled to 56 C, 12% (v/v) sterile rabbit Coagulase Plasma (BBL) was added and the mixture was incubated at 56 C for 15-20 minutes in order to precipitate the fibrinogen. The precipitate was uniformly suspended by mixing and approximately 20-ml amounts were poured into petri dishes. Isolates to be tested for fibrinolytic activity were inoculated onto FTA with a sterile loop or spot-inoculated with sterile toothpicks. Thus, the fibrinolytic activity of 50-60 colonies could be determined on a single plate. All plates were incubated 24-48 hours. Colonies of fibrinolysin positive (K+) strains were surrounded by zones of clearing.

Coagulase and Mannitol Fermentation

Coagulase-Mannitol Broth (BBL) containing 15% (v/v) Coagulase Plasma (BBL) was used in all coagulase tests. Both mannitol fermentation and coagulase production were determined at the same time. The medium was prepared and the tests were performed as directed by the manufacturer.

DNase Production

DNase-Test Agar (BBL) was prepared and used according to the manu-

facturer's instruction. Eight isolates were inoculated per plate. After overnight incubation, plates were flooded with 0.1% (w/v) toluidine blue, Fisher Scientific Company (Fisher), Fair Lawn, N. J., in H_2O, allowed to stand 1-2 minutes, and the excess poured off. DNase-positive strains were surrounded with bright rose-pink zones. The color developed immediately and persisted for a day.

Lipase Production

Lipolysis was determined by use of Bacto-Spirit Blue Agar (Difco) as a basal medium. An emulsion of sterile cotton seed oil and water was prepared and added to the basal medium as directed by its manufacturer. Four cultures were tested per plate. After incubation overnight, lipolytic organisms were recognized by the occurrence of a deep-blue color beneath and surrounding the colony.

Gelatinase

Nutrient Gelatin (BBL) was prepared and tests were performed according to directions of the manufacturer. Results were taken after a seven-day incubation period at room temperature.

Crystal-Violet Reaction

A basal medium was prepared by modification of the method of Chapman (1936). The medium contained the following ingredients:

agar (BBL)	15 gm
Proteose Peptone (Difco)	5 gm
Beef Extract (Difco)	3 gm
lactose (Fisher)	5 gm
water	1000 ml
pH 6.8	

A 0.1% (w/v) stock solution of crystal violet (99% pure, Fisher) was prepared and added to the basal medium to a concentration of 1/100,000. The

mixture was autoclaved and dispensed into plates. An inoculating loop was used to transfer a heavy inoculum from overnight TSA slant cultures of four strains onto each plate of the crystal-violet-test medium. No growth was obtained when organisms were streaked to produce isolated colonies. The growth of crystal-violet-negative strains appeared purple after 24-hour incubation while crystal violet-positive strains appeared yellow-gold. In some cases the outer edges of growth of crystal-violet-positive strains were purple.

Bacteriophages and Bacteriophage Methods

The bacteriophages used in this investigation were either of the International Phage Typing Series or were obtained by UV-induction of lysogenic S. aureus strains. Phages of the International Phage Typing Series were prepared for use by propagation (see below). Phages 491, 269, 756, and 509 were obtained by UV-induction of lysogenic S. aureus strains provided by K. C. Winkler (see Cultures) and were used undiluted without propagation. Phages L42E, L80, and L81 were obtained by UV-induction of propagating strains of the International Phage Typing Series and were used undiluted without propagation. These phages are identified by the donor strain number and are distinguished from the typing phages by the prefix "L", e.g. phage L42E was obtained from PS 42E.

Induction of Bacteriophages

Modification of the UV-irradiation technique of Gorrill and Gray (1956) was used for induction of bacteriophages. Cultures were prepared for induction by growing with continuous gentle shaking (80 cycles per minute in a Magic-Whirl Constant Temperature Bath, Blue M Electric Com-

pany, Blue Island, Illinois) in 160 X 15 mm tubes containing 10 ml of Trypticase Soy Broth, TSB, (BBL) -YE-CA for 3 hours at 37 C. Cultures were then placed in glass petri plates and irradiated with gentle shaking for 45 seconds under a 15-watt germicidal lamp (General Electric) that delivered 0.65 X 10^4 ergs/cm^2-sec. to the surface of the liquid. The UV dosage used was measured with a Radiometer (model 65, Yellow Springs Instrument Company, Yellow Springs, Ohio). After irradiation, cultures were incubated for 2 hours to permit lysis. The lysates were sterilized by filtration through a 0.45 μ membrane filter (Gelman, Ann Arbor, Michigan).

The filtrates obtained by UV-irradiation of strains 7-8, 879, and 57 were tested for presence of phages by screening for lytic activity on the following indicator strains of S. aureus: 879, 57, 491, and 509, and all propagating strains (19) of the basic set used by Blair and Williams (1961), with the exception of PS 6 and PS 7. All cultures were grown overnight in TSB-YE-CA. Each strain was spot inoculated with a sterile cotton swab onto plates containing TSA-YE-CA. Up to 8 strains could be inoculated onto a single plate. A 0.01 ml portion of the UV-irradiation filtrate from the culture being tested was added to the area of inoculation of each indicator strain. After incubation overnight, plates were inspected for lysis indicated by any clearing within the area of growth of an indicator strain.

