STUDIES ON THE ORIGIN OF STAPHYLOCOCCI FOUND IN EXTERNAL INFECTIONS OF THE EYE WITH SPECIAL EMPHASIS ON THE CORRELATION IN PHAGE TYPE BETWEEN EYE AND NOSE

by

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INTRODUCTION

Relationship of staphylococci to external infections of the eye. Acute and chronic infections of the lid margins, conjunctivae and meibomian glands are the most frequent external diseases treated by the ophthalmologist. That staphylococci play the prominent role in these infections has been shown by the studies of Thygeson[1, 2], Allen[3, 4] and Berens[5]. Thygeson[1] reported that 259 of 280 cases yielded pathogenic staphylococci. In a majority of his cases staphylococci were the only organisms found. In a subsequent study[2] of 350 military personnel with the diagnosis of blepharitis, he found pathogenic staphylocci in 130 cases, budding yeast cells alone in 100 cases and both staphylococci and yeast cells in 102 cases. The role played by the yeast in these infections has not as yet been clearly defined.

Allen[3, 4] demonstrated that toxic filtrates of Staphylococcus aureus cultures produced conjunctivitis in humans, rabbits, monkeys and baboons. Punctate ulcers of the cornea similar to those found in clinical cases of staphylococcic blepharitis were seen. In other experiments he was able to produce chronic meibomitis in rabbits by inoculation of S. aureus into the meibomian glands. Acute infections developed in 12 to 18 hours and lasted two to three weeks, then were followed by chronic meibomitis and conjunctivitis of six to eight months duration. Injection of S. albus into the glands resulted in acute infections which subsided in 48 to 60 hours.

Individuals without blepharitis have few if any pathogenic staphylococci on the lid margins or conjunctivae. Thygeson[1] found

them in only ten per cent of 2,534 unselected preoperative cases. Of 100 patients with normal eyes studied by Keilty[6], 43 per cent had sterile conjunctivae; 34 per cent had organisms on both sides. He noted hemolytic staphylococci in 70 per cent of the cases where organisms were found, but these staphylococci were described as producing large, white colonies with distinct zones of hemolysis typical of a type frequently found in the nose. No tests for pathogenicity were made, but the inference was that these staphylococci were nonpathogenic. Khorazo and Thompson[7] found 64 per cent of a total of 1,122 normal individuals to have staphylococci on their conjunctivae, but of 103 of their cultures tested for mannitol fermentation and coagulation of plasma, only 18 were positive for one or both tests. Their results indicated that pathogenic staphylococci are rare on the normal conjunctiva. Rodin[8] obtained no growth from 20 per cent of 42 clinically normal conjunctivae. Seventy-six per cent yielded coagulase negative staphylococci.

Possible sources of the staphylococci found in external infections of the eye. There is some disagreement among the ophthalmologists about the source of the staphylococci found in cases of blepharitis, meibomitis and conjunctivitis. Thygeson[2] suggested that the staphylococci carried normally in the nose may be carried to the eyes by way of the fingers. Swan[9] believes that infection passes from one member of the family to another by way of contaminated towels and other household articles used commonly by members of a family. Berens[5] suggested that retrograde infection from the nose through the naso-lacrimal duct may occur, or that a toxic condition of the conjunctiva may arise from exotoxin produced by the nasal staphylococci.

Numerous studies have shown that S. aureus is a common normal inhabitant of the nose, nasopharynx and, to a lesser extent, the skin. Several attempts have been made to associate the carrier state with staphylococcic infections elsewhere in the body. Other studies purport to show that nasal carriage of staphylococci correlates well with chronic focal infection. Hudson[10] found 40 per cent of 70 strains of staphylococci from 65 normal individuals to be S. aureus. Gillespie, Devenish and Cowan[11] reported 30.8 per cent nasal carriers of pathogenic staphylococci among 159 normal persons examined. The carrier state in his study correlated well with a history of chronic nasal disease. Three months after the original study, 17 of 20 nasal carriers still remained carriers, and in each case the infecting staphylococcus was of the same serological type. Miles, Williams and Clayton-Cooper[12] in a study of septic wounds, found coagulase positive staphylococci in 87.5 per cent of the wounds. Of 479 outpatients examined, 47.4 per cent were carriers of pathogenic staphylococci in the nose and 18.4 per cent carried pathogenic staphylococci on the skin. Forty-nine per cent of ward patients were found to be carriers on admission. Sixty-four per cent of the nursing staff were nasal carriers. Twenty-seven of the nurses were followed from 10 to 50 weeks. Of these nurses, 15 to 20 per cent never failed to carry staphylococci; 10 per cent were consistently negative and the remainder were transient carriers. In eight cases studied, Bloomfield [13, 14, 15] found diphtheroids and S. albus made up the constant flora of the nose. There was a variable flora of pathogens and nonpathogens including S. aureus. S. aureus was found constantly in one case associated with a chronic focal infection of the tonsil.

Hallman[16] found that 39.2 to 60.5 per cent of a group of 468 persons carried pathogenic staphylococci. The percentage of carriers varied in different age groups. McFarlan[17] found 34.4 to 57.5 per cent carriers among 165 healthy persons. Here again the percentage varied with different age groups. McFarlan felt that, because of the frequency with which pathogenic staphylococci occur in the noses of healthy persons, it is reasonable to assume that their presence in the noses of persons with infection is just a reflection of the general carrier rate and cannot be considered the source of infection. Jacobson and Dick[18] cultured the noses of 500 patients and found S. albus and diphtheroids in all cases. No S. aureus were reported by them, an observation which is at variance with other published findings. These authors stated that when organisms other than S. albus, diphtheroids and Micrococcus catarrhalis were found, abnormal changes existed in the nasal cavity or paranasal sinuses.

Both Keilty[6] and Berens[5] have pointed out the close relationship of nasal organisms to those found on the lid margins or conjunctivae. Keilty found that the type of colony from the eye bore a direct resemblance to that from the nose, and concluded that the majority of eye contaminants are of nasal origin. He was unable to distinguish specifically one strain from another. Berens studied 83 cases of conjunctivitis in which he obtained cultures from both nose and infected eye. Of the nasal cultures, 85.5 per cent yielded staphylococci of some sort, but only 54 per cent showed toxic staphylococci. Of the eye cultures, 84.3 per cent showed staphylococci of some sort, but only 29 per cent showed toxic staphylococci. Of 31 sets of cultures studied, the same organism was present in pure growth from both eye and nose in 22 instances. In 16

instances, toxic staphylococci were found in pure culture from both sites and in combination with streptococci in four instances. Berens emphasized that 28 of the 31 sets showed a suggestive similarity between the flora of the nasal and conjunctival membranes. In the case of the toxic staphylococci, the relationship was assumed because of the correspondence in species and toxicity. In 36 sets of cultures, the eye cultures were sterile or showed non-toxic organisms while the organisms from the nasal membranes were toxic. This circumstance was noted particularly in cases of recurrent conjunctivitis where toxic organisms were on the conjunctiva during the primary acute infection but were not found at the time of the recurrence. The same species of toxic organisms found originally were still present in the nose at the time of the recurrence. Berens feels that these findings support his contention that a toxic condition of the conjunctiva may arise from exotoxin produced by nasal organisms.

