Bacterial Interference Between Strains of *Staphylococcus aureus*, 1960 to 1970

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During the past ten years clinical experience has indicated that bacterial interference can be useful in curtailing staphylococcal disease among newborn babies in the nursery during epidemics and in patients with chronic furunculosis. Studies in a variety of animal models and in vitro have permitted investigations of mechanisms responsible for this ecologic relationship. However, to date, the explanation of this phenomenon in the human remains unknown.

During the past ten years, considerable data have been gathered by a number of investigators related to the phenomenon of bacterial interference between strains of coagulase-positive staphylococci. The present report summarizes the information as observed in humans, in animals, and in vitro.

Initial Observations

Our first observation suggesting that colonization of a single site with a single strain of coagulase-positive staphylococcus might inhibit colonization with a second strain of coagulase-positive staphylococcus was made in a nursery in the New York Hospital in 1959. Epidemiologic surveillence uncovered a nurse, a known nasal carrier of *Staphylococcus aureus* phage type 80/81, who had had repeated contacts with a group of 68 newborn infants of various ages. Our data revealed a 22% colonization rate with phage type 80/81 among 37 babies who were less than 24 hours of age when first handled by the index nurse. In contrast, not a single infant was colonized with *S aureus* 80/81 among the 31 babies who were more than 24 hours of age before first contact with the index nurse.[1] Examination of the nasal flora of all these infants disclosed that 84% of those more than 24 hours of age were nasally colonized with coagulase-positive staphylococci other than type 80/81 prior to contact with this nurse, while no baby less than one day of age was similarly infected. These data suggested that the presence of one strain of staphylococcus at a single site interfered with subsequent acquisition of another strain of staphylococcus at that particular site.

Detailed studies were then made of a strain of staphylococcus isolated from the nasal mucosa of a nurse found to colonize more than 100 infants during a short period of time. This coagulase-positive staphylococcus, labeled in our laboratory as strain 502A, was exquisitely sensitive to penicillin, resistant to tetracycline and was lysed by group 3 staphylococcal phages. The infants colonized with this strain were followed for more than a period of a year. No disease in them or their family members attributed to this staphylococcal strain could be uncovered.

Observations were then made on the number of bacterial cells of 502A needed to colonize particular sites. It was found that ten cells or less would colonize 50% of umbilical cords of newborn infants, while 250 cells

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were needed to colonize the nasal mucosa of 50% of newborn infants.[1] These preliminary data suggested that newborn infants could be safely and easily colonized within the first few hours of life with *S aureus* 502A and that this technique might be useful in curtailing epidemics due to pathogenic strains of coagulase-positive staphylococcus.

Bacterial Interference In Neonatal Hospital Infection

In order to establish the effectiveness of this technique in curtailing nursery epidemics, observations were made in a controlled fashion in several nurseries with high infant colonization and disease rates due to a single strain of staphylococcus. Previous attempts to control these epidemics with a variety of standard aseptic and antiseptic techniques had failed. Half of the infants in the nursery were artificially colonized on the nasal mucosa and umbilical stump with strain 502A while the remainder of the infants received a placebo consisting of saline solution. No other changes were made in the usual nursery procedures. Hospital personnel who carried epidemic strains were permitted to continue to work, and were not notified of their colonization status. Artificial colonization was performed within the early hours of life and the infants were followed at home for a period of a year to determine nasal colonization status and disease rates. Observations in the several control trials conclusively demonstrated the protection nasal colonization with 502A afforded newborn infants.[1]

To date there have been at least eight nursery staphylococcal epidemics in which colonization with *S aureus* 502A as a control measure was utilized[1-4] (H. Eichenwald, unpublished data). In no instance has this technique failed to curtail an epidemic. More than 4,000 infants have been colonized. There has not been a single case of serious disease in any of these infants or any household contact of the infants. Five percent to 15% of infants colonized with 502A developed tiny vesicles around the umbilical area in the first few days of life. These spontaneously disappeared and were not a cause of concern. In one nursery in a group of 50 infants the rate of these periumbilical lesions was reported to be 34%.[5] None of these infants developed any serious disease on careful follow-up during a period of more than one year (V. A. Fulginiti, oral communication, January 1968) although it is not clear whether the vesicular lesions were due to the epidemic strain in the nursery or strain 502A. Conjunctivitis has also been seen in the newborn associated with *S aureus* 502A.[1]

In summary, artificial colonization of newborn infants with 502A in nursery situations where there is a high colonization rate and disease rate due to a virulent hospital strain of staphylococcus has proved to be an effective and safe method for curtailing such epidemics.

Bacterial Interference in Furunculosis

A second situation where the concept of bacterial interference has been useful is in the treatment of patients with recurrent staphylococcal furunculosis. Here the technique has been one of recolonization rather than colonization. Prior to the nasal application of strain 502A, individuals with recurrent furunculosis are treated with antibiotics systemically and local application to the nasal mucosa of an antimicrobial cream. This technique eliminates the staphylococcal carrier strain related to the disease and is necessary to assure effective nasal colonization with the 502A strain.[6] In a controlled study Boris reported that this technique curtailed recurrent furunculosis in about 80% of treated individuals.[8] A number of other investigators have used this method to treat individuals and families who were afflicted with furuncles for years.[9-11]

Data on recolonization have been collected during a seven-year period on 587 patients with chronic recurrent furunculosis (M. Boris and H. R. Shinefield, unpublished data). Relapse rate defined as recurrence of the original staphylococcal strain on the nasal mucosa of treated individuals within 12 months was 21%. Of interest is a high relapse rate in patients with diabetes, eczema, and acne (Table 1). Relapse was also related to the antimicrobial agent used systemically in initial therapy prior to recolonization. In patients treated with dicloxacillin sodium monohydrate (Pathocil) the relapse rate was 15% while patients suspected of penicillin allergy and treated with lincomycin hydrochloride monohydrate (Lincocin) exhibited a relapse rate of 45% (Table 2).