Phage Propagation, Titration, and Typing

Phage methods were according to standard methods of Blair and Williams (1961) with the exception that undiluted phage stocks were used in the determination of phage sensitivities. All bacteriophages of the basic set

of Blair and Williams (1961) were used for typing except phages 6 and 7. Phage preparations were sterilized by filtration as above.

Comparative Morphology of Serological Group A and Group F Staphylococcal Phages

Phages were prepared for electron microscopic examination by a modification of the technique of Bradley and Kay (1960). The undiluted phage suspension in TSB-YE-CA was centrifuged at low speed to remove bacterial debris. The phage particles themselves were then sedimented at 8900 X g and the supernatant fluid discarded. The pellet was resuspended in a 1% (w/v) solution of phosphotungstic acid. Electron photomicrographs were taken on a Hitachi HS-8 electron microscope.

Phage Mutation

Three methods were employed for mutation of phage 42D. Firstly, a modification of the nitrosoguanidine (NTG) method of Novick (1967) was used. A mixture of 3 plaque-forming units (PFU'S) of phage 42D/colony-forming unit (CFU) of PS 42D in 10 ml of TSB-YE-CA containing 100 µg/ml NTG (Sigma Chemical Company, St. Louis, Mo.) was incubated at 30 C with gentle shaking for 20 min. The mixture was then diluted 1:10 into TSB-YE-CA and the cells allowed to lyse at 30 C with gentle shaking. The ethyl methanesulfonate (EMS) method was identical to the NTG method except that EMS (Eastman, Rodchester, N. Y.) was used at a concentration of 0.4 M. Finally, a modification of the 5-bromouracil (5-BU) method of Benzer and Freese (1958) was used. A mixture of 3 PFU'S of phage 42D/CFU of PS 42D in 10 ml TSA-YE-CA containing 50 µg/ml 5-bromouracil was incubated at 30 C with gentle shaking until lysis occurred. Lysates were sterilized by filtration as previously described. These treated phage

preparations were disignated by the phage strain number followed by the abbreviation of the mutagen, e.g. phage preparation 42D treated with NTG will be identified as 42D-NTG.

Lysogenization

Growth from a single β-hemolytic colony was used as initial inoculum for all experiments. Strains to be lysogenized were grown overnight in TSB-YE-CA and 0.1 ml volumes spread over the surfaces of TSA-YE-CA plates with a sterile glass applicator. About 0.2 ml undiluted phage preparation was spotted onto the seeded area and incubated overnight. Any secondary growth occurring within the area of confluent lysis was inoculated into TSB-YE-CA and incubated for 5 hours with constant shaking. Dilutions, of 1×10^{-6} and 1×10^{-7}, in sterile 0.85% (w/v) NaCl-H_2O, were streaked onto surfaces of sheep blood agar plates using a sterile glass applicator. After incubation overnight, as many as 15 well-isolated, non-β-hemolytic colonies were transferred onto TSA-YE-CA slants, incubated, and stored for further testing. Each of these isolates was identified by a letter and a number that follows the strain designation, e.g. strain 7-8(42D)W3 carries the isolate indication W3. A strain was considered lysogenized when, (A) it was resistant to the lysogenizing phage, (B) UV-induction of a broth culture resulted in a lysate which was lytic for an indicator strain.

All lysogenization procedures were carried out as above except those studies using the serological group A phage, 42E. Ten-fold dilutions of the preparation of phage 42E were spotted onto the seeded area and individual plaques containing lysogenized cells were excised from the agar

using sterile Pasteur pipets and treated as in other lysogenization experiments.

Lysogenization With Phage Mutants

Attempts to lysogenize strain 7-8 with mutagen-treated phage 42D preparations were identical to the above lysogenization methods. Identification of phage mutants converting either β-hemolysin or fibrinolysin alone, however, required that β-hemolytic and non-β-hemolytic colonies be tested on FTA for identification of β+K+ and β-K- colonies. All β+K+ colonies obtained were then streaked onto sheep blood agar plates for isolation of colonies to determine if β+K+ colonies were mixtures of β+K- non-lysogenic cells and β-K+ lysogenic cells. A number of the β-hemolytic secondary isolates were also transferred onto FTA medium to determine their fibrinolytic activity.

Curing of Lysogenic Strains

Curing of lysogenically converted non-β-hemolytic strains was attempted by several methods. All cultures used were obtained from single colony isolates of non-β-hemolytic strains.

(a) Sodium dodecyl sulfate, SDS, was used as a curing agent in a modification of the technique of Sonstein and Baldwin (1972). Lysogenic strains were incubated overnight with constant shaking in TSB-YE containing 0.002% SDS (Fisher).

(b) Ethidium bromide was used as a curing agent in a modification of the method of Boanchaud et al. (1969). Cultures were incubated overnight in the dark in TSB-YE containing 8×10^{-6} M ethidium bromide (Sigma).

(c) Exposure to ultraviolet light was identical to the UV-induction technique. The growth medium, however, was TSB containing 0.02 M sodium citrate.

Combinations of treatments (a) and (c), and (b) and (c) were also employed to effect curing. The rate of spontaneous loss of prophage was determined after incubating cultures in TSB containing 0.02 M sodium citrate for 18 hours.

After each of the above treatments, culture dilutions of 1×10^{-6} and 1×10^{-7} were streaked onto sheep blood agar using a sterile glass streaker, and incubated. As many as 15 well-isolated β-hemolytic colonies were transferred from the agar onto TSA slants, incubated, and stored for further testing.