The data reviewed strongly suggests that there is a direct relationship between the staphylococci found in eye infections and those carried in the nose by the same individual, but the evidence is at best presumptive since no reasonably accurate method of identifying strains was used. We have undertaken this study in an attempt to demonstrate by a more specific method the correlation between the strains of pathogenic staphylococci obtained from the nose and from the infected eye in an individual. Several methods to aid in identifying strains were considered. These methods included biochemical reactions, toxin production, agglutination and precipitation reactions and bacteriophage typing.

Consideration of methods available as aids in identification of strains. Early studies of the biochemical reactions of staphylococci were made in efforts to find stable reactions that would help in classifying staphylococci into groups rather than to separate one strain from another. Winslow, Rothberg and Parsons[19] recorded differences in final pH values of sugars fermented, while Julianelle[20] concluded that all strains of hemolytic staphylococci fermented certain sugars but not others. However, he did not determine final pH values. He did find differences in the amount of liquefaction of gelatin in a given time interval. On the other hand, Dudgeon[21] noted that subculturing daily caused changes in the biochemical reactions, and that for comparative study, only freshly isolated strains should be used, and the time interval between isolation and testing should be constant for all strains used. Chapman and Stiles[22] noted only an occasional reaction occurring after as long as 48 hours incubation and concluded that the reactions occurring after incubation periods of 5 to 30 days, did so because of the development of variants.

Serological methods have been used extensively in the classification of organisms such as the genera of Streptococci, Salmonella and Shigella, and for identification of types of organisms within a species such as the 40 or more types of Lancefield group A streptococci and the 112 O antigenic groups of Escherichia coli. Sears, Brownlee and Uchiyama[23] and Sears and Brownlee[24] used O group agglutination as a means of identifying resident and transient strains of E. coli in the human bowel. In 1922 Hine[25] attempted to classify staphylococci by agglutination reac-

tions, but found only three types among the yellow strains and two types among the white strains. By precipitation reactions, using a soluble specific substance from staphylococci, Julianelle and Weighard[26] divided staphylococci into two distinct immunological groups corresponding to the pathogens and nonpathogens. It was not until Cowan[27, 28] developed the slide agglutination technique in 1939 that strains of S. aureus were divided into a significant number of serological types. Christie and Keogh[29] identified nine serological types. Hobbs[30] added four more types to Christie and Keogh's nine and applied the method to epidemiological studies in the field.

It has long been known that the pathogenic staphylococci produce a soluble toxin called the alpha toxin, characterised by its hemolytic, skin necrotizing and lethal effects. Few reports in the literature describing the toxic properties of various strains of staphylococci mention the species of red cells used to determine the hemolytic effect. This may account for some of the discrepancies in reports by different investigators of the properties of alpha toxin. Alpha toxin hemolyses rabbit and sheep red cells but not those of other species. Since 1935, at least three other hemolysins have been described and confirmed by later workers. These toxins or hemolysins are produced by staphylococci in various combinations, a fact which might be made use of in identifying strains. However special conditions of culture are required to demonstrate the presence or absence of these hemolysins in any given strain, and specific antihemolysins are necessary to confirm the findings.

Since the description by Twort[31] in 1915 of an "ultramicroscopic virus" and the early investigations of the lytic properties of bacterial viruses by D'Herelle[32] culminating in his monograph in 1922, the study of bacteriophages has progressed rapidly. Craigie and his coworkers[33, 34, 35, 36, 37] developed specific phages for at least 11 types of virulent typhoid organisms, and these phages have been used extensively in epidemiological studies of typhoid fever. While bacteriophages for staphylococci have been studied by numerous workers, the use of these phages in the epidemiology of staphylococcic infections was not feasible until the reports by Fisk[38, 39] in 1942 of the occurrence of bacteriophage carriers among strains of S. aureus, and his description of methods for isolating the phages. In a subsequent report, Fisk and Mordvin[40] showed the practicability of identifying types of S. aureus by bacteriophage action. Wilson and Atkinson[41] extended Fisk's technique, developing 18 phages with which they identified 21 types or subtypes of S. aureus. Since 1945, other phages have been isolated bringing the total number of different staphylococcus phages to 32.

Bacteriophage typing of staphylococci has been used quite extensively in recent years in epidemiological studies of various kinds. Skin and nose carriage of bacteriophage types was reported by Williams[42] in 1946. Denton, Kalz and Foley[43] used the method in an investigation of an outbreak of phemphigus neonatorum in a hospital. McClure and Miller[44], Evan and Neven[45], and Saint-Martin, Charest and Desranleau[46] applied bacteriophage typing to epidemiological investigations of food poisoning outbreaks. The most widespread use of bacteriophage typing

has been in studies of the increasing frequency of staphylococcus infections caused by strains resistant to penicillin. Among the reports appearing in the literature are those of Barber and Rozwadowska-Dowzenko[47], Barber and Whitehead[48], Rountree and Thompson[49], Barber, Hayhoe and Whitehead[50], Elwood[51], and Wallmark and Laurell[52].

It has been shown in several studies that nasal carriage of certain bacteriophage types of staphylococci are common in hospitals. These types, often perdcillin resistant, may spread rather rapidly to new staff members or patients entering the hospitals. Often, too, infections occurring after entry into the hospital are of the same phage types as the predominant phage types carried by the hospital personnel. It has been demonstrated that the same phage types that are found in carriers are also found in the air of the wards where the carriers work[43, 47, 49, 50, 52]. Laurell and Wallmark[53, 54] found staphylococci most frequently on the upper lip, hands and clothes when they were present in the nose. All but one child of 26 children with skin lesions examined by these investigators had the same phage type isolated from both respiratory tract and skin.

Bacteriophage typing as the method of choice for this study. After consideration of all the methods described, bacteriophage typing seemed to be the method of choice for our problem from the standpoint of simplicity and reliability. Determination of biochemical reactions is time-consuming and unreliable. Hemolysin production as a means of identifying types has not been investigated; the reliability of the method is unknown. The necessity for specific antitoxins and special

equipment to identify the hemolysins precludes its use as a quick and simple method of typing. Precipitin reactions identify at most only six types. The agglutinin-absorption test identifies a much larger number of types, but the antigenic composition of the staphylococci has not been as well defined as in the enteric group of organisms, and the method has not been used extensively enough to determine its reliability.

Smith[55, 56] and Williams and Rippon[57] in independent studies showed that lysis of staphylococci by bacteriophage is not specific in the sense that a given strain of staphylococcus will be lysed by one particular phage only, even at high dilution. If however, a large number of phages are tested against a large number of strains, the occurrence of lysis will fall into a number of more or less distinct patterns. The phages producing these patterns are not randomly assorted, but certain phages are commonly associated in groups. On the basis of this association, Williams and Rippon[57] divided their phages into three groups which are quite or nearly distinct in their actions, that is staphylococci which are lysed by Group I phages are usually highly resistant to the phages constituting Groups II and III, and the same exclusiveness is manifested by Groups II and III. Any given strain of staphylococcus may be lysed by one or more phages within the group. The staphylococci lysed by phages in Groups I, II or III correspond broadly to Cowan's serological types I, II and III respectively.

Williams, Rippon and Dowsett[58] believed that phage patterns are too numerous to designate definite types of staphylococci and suggested that results be reported as "bacteriophage patterns." Most of the phage

typing studies reported have been studies of epidemics of staphylococcus infections among closely associated people with the idea in mind of finding a common source of infection. In these cases, variation in lytic patterns assumes major importance, and one must be certain that the variations in patterns actually denote distinct strains, and are not the result of conditions in the laboratory.

Smith[55, 56] has demonstrated that staphylococci may acquire resistance to [illegible] phage lysis, and suggested that this may account for the different patterns that occur within a group. He expressed the opinion that such changes in pattern may occur in the field among strains isolated in epidemiological investigations, but felt that such changes would be a rare occurrence in investigations of short duration. In spite of the ability to acquire resistance, Smith considered phage typing of staphylococci to be useful in epidemiological studies so long as the acquired resistance becomes a permanent and constant character. He then demonstrated that phage patterns are stable in character when the strains of staphylococci are maintained under laboratory conditions.

However, Williams and Rippon[57] demonstrated a gain or loss of weak reactions in 50 percent of duplicate plates seeded from the same broth culture. When phage testing of a culture was repeated on different days a greater degree of variation resulted. When multiple colonies were picked from the original plate, or when multiple cultures were made from the same broth, a gain or loss of a strong reaction sometimes occurred. Cultures taken in sequence from the same site on different days might show a gain or loss of one or more strong reactions. As a

result of these rather extensive studies, Williams and Rippon concluded that results of phage typing should be interpreted in the following manner. In sets of cultures tested on the same day, two strong differences in lysis, that is complete absence of lysis, indicates a distinct population in any of the three groups, but one strong difference in lysis indicates a distinct population in either of Groups I or II. A population was defined as a group of cocci and their progeny growing in one natural site. Because of the variations that could occur on strains of staphylococci maintained in the laboratory, these investigators felt that the staphylococci could be placed definitely in one of the three groups, but beyond that the most that could be done was to decide whether or not any pair of cultures was or was not identical. Wallmark and Laurell[52] independently concurred in this opinion, but added that consideration of the epidemiological data and the complicated phage patterns that sometimes occur increased the reliability of the method.

Phage filtrates used for typing are diluted in such a manner that any given filtrate will just lyse completely the strain of staphylococcus upon which it was propagated. Many strains of staphylococci isolated for epidemiological study are not lysed by any of the diluted phages. Williams and Rippon[57] tried undiluted phages upon these resistant strains and found that 49.4 per cent of 154 resistant strains were lysed by one or more undiluted phages. They pointed out, however, that interpretation of results was more difficult because the filtrates would vary greatly in titer and plaques were often obscured by secondary growth.

In a number of epidemiological investigations, particularly in

the study of acquired penicillin resistance, it has been found that in each instance one or at most two phage types were involved in a given epidemic. That this is not because of the existence of only two or three types in the general population has been shown by both Wallmark and Laurell[52] and Denton[43] who found that in outpatients and others not associated in any way with the hospital or epidemics under investigation, a large number of phage types existed.

Williams[42] studied the phage types of staphylococci obtained from 161 persons and from the frequency distribution of types found, he calculated the probability of chance duplications of any type. In a group of 36 persons from whom S. aureus was isolated from both nose and skin, 31 carried the same phage type both places. The number of duplications that occurred in this group was far in excess of the number to be expected on a chance basis and Williams concluded that duplication of types is unlikely to be a chance effect.

In spite of the very evident limitations of the bacteriophage method of typing, its reliability for the purpose of this study seems to be sufficient since it is proposed simply to show similarity or differences in strains of staphylococci obtained from the nose and from the lid margins, conjunctivae or meibomian glands of the same individual.

METHODS AND MATERIALS

Collection of cultures. Material from both nose and lid margins, conjunctivae or meibomian glands was obtained from infected persons. For purposes of comparison, similar material was obtained from a group

of medical students who gave no history or symptoms of infection. This group was considered to be representative of the general population.

Sterile cotton swabs moistened with sterile, 0.9 per cent saline were used for obtaining cultures. Two cultures were taken from each patient, one from the nose, and one from the lid margin, conjunctiva or meibomian glands. Lid margin cultures were obtained by rubbing the moist swab vigorously along the lash line. Cultures from the conjunctiva were made by everting the lower lid and passing the moist swab over the exposed palpebral conjunctiva. Cultures of meibomian glands were obtained by expressing the secretion from the glands and picking up this material with a moist swab. Cultures from the nose were made by inserting the moist swab into the anterior part of the nostril, rotating the swab and withdrawing it. The swabs were streaked over the surface of blood agar plates on the same day. During the course of routine work in the eye laboratory it had been observed that swabs used in making cultures of the eye could be streaked over the entire blood plate without danger of obtaining confluent growth. However swabs used for taking cultures from the nose were streaked over a third of the plate only, then a straight inoculating needle was used to streak out the culture over a third of the plate at right angles to the initial inoculum. The needle was flamed and cooled and the second inoculum streaked out at right angles to it.

Blood plates were made fresh each day when needed. Human citrated blood from the blood bank was used throughout the experimental work. Plates were prepared by melting resuspended Difco dehydrated blood agar

base at 100°C., cooling to 45°C. and pouring into 10 cm. Petri dishes containing one c.c. of blood.

Culture plates were examined for the presence of staphylococci after 24 hours incubation at 37°C., and if they were present a notation was made of the approximate number of colonies as follows:

1+ 50 colonies or less
2+ 50 to 300 colonies
3+ more than 300 colonies but without confluent growth
4+ confluent growth

One to five or more colonies were picked from each plate and transferred to extract agar slants. The number of colonies picked depended upon the total number of colonies present on the plate. The extract slant cultures served as the stock cultures. A record was kept of the color on blood agar and the hemolytic action of each colony picked. If the colonies differed in appearance, at least one of each kind was picked.

A code number was assigned to each stock culture. This number indicated the patient from whom the culture was obtained, the number of the culture if more than one culture was obtained from the same site at different times, the site from which the culture was obtained, and the number of the colony picked from the plate. Thus ABC(2)a 1 indicated patient ABC, culture number two from the conjunctiva and the first colony picked from the plate. The extract slant cultures were examined after 24 to 48 hours incubation at 37°C. for extent and color of growth. They were then stoppered with rubber stoppers and stored in the refrigerator until used.

Biochemical tests. The biochemical tests outlined in Bergey's Manual, 6th ed., 1948[59], were used to identify species. Included are

reactions to such tests as mannitol fermentation, reduction of nitrate to nitrite, and ability to utilize $NH_4H_2PO_4$ as the sole source of nitrogen. Ability to liquefy gelatin is included in the necessary tests for defining species, but this test was omitted in our study. Because the range of tests used was incomplete, the results are treated in an appendix.

Stock cultures were inoculated onto the surface of nitrate agar slants, incubated for 48 hours at 37°C. and tested for nitrate reduction by adding a few drops each of sulfanilic acid and alpha-naphthylamine. A distinct pink or red color indicated the presence of nitrite. Uninoculated tubes of nitrate agar were incubated under the same conditions and tested in the same manner. Results were recorded as positive if nitrite was produced and negative if not produced under the conditions of the experiment.