RESULTS

The Non-lysogenic Nature of S. aureus strain 7-8

The parent culture of S. aureus strain 7-8 was repeatedly tested for the presence of bacteriophage. The filtrate obtained after UV-irradiation treatment did not cause lysis of any indicator strain tested.

Lysogenization

The effects of infection with phages upon S. aureus strains 7-8 and 57 are summarized in Table 1. Colonies of S. aureus strain 7-8 that had lost the ability to produce β-hemolysin were recovered from sheep blood agar plates after treatment with serological group F phages 42D, L42E, and serological group A phage 42E. Non-β-hemolytic colonies were also isolated upon treatment of strain 57 with phage 42E.

Serological group F phages L80, L81, 491, 269, 756, and 509 were not lytic for strain 7-8 and no non-β-hemolytic colonies were recovered after treatment with any of these phages. Serological group F phage 77, serological group B phages 52A and 79, and serological group A phage 75 were lytic for strain 7-8, however, 3000-5000 colonies were inspected after treatments with each phage and no non-β-hemolytic colonies were recovered.

Identification of Bacteriophages

Phage L42E was obtained by UV-induction of PS 42E. This phage did produce conversion to loss of β-hemolysin production. The identity of L42E was established morphologically. Phage L42E is shown in Figure 2

TABLE 1. Effect of Lysogenization on β-hemolysin Production of S. aureus Strains 7-8 and 57.

S. aureus	Lytic Phages			β-hemolysin[a] Production
	Identity	Origin	Serological Group	
7-8				+
7-8	42D	Basic Set	F	+ ; -
7-8	L42E	PS 42E	F	+ ; -
7-8	77	Basic Set	F	+
7-8	79	Basic Set	B	+
7-8	52A	Basic Set	B	+
7-8	75	Basic Set	A	+
7-8	42E	Basic Set	A	+ ; -
57				+
57	42E	Basic Set	A	+ ; -

[a] + ; - = recovery of beta-hemolytic and non-beta-hemolytic colonies after phage treatment

to bear round-shaped heads characreristic of phages of serological group F (Rosenblum and Tyrone, 1964).

Phage 42E (one of the basic set of S. aureus bacteriophages) is known to be a serological group A phage. The preparation of phage 42E used in this investigation is shown by Figure 1 to bear oval-shaped heads characteristic of phages of serological group A (Rosenblum and Tyrone, 1964).

The Lysogenic Nature of the Non-β-hemolytic Isolates

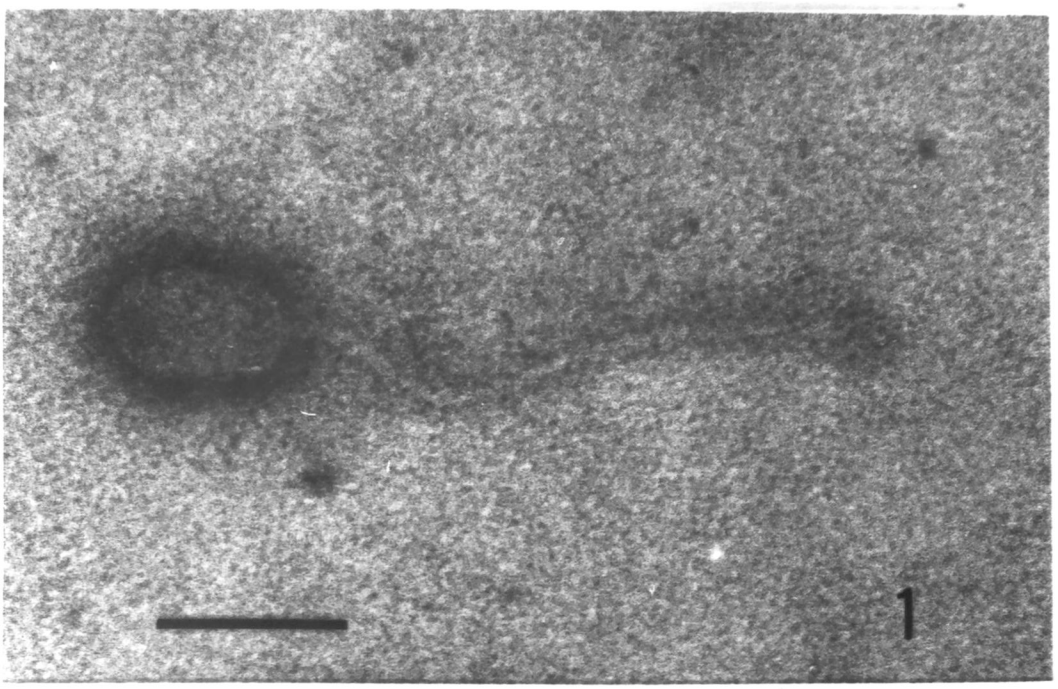
The results of tests defining the lysogenic nature of the non-β-hemolytic isolates are presented in Table 2. Twenty-nine non-β-hemolytic isolates obtained from attempts to lysogenize strains 7-8 and 57 were tested. Each non-β-hemolytic isolate was immune to lysis by the lysogenizing phage and the filtrate of each, obtained after UV-irradiation, contained phage capable of lysis of a sensitive strain. Filtrates obtained after UV-induction of strain 7-8(42D) were not lytic for strain PS 42D, but did lyse strain 7-8.