To determine ability to utilize ammonium dihydrogen phosphate, stock cultures were streaked onto the surface of a synthetic carbohydrate agar medium containing the phosphate and incubated for three weeks at 37°C. Results were recorded as positive or negative growth at the end of this period.

Mannitol broth was used to determine ability to ferment mannitol. Stock cultures were inoculated into tubes of mannitol broth and incubated for 48 hours at 37°C. A red color indicated fermentation; no test was made for gas production. Results were recorded as positive if an acid change in the indicator appeared and negative if such indicator change did not occur.

Coagulase test for pathogenicity. Coagulase tests were performed on all strains isolated to determine pathogenicity. Several lots of human plasma removed from outdated blood bank blood were tested for coagulability by a known coagulase positive strain of staphylococcus. One lot of plasma, used in a one to five dilution, was found to coagulate within three hours with the test strain. All stock strains were tested with this one lot of plasma. The plasma was used in a one to five dilution in 0.9 per cent saline. One-half ml. amounts of the diluted plasma were distributed in small tubes. A large loopful of an 18 to 24 hour agar slant culture of the organism to be tested was added to one-half ml. of the diluted plasma and incubated in a water bath at 37°C. Results were recorded as positive if coagulation took place within three hours. An uninoculated tube of plasma and a tube inoculated with a known coagulase positive strain were used as controls each time the test was performed.

Sources of staphylococcus bacteriophages. Phage filtrates together with their propagating strains of staphylococci were received from Dr. G. G. Kalz, Department of Bacteriology, McGill University, Montreal, Canada. Phages received were designated 3A, 3B, 3C, 47, 47A, 47B, 47C, 7, 6, 52, 52A, W, 29, 31, 44, 42. Initially, propagation was tried by the method of Wilson and Atkinson[41] in which a few drops of purified phage filtrate and six drops of an 18 hour broth culture of the propagating strain of staphylococcus were added to ten ml. of extract broth and incubated at 37°C. until lysis took place. Attempts to propagate phage in this manner resulted in the loss of several phages

including 29, 31, 42, 44, 52A, 6 and 51. Phages 42 and 44 were reported by Dr. Kalz to be very weak, and the vials containing phages 6 and 51 were dry on arrival.

Subsequently two phages were found by the cross culture method of Fisk(38). Staphylococcus strain CRy when spotted on strain 3, the propagating strain for phage 6, produced plaques. The plaques were picked and propagated on strain 3 until a filtrate was obtained which lysed strain 3 at a dilution of 1:10,000. In the same manner a phage lysing strain 1163 was propagated. This was believed to be phage 42B.

In testing all undiluted phage filtrates against all propagating strains, it was noted that some phages attacked strains other than their propagating strains although to a lesser degree. When lysis of a propagating strain for one of the missing phages was observed, propagation was attempted. In this way phages 44A and 52A were propagated from phage 52 on staphylococcus strains 373 and 925 respectively. Phage 51 was propagated from phage 3C using staphylococcus strain 145, the propagating strain for phage 51. Phages 42C and 42E were obtained by propagating 42B on staphylococcus strains 1307 and 1670 respectively. Phages 29, 31 and 44 were not recovered. There is no certainty that the recovered phages are identical to those lost, but when they lysed the propagating strains only at the routine test dilutions, or showed the same pattern of reactions on the propagating strains, they were so designated.

Propagation of phages. For routine phage typing, 17 phages were used. These phages were 3A, 3B, 3C, 47, 47A, 47B, 47C, 52, 52A, 7, 6,

51, W, 42B, 42C, 42E, and 44A. Phages were propagated by the plate method of Williams and Rippon[57] with some modifications. Extract agar plates were spread evenly with 18 hour broth cultures of the propagating strains of staphylococci and allowed to dry at 37°C. for one-half to one hour. Wedge-shaped areas were marked with a wax pencil on the bottom of the Petri dishes. Two or three drops of the phage to be propagated were spread over the surface of the agar plate previously inoculated with the phage's specific propagating strain, with the exception of the wedge-shaped area used for control. This procedure was repeated for each phage to be propagated. The plates were incubated at 37°C. overnight or until lysis had occurred. Growth of staphylococci was apparent only in the wedge-shaped areas if phages were present. After incubation these areas were cut out with a sterile instrument and the plates placed in a freezer at -40°C. for several hours. Plates were then thawed at room temperature and the resulting fluids pipetted off. These fluids contained the phage particles. The fluids were filtered through sintered glass filters and stored in screw-capped tubes in the refrigerator.

Titration of phages. Each phage filtrate was titrated against its propagating strain. Ten-fold dilutions of the phage filtrate to be titrated were prepared in broth. An extract agar plate was inoculated with the specific propagating strain of the phage and dried for one-half to one hour at 37°C. One drop of each of the dilutions was then spotted on the plate. The plate was incubated at 37°C. for three to five hours then overnight at room temperature. The titer and routine test dilution was the highest ten-fold dilution causing complete lysis of the propagating

strain. All phage filtrates were tested both undiluted and at the routine test dilution for phage patterns as suggested by Williams and Rippon[57]. The staphylococcus phages are identified by the patterns of lysis shown by the phages when tested upon all of the propagating strains. The lysis becomes more specific when the phages are diluted to titer. To illustrate, phage 3A when tested undiluted will lyse strains 284, 211, 1339, 1307, 373 and 145, but when diluted to titer it will lyse only strain 284, its propagating strain. When new batches of phage are prepared they must show the same lytic patterns as previous batches; any change in pattern may indicate contamination with another phage or possibly a mutation of the phage in question. Tables 1 and 2 indicate the lytic patterns of the phages used in this study.

Method for routine phage testing of staphylococci. Dilutions of phage filtrates used in this study were tested against the propagating strains of staphylococci not more than four days before using, to be sure that there was no loss of titer. All strains of staphylococci isolated for study that showed weak or no lysis with diluted phages were tested with undiluted phages following the suggestion of Williams and Rippon[57].

Extract agar plates were inoculated evenly over the surface with 18-hour extract broth cultures of the staphylococcus strains to be typed, and were dried at room temperature with the lids open for one hour. Phages were spotted in a constant order on the plates using a tuberculin syringe with a 30-gauge needle. Each drop contained approximately 0.01 c.c. of phage filtrate. Plates were incubated three to five hours at

37°C. then at room temperature overnight. After incubation the phages lysing a given strain were noted and the degree of lysis indicated as follows:

+++ confluent lysis or semiconfluent lysis or confluent lysis with secondary growth
++ more than 50 discrete plaques
+ less than 50 discrete plaques

Only three plus reactions were used in designating phage patterns. Where the three plus reactions occurred with undiluted phages only, the designated phages were enclosed in parentheses. If additional weak reactions were present, a plus sign was placed after the numbers of the phages giving three plus lysis. The phage reactions were also grouped according to those suggested by Williams and Rippon(57). The phage filtrates included in each group are as follows:

Group I 52, 52A, 29, 31, 31A, 44, 44A
Group II 3A, 3B, 3C, 51
Group III 6, 7, 42B, 42D, 42E, 47, 47B, 47C

Phages 42C, 47A, are unclassified. We had available for our study only three of the group I phages, 52, 52A and 44A. Phage W was one isolated by Dr. Kalz and was not included in Williams and Rippon's classification.