Curing

Every lysogenized strain obtained was subjected to one or more of the curing techniques employed. Numbers of β-hemolytic derivatives of the lysogenic non-β-hemolytic isolates were obtained by various techniques. All techniques except ethidium bromide (EB) treatment enhanced the recovery of β-hemolytic colonies of S. aureus 7-8(42D) compared to the numbers of colonies showing spontaneous loss of β-hemolytic activity (Table 3). Variation among strains was observed in the rate of curing

Figure 1. Phage 42E. Bar is .1 μ.

Figure 2. Phage L42E. Bar is .1 μ.


1

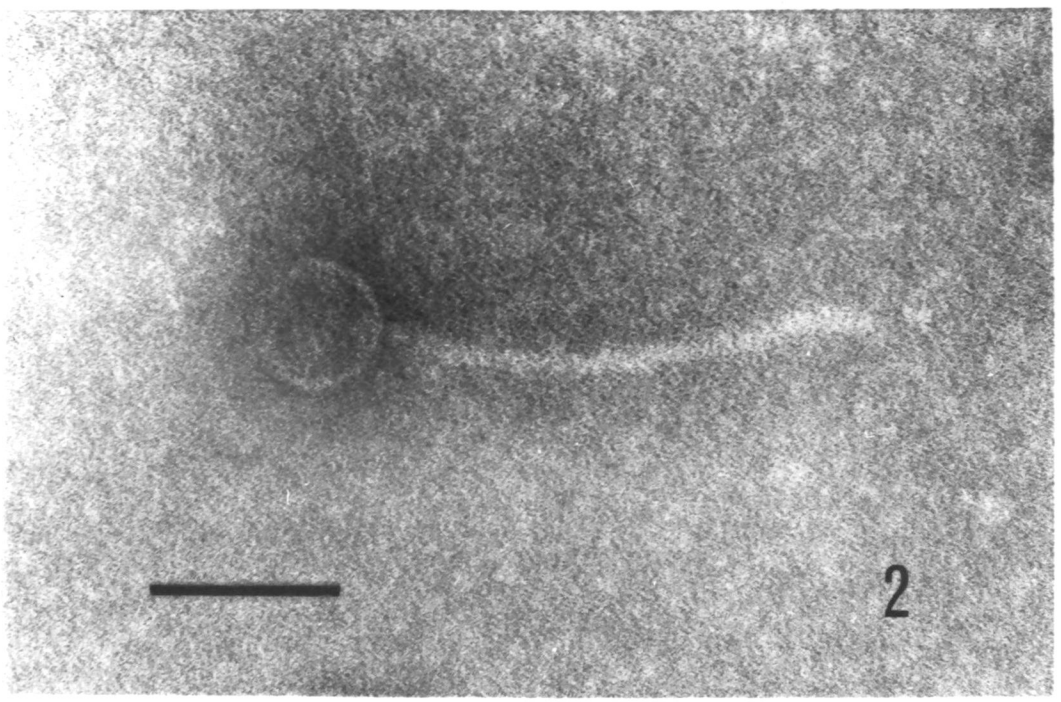

2

TABLE 2. Definition of the Lysogenic Nature of the Non-β-hemolytic Isolates.

S. aureus	Number β-Isolates Tested	Lysis of Sensitive Strains	Immunity To Lysogenizing Phage
7-8(42D)	13	7-8+(13);PS 42D-(13)	13
7-8(L42E)	5	7-8+(5)	5
7-8(42E)	9	7-8+(9);PS 42E+(9)	9
57(42E)	2	57+(2);PS 42E+(2)	2

(+)=lysis; (-)=no lysis

by any single method employed. Strains PS 80 and PS 81, known to be lysogenic for serological group F phages (Winkler et al. 1965), exhibited a low rate of curing upon exposure to 0.002% sodium dodecyl sulfate (SDS) and 45 sec. UV-irradiation while PS 42E, also carrying a group F phage, was not cured by this treatment. Strains 879(491) and 7-8(42E) were cured by the SDS + UV treatment at much higher rates than strains PS 80 and PS 81. The rate of curing also varied with the treatment, e.g. the percent curing (β- →β+) for strain 7-8(42D) was 0.012 with SDS treatment, 0.0 with EB treatment, 0.2 with UV-irradiation, 0.19 with EB and UV, and 0.05 with SDS and UV.

Change in Phage Sensitivity Patterns Associated with Phage Conversion

Phage typing was performed to identify contaminants and to detect changes in phage-sensitivity patterns upon lysogenic conversion to loss of β-hemolysin production. Changes in the phage-sensitivity patterns involving loss or gain of lytic activity by phage of the International Phage Typing Set for Staphylococci are given in Table 4A and 4B. All three types of change in phage-sensitivity patterns as described by Duval-Iflah (1972) were observed (Table 4A). Gain in specific prophage immunity is demonstrated by strain 879 when lysogenized with phage 491. Strain 879 (491) is resistant to phage 491 and does not demonstrate any other change in phage-sensitivity pattern in relation to parent strain 879. Gain in less-specific phage immunity is demonstrated by strain 7-8 when lysogenized with phages 42D or 42E and by strain 57 when lysogenized with phage 42E. The lysogenic isolates of strain 7-8(42D), 7-8(42E), and 57(42E) are resis-

TABLE 3. Effect of Physical and Chemical Agents on β-hemolysin Production by Lysogenic Strains of S. aureus.

[a] β-lysogenic Strain	Treatment	Colonies Inspected	Number β+ Colonies	% β-→β+
7-8(42D)	NONE	22,500	2	0.009
7-8(42D)	SDS[b]	8,046	1	0.012
7-8(42D)	EB[c]	677	0	0.0
7-8(42D)	UV[d]	5,508	11	0.2
7-8(42D)	UV + EB	2,058	4	0.19
7-8(42D)	UV + SDS	8,293	4	0.05
7-8(42E)	UV + SDS	3,196	19	0.6
879(491)	UV + SDS	3,986	69	1.7
PS 80	UV + SDS	1,745	2	0.11
PS 81	UV + SDS	1,383	2	0.14
PS 42E	UV + SDS	1,312	0	0.0

[a] β- = non-beta-hemolytic
[b] SDS=sodium dodecyl sulfate (0.002%)
[c] EB=ethidium bromide (8×10^{-6} M)
[d] UV=ultraviolet irradiation (45 sec.)

Key: Table 4A & 4B
Phage-Sensitivity Pattern of the Parent Strains

S. aureus	Phage Sensitivity	Phage Groups
7-8	42E/53/77/79/52A/80/42D	I, III, IV
57	29/52/52A/79/80/81/47/54/75/77/42E/42D	I, III, IV
879	3A/3B/3C/55/71	II
PS 80(L80)[a]	80/81	I
PS 81(L81)[a]	80/81	I

[a] *S. aureus* strains PS 80 and PS 81 are naturally lysogenic for serological group F phages.

TABLE 4A. Changes in Sensitivity to Phage of the International Phage Typing Set for Staphylococci Associated with Lysogenic Conversion to Loss of β-Hemolysin Production by S. aureus.

S. aureus[a]	Changes In Sensitivity[b]		
	Gain	Loss	Phage Groups
7-8(42D)W3	None	53/77/79/52A/42D	I, III, IV
7-8(42D)W4	"	53/77/79/52A/42D	"
7-8(42D)W5	"	53/77/79/52A/80/42D	"
7-8(42D)W6	"	53/77/79/42D	"
7-8(42D)W7	"	53/77/79/52A/42D	"
7-8(42D)W8	"	53/77/79/52A/42D	"
7-8(L42E)1	187/81/75/54/29/52/3A/3B/3C/55/71/47	42D	I, II, III, IV
7-8(L42E)2	"	"	"
7-8(L42E)3	"	"	"
7-8(I42E)4	"	"	"
7-8(L42E)5	"	"	"
7-8(42E)1	75	42E/80/42D	I, III, IV
7-8(42E)11	75	42E/80/53/42D	"
57(42E)1	None	29/52/79/81/47/54/42E/42D	I, III, IV
879(491)W	None	None	

[a]All lysogenized strains carry the phage strain number in parenthesis; phages 42D, L42E, and 491 are serological group F phages; phage 42E is a serological group A phage.

[b]All changes in sensitivity are relative to the phage sensitivity pattern of the parent strains (see Key).

TABLE 4B. Changes in Sensitivity to Phage of the International Phage Typing Set for Staphylococci of β-Hemolytic Cured Isolates of Lysogenically Converted Non-β-Hemolytic Strains.

S. aureus	Source	Changes in Sensitivity[a]		
		Gain	Loss	Phage Groups
7-8/W31	7-8(42D)W3	54/75/29/52	None	I, III
7-8/W57	7-8(42D)W5	75	53/80/42D	I, III, IV
7-8/W519		75	53/80	I, III
7-8/W526		47/75/52/29	42D	I, III, IV
7-8/W533		54/75/29/52	53	I, III
7-8/W534		54/75/29	42D/53	I, III, IV
7-8/W516		47/54/75/29/52	42D	I, III, IV
7-8/C3	7-8(42E)1	75	80	I
879/C2	879(491)W	None	None	None
879/C3	879(491)W			
PS 80C1	PS 80(L80)	None	None	None
PS 81C1	PS 81(L81)	42E	None	III

[a] All changes in sensitivity are relative to the phage-sensitivity pattern of parent strains (see Key).

tant to several phages which were lytic for their non-lysogenic parent strains. Gain in sensitivity to certain phages was demonstrated by strain 7-8 when lysogenized with phages L42E or 42E. Each lysogenized isolate of strains 7-8(L42E) and 7-8(42E) demonstrated sensitivity to phages which did not lyse the parent strain 7-8.

The phage sensitivity patterns of cured isolates of the lysogenized strains 7-8(42D)W3, 7-8(42D)W5, and 7-8(42E)1 were observed to return to patterns similar to the parent strain (Table 4B). However, slight differences did occur between the phage sensitivity patterns of the parent culture and the cured isolates obtained by UV-irradiation, e.g. strain 7-8/W31, a cured isolate of 7-8(42D)W3, gained sensitivity for Group I and Group III phages 54/75/29/52 that were not lytic for the parent strain 7-8. No changes in phage-sensitivity patterns were observed for strains 879/C2 and 879/C3, cured isolates of 879(491)W. Any changes in sensitivity after curing (compared to the phage type of the parent strain) were always within the same groups. For example, several cured isolates of 7-8(42D) strains gained sensitivity to phages in groups I and III. The parent strain 7-8 was initially sensitive to phages of each of these groups.