TABLE 1. PHAGE PATTERNS OF 17 PHAGES AT ROUTINE TEST DILUTION ON 16 PROPAGATING STRAINS

Propagating strains	Phages and dilutions used																
	3A 10^{-5}	3B 10^{-5}	3C 10^{-4}	6 10^{-4}	7 10^{-1}	42B 10^{-2}	42C 10^{-1}	42E 10^{-1}	44A 10^{-2}	47 10^{-4}	47A 10^{-2}	47B 10^{-2}	47C 10^{-1}	51 10^{-4}	52 10^{-3}	52A 10^{-4}	W 10^{-2}
284	++*	++	+											++			
211		++												++			
1339			++											+			
3				++						++		++					
4					++												
1163						++							++				
1307							++										
1670								++				++					
373									++								
36										++		++					
761											++						
987												++					
145														++			
144															++	+	
925																++	
W																	++

* ++ = Complete lysis, + = incomplete lysis

TABLE 2. PHAGE PATTERNS OF 17 UNDILUTED PHAGES ON 16 PROPAGATING STRAINS

Propagating strains	Phages used																
	3A	3B	3C	6	7	42B	42C	42E	44A	47	47A	47B	47C	51	52	52A	W
284	++*	++	++								+			++			
211	++	++												++			
1339	++	+	++											++			
3				++						++	+	++					
44					++												
1163						++				+			++		+		
1307	++	++					++							++		+	
1670								++			++	++					
373	++				++				++					+		++	+
36										++		++					
761											++						
987												++					
145	++	++												++			
144															++	++	
925															+	++	
W																	++

* ++ = Complete lysis, + = incomplete lysis

MEDIA

Extract broth.

Bacto-peptone	10.0 grams
Sodium chloride	0.5 grams
Bacto-beef extract	0.3 grams

The above materials were added to 1000 ml. cold tap water and brought to a boil to dissolve. The medium was adjusted to pH of 7.0 with normal NaOH and dispensed in approximately 3.5 ml. amounts in 4 x 5/8 inch tubes, or 15 ml. amounts in 6 x 3/4 inch tubes. The tubed media was autoclaved at 15 lbs. pressure for 6 to 8 minutes, cooled and stored in the refrigerator.

Extract slants.

Fifteen grams of agar was added to 1000 ml. of extract broth, brought to a boil, then dispensed in approximately 3.5 ml. amounts in 4 x 5/8 inch tubes. The tubed media was autoclaved at 15 lbs. pressure for 6 to 8 minutes and slanted to cool. Media was stored in the refrigerator until used.

Mannitol broth.

The medium was prepared by adding 10.0 grams of mannitol and 10.0 c. c. Andrade's indicator to 1000 ml. of extract broth. The broth was tubed in approximately 3.5 ml. amounts in 4 x 5/8 inch tubes and autoclaved at 15 lbs. pressure for 6 minutes. The tubed media was stored in the refrigerator until used.

Synthetic carbohydrate medium.

$NH_4H_2PO_4$	1.0 grams
Potassium chloride	0.2 grams
Magnesium sulfate	0.2 grams
Glucose	10.0 grams
Agar	15.0 grams

The above ingredients were dissolved in cold water and brought to a boil. The pH was adjusted to 7.0 with normal sodium hydroxide and brom cresol purple indicator in a 0.04 per cent aqueous solution was added. The medium was tubed in approximately 3.5 ml. amounts in 4 x 5/8 inch tubes, autoclaved at 15 lbs. pressure for 6 to 8 minutes, slanted and cooled. The tubed medium was stored in the refrigerator until used.

Nitrate agar.

Bacto-beef extract	3.0 grams
Bacto-peptone	5.0 grams
Potassium nitrate	1.0 grams
Bacto-agar	12.0 grams

Twenty-one grams of Difco's dehydrated medium in the above proportions was dissolved in 1000 ml. cold tap water and brought to a boil. The medium was dispensed in approximately 3.5 ml. amounts in 4 x 5/8 inch tubes and sterilized by autoclaving at 15 lbs. pressure for 6 to 8 minutes, then slanted and cooled. The sulfanilic acid reagent was prepared by dissolving 0.8 grams powdered sulfanilic acid in 100 ml. 5 N acetic acid. Alpha naphthylamine reagent was prepared by dissolving 0.5 grams powdered alpha naphthylamine in 100 ml. 5 N acetic acid.

EXPERIMENTAL

In reporting the results of our study, it is important to keep in mind that the objective was to obtain evidence as to the origin of the staphylococci found in a high proportion of cases of external infections of the eye. The staphylococci carried in the normal nose have been cited as one possible source of the infections. By use of the coagulase test and phage typing we have attempted to find evidence that the staphylococci from these two sites may or may not be progeny of a single strain. It is obvious that if the staphylococci from the two sites differ in pathogenicity or phage type, they are not the same strain. The staphylococci at one site cannot then be considered the source of the staphylococci at the other site.

The data as presented shows first the correlation in pathogenicity of the cultures from the two sites as indicated by the coagulas test, and second where cultures from both sites are coagulase positive, the correlation in phage type between the two sites. The term experimental group has been applied to that group of individuals with external infections of the eye, while the term control group has been applied to that group of individuals who are normal as far as external infections of the eye are concerned.

Basis of comparison of cultures from eye and nose. In the control group, 55 sets of cultures were obtained from 55 individuals with a total of 135 separate cultures. There was an average of 2.5 cultures per set. A set consisted of cultures from eye and nose of the same individual taken at the same time.

In the experimental group, 59 sets of cultures were obtained from 55 individuals. Duplicate cultures were taken from three of these people. The interval between cultures was three weeks in two cases and five months in one case. In the latter instance, cultures were taken from both eyes and both nostrils on the same date. These cultures were counted as two sets. In addition, in three instances, the author was able to compare the culture sets with eye cultures taken previously at six, seven and ten months respectively. Two of the individuals from whom duplicate cultures were made were mother and daughter. The 59 sets included 483 separate cultures, an average of 8.2 cultures per set.

While the number of sets in each group is approximately the same, there is a marked difference in the number of cultures from each group. Up to the present time only those staphylococci considered to be pathogenic have shown susceptibility to lysis by phage. These pathogenic staphylococci are the organisms with which we are primarily concerned in this study. It was thought advisable to determine the constancy of phage patterns of staphylococci from any one site. For this reason more colonies were selected from plates with S. aureus than were selected from plates with nonpathogens only. The incidence of S. aureus was much higher in the experimental group than in the control group thus accounting for the discrepancy in the total number of cultures in each group. The proper basis for comparison then is not on the total number of cultures, but on sets of cultures.

Incidence of coagulase positive and coagulas negative sets of cultures. When a comparison is made of the incidence of coagulase

positive and coagulase negative sets of cultures in the two groups, a marked difference is noted. Table 3 illustrates this difference.

TABLE 3. INCIDENCE OF COAGULASE POSITIVE AND COAGULASE NEGATIVE SETS OF CULTURES FROM EYE AND NOSE

Results of coagulase test	Experimental group		Control group	
	Number of sets	Per cent of total	Number of sets	Per cent of total
Coagulas positive both eye and nose	24	40.6	1	1.8
Coagulase positive eye only	11	18.7	0	0
Coagulase positive nose only	13	22.0	17	30.9
Coagulase negative only, eye and nose	11	18.7	37	67.3
Total	59	100.0	55	100.0

Coagulase positive staphylococci were isolated from 48, or 81.3 per cent of the persons in the experimental group, while only 18, or 32.7 per cent of the control group carried coagulase positive staphylococci. The incidence of nasal carriage alone, without coagulase positive staphylococci from the eye was more nearly the same in both groups. However, a total of 62.6 per cent of the entire experimental group carried coagulase positive staphylococci in the nose as compared with 32.7 per cent of the control group.

Correlation between clinical diagnosis and results of the coagulase test. It is difficult to correlate the presence or absence of coagulase positive staphylococci with the clinical diagnosis since standard terminology was not used by the clinical staff. An effort has been made to tabulate this data nevertheless. Table 4 shows the coagulase negative cultures in relation to clinical diagnosis.

TABLE 4. CORRELATION BETWEEN CLINICAL DIAGNOSIS AND CULTURES SHOWING COAGULASE NEGATIVE STAPHYLOCOCCI FROM THE EYE

Clinical diagnosis	Coagulase negative cultures from the eye	
	With coagulase positive cultures from the nose	Without coagulase positive cultures from the nose
Acute conjunctivitis and keratitis	4	6
Acute exacerbation of chronic meibomitis or blepharitis	3	2
Chronic meibomitis, blepharitis or dacryocystitis	6	0
Normal	0	1
No diagnosis	0	2
Total	13	11

It should be pointed out that of the 24 cases of eye infections in which coagulase positive organisms were not found in cultures from the eye, ten were diagnosed as acute conjunctivitis. Acute conjunctivitis is not a disease caused by a single agent. Many organisms have been implicated, including Diplococcus pneumoniae, Streptococcus pyogenes, Hemo-

philus influenzae, Neisseria gonorrhea, N. meningitidis and certain of the viruses. It is not surprising then that coagulase positive staphylococci were not found in every instance.

In nine cases of chronic meibomitis and blepharitis and acute exacerbations of these chronic diseases, no coagulase positive staphylococci were isolated in cultures from the eye, but six of these cases proved to have these organisms in the nose.

Table 5 indicates the distribution according to clinical diagnosis of those cases in which coagulase positive cultures were isolated from the eye.

TABLE 5. CORRELATION BETWEEN CLINICAL DIAGNOSIS AND CULTURES SHOWING COAGULASE POSITIVE STAPHYLOCOCCI FROM THE EYE

Clinical diagnosis	Coagulase positive cultures from the eye	
	With coagulase positive cultures from the nose	Without coagulase positive cultures from the nose
Acute and subacute conjunctivitis and keratitis	9	3
Chronic conjunctivitis	2	0
Blepharitis, acute and chronic	9	4
Meibomitis	1	1
Miscellaneous diagnoses	3	3
Total	24	11

Coagulase positive staphylococci were isolated from 17 cases of acute and chronic cases of blepharitis and meibomitis and from 12 of these cases coagulase positive staphylococci were isolated also from the nose.

Correspondence in phage patterns of cultures from a single site. In all but two instances, the phage patterns of cultures from a single site, eye or nose, were alike. Table 6 indicates the correspondence in phage patterns, including weak lysis and those instancesin which no lysis occurred with coagulase positive organisms.

TABLE 6. CORRESPONDENCE IN PHAGE TYPES OF CULTURES FROM ONE SITE

Type of reaction	Number of sites in which multiple cultures were of same phage pattern		Number of sites in which multiple cultures were of different phage patterns	
	Eye	Nose	Eye	Nose
Strong lysis	22	20	0	0
Weak lysis only	12	11	0	1
No lysis	4	5	0	0
Strong lysis and no lysis	0	0	0	1
Total	38	36	0	2

The similarity in phage patterns of staphylococci isolated from a single site simplified the problem of comparison. Where phage patterns were identical, the staphylococci were treated as single strains.

Phage patterns of staphylococci isolated from the experimental group. Table 7 lists the individuals from whom typable staphylococci were isolated in this study, and the phage reactions of each strain of staphylococcus. If more than one phage pattern occurred in strains isolated from any one individual, all patterns are listed. The strains listed include only those showing strong lysis.

More than one culture was obtained at different times from four of these individuals. The results of phage typing varied somewhat among these four individuals. In case 3, (Table 7) two cultures were taken about six months apart. The phage patterns of the cultures differ by one phage. If Williams and Rippon's[57] conclusions are correct, one might assume that these cultures are from the same strain. However, since so much time had elapsed between the first and second cultures, the difference in patterns may indicate that a new strain had been acquired in the interim.

In case 12 (Table 7) two cultures taken ten months apart are exactly alike in pattern. In cases 13 and 14 (Table 7), a mother and her daughter, all cultures showed the same phage patterns. In case 17 (Table 7) three cultures taken at intervals of several months proved to differ in phage patterns in each instance. The first culture was non-typable. The second culture showed weak reactions only, while the third culture was typable, but cultures from nose and eye differed in phage patterns. This individual had close contact with many patients suffering from staphylococcus infections and thus had ample opportunity to acquire new strains.

TABLE 7. PHAGE PATTERNS OF STAPHYLOCOCCI FROM THE EXPERIMENTAL GROUP SHOWING STRONG LYSIS

Case No.	Date	Patient	Phage Pattern
1	7/10/52	GePe(1)	52, 52A, (+)*
2	7/18/52	DoMi(1)d 1 DoMi(1)d 2	(52, 52A, +) (7, +)
3	2/6/52 8/8/52	LeGo(1) LeGo(2)	47, 47B 6, 47, 47B
4	9/12/52	JaGi(1)	3C, 51, (+)
5	9/10/52	MaCr(1)	47C, (+)
6	9/19/52	EdHi(1)	52, 52A, (+)
7	9/25/52	RJM(1)	(3C, 51, +)
8	9/26/52	MacC(1)	51, (+)
9	9/29/52	ThKe(1)	52, 52A, (+)
10	10/1/52	GrEm(1)a 1 GrEm(1)d 2	7 52, 52A
11	12/23/52	RiGr(1)	(47, 47B)
12	12/17/51 10/25/52	WiDa(1) WiDa(2)	(3C, 51, +) (3C, 51, +)
13	11/8/52 12/1/52	SaHa(1) SaHa(2)	3B, 51, (+) 3B, 51, (+)
14	11/8/52 12/1/52	MrHa(1) MrHa(2)	3B, 51, (+) 3B, 51, (+)
15	11/14/52	MaSe(1)b 1 MaSe(1)b 3	7, 47, 47B, (+) 7, 47B

* (+) = additional weak reactions present
(52, 52A) = lysis by undiluted phages only
NT = nontypable cultures

TABLE 7 (Continued)