Additional Characteristics of the Lysogenic Non-β-hemolytic Isolates

Table 5 contains additional characteristics of the β-hemolytic parent strains, the non-β-hemolytic strains (lysogenic) and the cured strains. Strains that naturally carry group F phages, PS 80, PS 81, and PS 42E, are non-β-hemolytic and fibrinolytic. Conversion to the loss of

TABLE 5. Additional Characteristics of *S. aureus* Converted to Loss of Beta-hemolysin Production

Strain Name[a]	Lysogenic Nature[b]	Number of Isolates Tested	β-hemolysin Production	Fibrinolysin Production	Crystal-Violet Reaction
7-8	NL	1	+	-	+
7-8(42D)	L	13	-	+	+
7-8/C42D	C	7	+	-	+
7-8(L42E)	L	5	-	+	+
7-8(42E)	L	9	-	-	+
7-8/C-42E	C	4	+	-	+
879	NL	1	+	-	-
879(491)	L	1	-	+	-
879/C-491	C	5	+	-	-
57	NL	1	+	-	-
57(42E)	L	1	-	-	-
PS 80(L80)	L	1	-	+	-
PS 80C1	C	1	+	-	-
PS 81(L81)	L	1	-	+	-
PS 81C1	C	1	+	-	-
PS 42E(L42E)	L	1	-	+	-

[a] Lysogenic strains carry the phage strain number in parenthesis. Phages 42D, L42E, 491, L80, and L81 are serological group F phages. Phage 42E is a serological group A phage.

[b] NL=non-lysogenic, L=lysogenic, C=cured of converting phage.

β-hemolysin production by the serological group F phages 42D, L42E, and 491 was always associated with a gain in fibrinolysin production. There was no change in fibrinolysin production associated with conversion to the loss of β-hemolysin production by the group A phage 42E.

There was no change in the crystal-violet (CV) reaction of strains 7-8, 879, 57, PS 80, PS 81, or PS 42E, whether non-lysogenic (β+K-), lysogenic (β-K+), or cured (β+K-). All strains listed in Table 5 fermented mannitol and produced coagulase, DNase, lipase and gelatinase.

Lysogenization with Phage Mutants

Preparations of phage 42D were obtained after treatments with NTG, EMS, and 5-BU. These treated phages had more lytic activity on strain 7-8 than did the untreated phage 42D preparation. Beta-hemolytic and non-β-hemolytic colonies were isolated during lysogenization experiments using each of the above phage preparations and tested for fibrinolysin production.

After treatment of S. aureus strain 7-8 (β+K-) with phage 42D-5-BU, 104 β-hemolytic and 104 non-β-hemolytic colonies were selected and tested for fibrinolysin production. All of the β-hemolytic isolates were non-fibrinolytic (β+K-) and all of the non-β-hemolytic colonies were fibrinolytic (β-K+).

Over 200 non-β-hemolytic colonies selected after treatment of S. aureus strain 7-8 (β+K-) with either phage 42D-NTG (104 colonies) or 42D-EMS (104 colonies) were shown to be fibrinolytic (β-K+). Fifty of the 104 β-hemolytic colonies selected after treatment of S. aureus strain 7-8 (β+K-) with phage 42D-NTG (25) or 42D-EMS (25) were also fibrinolytic

(β+K+). All of these β+K+ isolates proved to contain mixtures of β+K- and β+K+ types. No β-K- colonies were isolated from any treatment.

DISCUSSION

Non-β-hemolytic isolates of the naturally non-lysogenic, β-hemolytic strain 7-8 were obtained after treatments with the serological group F phages 42D and L42E, and the serological group A phage 42E. Non-β-hemolytic isolates of the β-hemolytic strain 57 were also recovered after treatment with the serological group A phage 42E (Table 1). This was due to lysogenic conversion since all of the non-β-hemolytic isolates were shown to be lysogenic, i.e. were insensitive to the lysogenizing phage and the filtrates caused lysis of sensitive indicator strains (Table 2).

Serological group F phages L80, L81, 491, 756, and 269 that are known to be converting phages (Winkler et al., 1965) were not lytic for strain 7-8 and no evidence of lysogenic conversion of β-hemolysin was obtained after treatments with any of these phages. Since these phages did not lyse strain 7-8, possibly, it was not infected, therefore, lysogenization would not occur. Alternatively, lysogenization could be produced by non-lytic phages, but to detect this the entire cell population would have to be screened contrasted with the small number of lysogenized lysis-resistant cells surviving infection by the lytic phages. The group F phage 77 was lytic for strain 7-8, however, no non-β-hemolytic isolates were obtained from its treatment on strain 7-8. This confirms the opinion of Winkler et al. (1965) that phage 77 is not a β-hemolysin converting phage. Apparently, the gene(s) affecting β-hemolysin production are not present or are permanently inactive (repressed) on the chromosomes of the non-converting phages.

The filtrates of the 7-8(42D) isolates were lytic for the sensitive

strain 7-8 but were not lytic for PS 42D (Table 2). This suggests that the lysogenizing phage 42D becomes modified in passing through host strain 7-8. According to Hayes (1968) a single cycle of growth in a particular bacterial host may alter the host range of all the phage progeny. This type of variation is imposed by the host bacteria and is inheritable; when the phage is grown in some other host, the modification is lost (Lauria and Human, 1952). Mutational modifications of phages also occur that affect host range and are heritable (Hayes, 1968). No attempt was made to determine the type of modification affecting phage 42D in passage through strain 7-8.