Case No.	Date	Patient	Phage Patterns
16	11/13/52	AlJo(1)	3C, 51
17	12/11/51	JEH(1)	NT
	7/25/52	JEH(2)	NT (+)
	12/11/52	JEH(3)b 1	51 (+)
		JEH(3)d 1	(7, +)
18	12/14/52	HaKe(1)	W
19	12/18/52	MaSc(1)	(47, 47B, 47C, +)
20	12/20/52	NeHe(1)	7, 47, (+)
21	1/16/53	BAr(1)	6, 47, 47B
22	1/24/53	ZeHa(1)	(3A, 3C, 51, +)
23	2/20/53	KnIr(1)	6, 47, (+)
24	2/3/53	MrRi(1)	6, 7, 47, 47B
25	2/12/53	ViHe(1)	6, 47, 47B, 47C, (+)
26	3/2/53	JeCu(1)	7, (+)

Table 8 lists the phage patterns found in each phage group. Group I phage patterns occurred six times with only one distinct pattern. Group II phage reactions appeared twelve times with four distinct patterns. Group III phage reactions appeared 21 times with 14 distinct patterns. Any given pattern, such as the pattern in Group I in this study, may have occurred with diluted or undiluted phage only. Strains showing the same phage patterns but differing in their resistance to lysis by these phages were considered to be different strains.

The variety in phage patterns of cultures from different individuals is in complete contrast to patterns of cultures isolated from a single individual. As a general rule all cultures obtained from any one individ- were of the same phage pattern. Had this not been true, no comparisons between cultures from nose and eye would have been possible. There would have been no indication that one strain was similar to or differed from another in any way.

Phage patterns of staphylococci showing weak lysis only. Where only weak reactions to undiluted phages occurred, the cultures were considered non-typable. With one exception, the cultures from a single site showed the same weak reactions. Table 9 lists the individuals from whom these cultures were isolated and the weak reactions shown. There was some variation from one individual to another in the patterns of weak lysis shown by the staphylococci isolated. Because of this variation we felt that in each instance we were dealing with a single strain and interpreted the results in the same manner as those strains showing strong lysis. It is possible that if more bacteriophages had been available, some of these cultures might have proved typable.

TABLE 8. PHAGE PATTERNS FOUND IN EACH OF THE THREE MAJOR GROUPS

Group I		Group II		Group III	
No.	Pattern	No.	Pattern	No.	Pattern
6	52, 52A	5	3C, 51	3	7
		2	51	2	47, 47B
		4	3B, 51	2	6, 47, 47B
		1	3A, 3C, 51	1	47C
				3	47
				1	7, 47, 47B
				1	7, 47B
				2	6, 47
				1	47
				1	47, 47B, 47C
				1	6, 47, 47B
				1	6, 7, 47, 47B
				1	47, 47B, 42B
				1	6, 47, 47B, 47C

Phages W and 47A are not classified in any of the three major groups.

TABLE 9. PHAGE PATTERNS OF STAPHYLOCOCCI FROM THE EXPERIMENTAL GROUP SHOWING WEAK LYSIS

Case No.	Date	Patient	Phage Patterns
1	7/11/52	RoMi(1)	47C++, 42B+ *
2	7/17/52	JoFr(1)	47++, 47B++
3	7/25/52	JEH(2)	3B+
4	8/18/52	ArBr(1)	47C++, 42B++
5	9/4/52	GrBe(1)	47+
6	9/5/52	ChWa(1)	47+
7	9/26/52	DrHo(1)	47++, 47B++, W++
8	11/25/52	HoLy(1)	7+
9	11/30/52	MaDi(1)	6++, 47++, 47B+
10	1/22/53	DMi(1)	3A+, 3C+
11	2/19/53	MrLa(1)	6+, 47++, 47B+
12	2/13/53	AgCr(1)	47+

* ++ = more than 50 discrete plaques with undiluted phage, + = less than 50 discrete plaques with undiluted phage.

Correspondence in phage type of cultures from nose and eye. In 24 of the 59 sets of cultures, coagulase positive staphylococci were isolated from both nose and eye. Eleven of these sets showed strong lysis with one or more phages. Eight of the sets consisted of non-typable cultures with weak reactions. All of the cultures in each of these sets showed the same weak reactions. Two sets showed no phage lysis. Table 10 illustrates the correlation in phage patterns between the nose and the eye.

TABLE 10. CORRESPONDENCE IN PHAGE TYPES OF CULTURES FROM NOSE AND EYE

Type of reaction	Number of sites in which eye and nose cultures yielded organisms	
	Of same phage type	Of different phage type
Strong lysis	11	3
Weak lysis only	8	0
No lysis	2	0
Total	21	3

The correlation shown by phage typing is much greater than one would expect judging from the number of sets that did not correspond in pathogenicity as evidenced by the coagulase test. In all, 21 or the 24 sets showed exact correspondence in phage lysis between nose and eye.

DISCUSSION

From the data presented, it can be seen that coagulas positive staphylococci are much more common on the lid margins and conjunctivae and in the meibomian gland of individuals with external infections than in the same tissues of normal individuals. These findings corroborate those of previous investigators. An absolute agreement in type between staphylococci from the nose and from the eye in infections of the latter is not shown in this investigation. However, the number of instances in which coagulase positive staphylococci were found from both the nose and the eye was much higher in the experimental group than in the control group. A coagulase positive staphylococcus was isolated from the eye of only one of 55 persons in the control group as compared with 35 of 55 persons in the experimental group.

It may be argued that if the source of the staphylococci found in eye infections is from those normally carried in the nose, one should be able to isolate coagulase positive staphylococci from the nose in all infected cases. In this study, cultures were taken from the anterior nares only. Berens and Cumming[60] have shown that when nasal cultures only are taken, some pathogenic organisms are missed. His study of the incidence of toxigenic staphylococci in the nose and nasopharynx showed that of 277 sets of cultures, 28.5 per cent had toxigenic staphylococci in both nose and nasopharynx, 28.5 per cent in the nose only, while 10 per cent had toxigenic staphylococci in the nasopharynx only. Had it been possible to obtain cultures from the nasopharynx as well as the nasal cavity in our study, the incidence in which the coagulase positive

staphylococci were found from both eye and nose might have been higher. On the same basis it must be admitted that the incidence of the carriage of coagulase positive staphylococci in the control group might have been higher also.

The significance of the absence of coagulase positive organisms from the eye in the experimental group is harder to evaluate. However, in ten cases diagnosed as acute conjunctivitis or keratitis, viruses or organisms other than staphylococci may have been the cause of the inflammations. Other pathogenic organisms were not isolated in this study. Of 11 cases of chronic meibomitis, blepharitis or acute exacerbations of these conditions, nine carried coagulase positive staphylococci in the nose. These findings may be of some significance in view of Berens' belief that exotoxin produced in the nose or nasopharynx may give rise to a toxic condition of the conjunctiva. His hypothesis might be extended to include lid margin and meibomian gland inflammations, particularly where an active infection existed previously. In the eye clinic, where the cultures for the present study were obtained, it is generally assumed that chronic infections of the lid margin or meibomian glands follow acute primary infections caused by pathogenic staphylococci. The assumption is based on the fact that the majority of acute infections of this type have been shown to be caused by the pathogenic staphylococci.

The results of bacteriophage typing demonstrate rather strikingly the close relationship of the staphylococci from the nose with those from the eye. Of the 59 sets of cultures in the experimental group, 24 corresponded in that coagulase positive organisms were isolated from both

eye and nose. Twenty-one of the 24 sets showed complete correspondence in phage reactions. The experimental group in this investigation differed from most groups studied in that the individuals in the group for the most part had had no contact with each other. Therefore the question was whether or not pairs of cultures from the same individual were identical in phage patterns, a problem that fell well within the range of reliability of the method.

One cannot say with certainty from the data presented that the staphylococci found in the nose of an individual are the source of those causing the infections of the eye. The findings of other investigators lend support to the belief that the staphylococci found in infections of the eye came originally from the nose. Infections of clean surgical wounds were found to be caused by staphylococci with the same phage patterns as those found in nasal carriers among the hospital staff. Children with skin lesions were found to have staphylococci of the same phage patterns from both lesions and the nose, suggesting that the nasal organisms were the source of those in the skin lesions. An epidemic of pemphigus neonatorum in a hospital was caused by staphylococci of the same phage pattern as attendants carried in the nose.

Staphylococci have been found frequently on hands and clothes when found in the nose. Both hands and certain articles of clothing would be ready means of transmitting staphylococci from the nose to the eye. Since many people without any evidence of staphylococcus infections are nasal carrier of pathogenic staphylococci, the presence of a staphylococcus infection elsewhere on the body does not appear to be

essential for nasal carriage. It seems more likely that the staphylococci found in eye infections come from the nose rather than the reverse.

In the present investigation, one person, WiDa (case 12, Table 7) and possibly a second, LeCo (case 3, Table 7) show the same phage patterns in cultures taken at widely separated intervals. Two others, MrHa and SaHa (cases 13 and 14, Table 7) members of the same family, show the same phage patterns. These findings are suggestive that nasal carriage of the same phage type may persist over a long period, and that staphylococci may be passed from one member of the family to another. However, too few individuals or families have been studied to allow definite conclusions to be drawn.

It would be of definite value to determine the constancy of nasal carriage of staphylococci of a given phage pattern over a long period of time. Such a study would be of particular interest in those people who present themselves to the ophthalmologist with acute lid infections. In these cases it is desirable to know whether or not the same type of organism originally isolated persists in the nose, and whether or not reinfection or exacerbation occurs with the type found originally.

SUMMARY

Cultures were obtained from nose and eye of 55 persons with lid infections or conjunctivitis and from 55 persons with no history of lid infections. The latter group served as a control group.

Coagulase positive staphylococci were isolated from both nose and

lid margin of 40.6 per cent of the experimental group, but from only 1.8 per cent of the control group.

In the experimental group, 21 of 24 sets of coagulase positive cultures showed complete correspondence in phage patterns.

No absolute proof is offered that the nose serves as a reservoir of infection for the eye, but the results are strongly suggestive that this is the case. It has been demonstrated in this investigation that bacteriophage typing will serve as a useful method in tracing sources of infections of the eye.

Suggestions are made for further investigative work that might be undertaken.

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APPENDIX

Biochemical Reactions of the Staphylococci Isolated for This Study

A marked difference in colonial appearance of the white staphylococci had been observed during the course of this work. In the literature, these white staphylococci, isolated from external infections of the eye, have invariably been called Staphylococcus albus. We thought it would be interesting to determine the species of these organisms, thinking some might be other micrococci.

The biochemical tests used in classification of the micrococci include ability to reduce nitrate, ability to utilize $NH_4H_2PO_4$ as a sole source of nitrogen, ability to ferment mannitol and ability to liquefy gelatin. Gelatin was not used in this study and, for that reason, the exact classification of some of the organisms isolated is in doubt. No micrococci other than S. aureus and S. albus are known to coagulate plasma. We felt confident, therefore, in classifying all coagulase positive organisms as S. aureus or S. albus.[1] A few cultures fermented mannitol but did not coagulate plasma. Without knowing their action on gelatin, these organisms could be classified either as S. aureus and S. albus or as Micrococcus aurantiacus.

Tables 11 and 12 show the biochemical reactions of the staphylococci isolated from the experimental and control groups respectively and the tentative classification of these organisms.

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[1]These organisms are now classified as Micrococcus pyogenes, var. aureus or var. albus (Bergey, 6th ed., 1948). We have used the older terminology throughout this paper since these terms are still in common use.

TABLE 11. BIOCHEMICAL REACTIONS SHOWN BY CULTURES FROM PERSONS IN THE EXPERIMENTAL GROUP

Species	Number of cultures	Per cent of total	Reduce nitrate	Utilise $NH_4H_2PO_4$	Ferment Mannitol	Yellow pigment	Coagulase positive	
							No.	Per cent
Staphylococcus aureus	303	64.8	+	-	+	+	303	100
Staphylococcus albus	24	5.1	+	-	+	-	24	100
Micrococcus aurantiacus	4	0.9	+	-	+	±	0	0
M. epidermidis	104	22.2	+	-	-	-	0	0
M. candidus	30	6.5	-	-	+	-	0	0
Unclassified	2	0.5	+	-	-	+	0	0
Total	467	100.0						

TABLE 12. BIOCHEMICAL REACTIONS SHOWN BY CULTURES FROM PERSONS IN THE CONTROL GROUP

Species	Number of cultures	Per cent of total	Reduce nitrate	Utilize $NH_4H_2PO_4$	Ferment mannitol	Yellow pigment	Coagulase positive	
							No.	Per cent
Staphylococcus aureus	24	17.8	+	-	+	+	24	100
S. albus	7	5.3	+	-	+	-	7	100
Micrococcus aurantiacus	14	10.4	+	-	+	±	0	0
M. epidermidis	64	47.4	+	-	-	-	0	0
M. flavus	1	0.6	-	-	-	+	0	0
M. candidus	25	18.5	-	-	-	-	0	0
Total	135	100.00						

It should be noted that none of the micrococci classified as other than S. aureus or S. albus showed ability to coagulase plasma.

In Table 13 are recorded the species of micrococci isolated from eye and nose of members of the experimental and control groups.

TABLE 13. SPECIES OF STAPHYLOCOCCI ISOLATED FROM THE EXPERIMENTAL AND CONTROL GROUPS AND THE NUMBER OF PERSONS FROM WHOM EACH SPECIES WAS ISOLATED

Species	Experimental group		Control group	
	Eye	Nose	Eye	Nose
S. aureus	33	34	0	11
S. albus	2	4	1	5
M. aurantiacus	2	0	5	4
M. epidermidis	20	23	20	20
M. candidus	6	8	10	11
M. flavus	0	0	1	0
Unclassified	0	0	0	1
Total	63	69	37	61

A high proportion of the micrococci from both eye and nose in the experimental group are S. aureus, while the reverse is true in the control group. Persons in the experimental group who carried S. aureus often carried M. epidermidis in addition. This fact accounts for the similarity in numbers of that species in the two groups.

In view of these findings one should be cautious in classifying any white micrococci as S. albus and assuming them to be nonpathogenic. The micrococci that are truly S. albus are often pathogenic. Those that are nonpathogenic generally will be found to be other micrococci. The coagulase test should be done on all micrococci found in infections before calling them nonpathogenic.