Each of the non-β-hemolytic isolates of strain 7-8 that was lysogenic for a serological group F phage was also shown to be fibrinolytic (β-K+) (Table 5). This verifies the report of Winkler et al. (1965). The non-β-hemolytic isolates of strains 7-8 and 57 obtained after treatments with group A phage 42E did not produce detectable amounts of fibrinolysin and were β-K- (Table 5). Lysogenic conversion by serological group A phages affecting β-hemolysin production had not been previously reported. The loss of β-hemolysin production after treatment with serological group A phage 42E was the result of lysogeny and not transduction since: (1) all reported transducing phages belong to serological group B (Pattee and Baldwin, 1961; Dowell and Rosenblum, 1962; and, Novick, 1967); (2) 7-8(42E) strains are resistant to phage 42E; and (3) 7-8(42E) strains carry a phage that is lytic for host strain PS 42E.

The serological group A phage 42E capable of producing phage conversion of β+K- to β-K-, is related to the serological group F phage 42D capable of producing the double conversion of β+K- to β-K+. Phages 42E

and 42D were both obtained from phage 42 by adaptation to different host strains (Rountree, 1942). Therefore, the mechanisms of conversion by the group F phages and group A phage 42E are probably similar but the gene(s) that affect fibrinolysin activity are altered or not present in phage 42E.

The correlation between loss of β-hemolysin production and gain in fibrinolysin production might be due to two independent loci on the chromosome of the converting phage or one regulator gene on the phage with a supressive effect on β-hemolysin production and a derepressive effect on fibrinolysin production. The theory that separate loci on the phage genome are involved was substantiated by the conversion of strain 7-8 (β+K-) to β-K- by phage 42E and to β-K+ by the serological group F phages. Winkler et al. (1965) treated serological group F phage 269 with UV-irradiation and used this phage preparation in lysogenization experiments. A single S. aureus strain was obtained that appeared to be β+K+, however, it easily lost its phage and reverted to β+K-. Winkler et al. (1965) suggested this implied separate loci on the phage chromosome responsible for conversion to β-K+.

In our investigations no serological group F phage preparations capable of causing phage conversion of β-hemolysin independent of fibrinolysin or vice versa were obtained with 5-BU treatments. However, approximately 50 β+K+ colonies were isolated from strain 7-8 (β+K-) after exposure to NTG or EMS treated phage preparations. From each β+K+ appearing colony tested, β+K- variants were easily obtained. Since no stable lysogenic β+K+ or β-K- isolates were obtained, the theory of Winkler et al. (1965) could not be confirmed by the use of phage mutants. Alternatively,

our results would seem to confirm the β+K+ isolates obtained after treatment with phage exposed to mutagens actually consisted of lysogenic (β-K+) and non-lysogenic (β+K-) cells. The β-K+ cell, however, would produce a β+K+ colony indicative of the unstable nature of the lysogenic conversion by these phage variants.

Lysogenically converted strains are expected to revert to their original characteristics upon loss of their prophages. All lysogenically converted β-K+ isolates were cured by one or more of the curing methods. Each isolate was shown to become β+K- upon loss of prophage. Curing rates of the SDS + UV technique varied with different host-phage relationships, e.g. the percent β- →β+ for 7-8(42D) was 0.05 and for 7-8(42E) was 0.6 (Table 3). These results suggest the curing rate may be a property of the prophage as is the case with induction (see Gorrill and Gray, 1956).

Gain and loss of prophages are known to produce changes in phage sensitivity patterns. Three types of changes in phage-sensitivity patterns (Tables 4A and 4B) were observed: (1) specific-prophage immunity (resistant to specific-lysogenizing phage); (2) increased less-specific immunity (resistant to several phages); and (3) loss of immunity to several phages (gain in phage sensitivity). The data in Tables 4A and 4B show no correlation between changes in phage-sensitivity patterns involving phage of groups I, II, or III and phage conversion to loss of β-hemolysin production. Every lysogenized non-β-hemolytic strain was resistant to phage 42D of phage group IV. This, however, is not conclusive evidence that resistance to phages of group IV accompanies lysogenic conversion to loss of β-hemolysin production, since phage 42D is the only phage of group IV used in routine phage typing. Determining the sensitivity of the lysogenic

non-β-hemolytic strains to additional group IV phages should clarify this.

Cured isolates of lysogenized strains were shown to have similar phage-sensitivity patterns compared to their untreated non-lysogenic-parent strains (Table 4B). Any variations in sensitivity were always "within the group" changes. These variations may be due to cell wall changes. Escherichia coli strain B, sensitive to phages T_2 and T_4, has been shown to yield mutant strains resistant to T_2 or T_4 as a result of specific cell-wall changes (Hayes, 1968). The use of UV-irradiation in curing techniques may have produced cell-wall changes in cured isolates accounting for the variation in phage-sensitivity patterns.

Generally, pathogenic strains of S. aureus of human and animal origin differ in certain characteristics. Strains of human origin are likely to be fibrinolytic, non-β-hemolytic and sensitive to phage other than those of phage group IV, while bovine isolates are likely to be non-fibrinolytic, β-hemolytic, and sensitive to phage of phage group IV. This pattern of characteristics has been shown to be subject to phage conversion (Cohen, 1972). This implies that phage conversion accounts for the difference between human and animal strains in nature.

Pathogenic S. aureus strains of bovine and human origin are likely to be coagulase positive, DNase positive, yellow pigmented, and gelatinase positive (Victor et al., 1969; and Cohen, 1972). Since these properties are characteristic of pathogenic isolates of both bovine and human origin, changes in these properties were not expected to accompany lysogenic conversion to loss of β-hemolysin production. No detectable change in coagulase activity, DNase activity, gelatinase activity, lipase activity, or mannitol fermentation could be correlated with lysogenic conversion to

loss of β-hemolysin production in any of the strains tested.

Meyer (1967) used crystal-violet reactions to distinguish S. aureus strains of bovine origin from strains of human origin. Of 2471 cultures of human origin, 84.9% were crystal-violet negative, while 81.2% of 618 bovine isolates were crystal-violet positive. These results suggested crystal-violet reactions may have a relation to the phage conversion for β-hemolysin and fibrinolysin. Strain 7-8 (β+K-), of bovine origin, remained crystal-violet positive whether lysogenic (β-K+) or non-lysogenic (β+K-). Strains 879 (β+K-), 57 (β+K-), PS 80 (β-K+), and PS 81 (β-K+) of human origin, were crystal-violet negative whether lysogenic or not (Table 5). Therefore, our results show the crystal-violet reaction has no correlation with phage conversion to β-K+. The CV trait may depend on gene(s) usually present in bovine strains and absent in human strains or may be subject to repression by phages not converting to β-K+.

Omenn and Friedman (1970) reported evidence supporting a common control mechanism regulating production of nuclease, coagulase, and β-hemolysin. Forsgren et al. (1971) reported evidence supporting a common control mechanism for protein A, nuclease, coagulase, β-hemolysin, fibrinolysin, and mannitol fermentation. DNase production and mannitol fermentation are correlated to coagulase production of pathogenic staphylococcal strains (Victor et al., 1969; and Cohen, 1972). According to Victor et al. (1969) investigations relating hemolysin, fibrinolysin, or lipase have failed to demonstrate a high correlation of any of these characterisitics with coagulase activity. Alpha-hemolysin, β-hemolysin, δ-hemolysin, fibrinolysin, and lipase have all been lysogenically converted (Blair and Carr, 1961; Waart, Winkler, and Grootsen, 1961; Winkler et al., 1965;

Clecner and Sonea, 1966; Rosendal and Bülow, 1965; and Duval-Iflah, 1972). Considering that each of these properties has been lysogenically converted and that Rountree (1949), Gorrill and Gray (1956), and Blair and Carr (1961) suggested that most strains of S. aureus are lysogenic in nature, it seems probable that coagulase-positive staphylococci may also carry any one, a combination, or all of the genes for α-hemolysin, β-hemolysin, δ-hemolysin, fibrinolysin, and lipase, however, in a given strain any of these genes may be expressed or repressed by the controlling prophage(s). This would be in accord with the results of those proposing common control mechanisms and would explain why efforts to correlate these properties to coagulase activity have failed.

SUMMARY

Staphylococcus aureus strain 7-8 that is β-hemolytic and non-fibrinolytic (β+K-) was lysogenically converted by certain serological group F bacteriophages to loss of β-hemolysin production and gain in fibrinolysin production (β-K+). This is in accord with the work of Winkler, Waart, and Grootsen (1965). Serological group A phage 42E was found to convert S. aureus strains 7-8 (β+K-) and 57 (β+K-) to β-K-. Several characteristics associated with virulence of staphylococci of human or animal origin other than fibrinolysin production (coagulase, DNase, lipase, gelatinase, and mannitol fermentation) were not correlated with lysogenic conversions to loss of β-hemolysin.

S. aureus strains of bovine origin are usually β-hemolytic, non-fibrinolytic, crystal-violet positive, and sensitive to group IV phages. Strains of human origin are usually non-β-hemolytic, fibrinolytic, crystal-violet negative, and insensitive to group IV phages. Beta-hemolytic and non-fibrinolytic strains can be lysogenically converted to loss of β-hemolysin production and gain in fibrinolysin production. Crystal-violet reactions were not affected by lysogenic conversion of β-hemolysin. Every lysogenically converted β- isolate was insensitive to group IV phage 42D. Further testing with additional group IV phages would be necessary to determine if loss in sensitivity to group IV phages always accompanied loss of β-hemolysin production.

Beta-hemolytic and non-fibrinolytic (β+K-) variants were regularly obtained from each of the 50 β+K+ appearing colonies of strain 7-8 (β+K-) isolated after treatment with nitrosoguanidine or ethyl methanesulfonate

treated phage preparations. Since no stable β+K+ isolate was obtained from any of the β+K+ appearing colonies tested and since no β-K- colonies were found, the theory of Winkler et al. (1965) that conversion to β-K+ involves two separate loci on the phage chromosome was not confirmed by use of phage mutants.

Conversion of β-hemolysin by lysogenization of a serological group A phage has not previously been reported. Phage 42E conversions differed from the group F conversions since fibrinolysin was not affected. This indicates that conversion to β-K+ involves two separate loci on the phage chromosome.

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