The lesions associated with 502A in the 587 patients are listed in Table 3. Of the 11 patients with lesions three were diabetics and two had extensive eczema. In only one case could the lesion be classified as more than mild. This was a diabetic patient with a 502A pyarthrosis. Response to penicillin was excellent. This patient had been suffering from episodes of chronic furunculosis every one to two months for more than two years prior to therapy. Following recolonization, aside from this one complication, she has been free

Table 1.—Relapses in 587 Patients Colonized With 502A

Underlying Disease	No. Treated	Relapsed
Diabetes	5	4
Eczema	3	3
Acne	11	5
Total Relapse	122/587	21%

Table 2.—Relapses Related to Initial Therapy

	Total Treated	Relapse	%
Dicloxacillin	470	69	15
Lincomycin	117	53	45
Total	587	122	21

Table 3.—502A Lesions in 587 Recolonized Patients

Lesion	Underlying Disease	Patients
External otitis	Diabetes	1
Impetigo	Eczema	2
Pyarthrosis	Diabetes	1
Pustules	Diabetes	1
(one or two)	None	3
Stye	None	3
Total		11

from staphylococcal disease for the past three years. Lesions in the patients without underlying disease consisted of three episodes of pustules and three styes in six individuals.

Other workers have reported lesions associated with 502A strain of staphylococcus. An occasional pustule was seen by Maibach and his group.[12] Drutz and co-workers reported a patient with primary skin disease who developed a 502A abcess while on steroids. Despite this, the authors commented on the marked improvement after recolonization.[13] After the abcess the patient was followed for two years and remained lesion-free (G. Koening, oral communication, February 1968).

In summary, individuals can be protected from recurrent staphylococcal disease by colonizing the nasal mucosa with *S aureus* 502A following antibiotic therapy. There is no doubt that *S aureus* 502A may occasionally be associated with lesions, particularly in certain compromised hosts. As in all therapeutic approaches, potential values must be weighed against potential risks.

Staphylococcal Interference in Experimental Animals and in vitro

Clinical experience has indicated that bacterial interference can be useful in prevention of staphylococcal disease. It has been difficult to study, in human subjects, many of the variables that influence bacterial interference and, therefore, animal models and in vitro experiments have been devised.

Staphylococcal Interference in Experimental Animals

Chick Embryos.—Chick embryos were used to study bacterial interference because lethal infection can be produced by the injection of as few as 10 colony-forming units of certain strains of coagulase-positive staphylococci. Moreover, strains of coagulase-negative staphylococci have low virulence for chick embryos which makes it possible to use them as the protecting or interfering strains. Growth of staphylococci and production of toxins can be conveniently studied in the easily accessible allantoic fluid.

With coagulase-negative staphylococci we were able to protect embryos against the lethal effect of coagulase-positive staphylococcal infection.[14] Ten-day old chick embryos were injected intraallantoically with a strain of coagulase-negative staphylococcus, or in the control group with sterile broth. After 24 hours of incubation, the same groups of embryos were injected with the challenge coagulase-positive strain. The embryos were incubated and "candled" each day to determine if they were still living. A lethal infection developed in control embryos inoculated with broth prior to the administration of the challenge strain. By the third day after challenge there were only 19% survivals. In contrast, injection of the coagulase-negative strain prior to challenge resulted in protection. The survivals on the third day, in doubly infected embryos were 77%.

The protecting injection of coagulase-negative staphylococci had a quantitative effect on the growth of the challenge strain in the allantoic fluid. In the embryos infected with the challenge strain alone, the bacterial concentration after 24 hours of incubation was 10^7 to 10^9 bacteria/ml, whereas in the doubly infected embryos the concentration was about 10^5 bacteria/ml.

The decreased bacterial growth of the challenge strain in doubly infected embryos was accompanied by the production of smaller amounts of toxic and hemolytic substances. Probably this factor is the most important aspect of protection inasmuch as death in chick embryos infected with staphylococci in the allantoic cavity is due primarily to the toxin and is not directly related to the bacterial infection.[15] However, the protection was influenced by the number of bacteria in the protecting and challenge inocula and by the interval between the administration of the protecting and challenge strains. Protection could not be transferred by the administration of sterile filtrates of allantoic fluid in which the protecting strain had grown.

McCabe[16] found that infection with avirulent staphylococci could protect chick embryos not only against challenge with staphylococci, but also

could prevent or modify the lethality of challenge with pneumococci, salmonellae, Proteus, *Escherichia coli*, and one strain of influenza virus. He found that protection was sometimes accompanied by decreased growth of the challenge strain, but in other experiments growth was decreased only slightly. The production of toxic substances was not measured.

Rabbits.—Anthony and Wannamaker[17] were able to demonstrate bacterial interference in full-thickness burns of the skin of rabbits. Inoculation of the burns with staphylococcus 502A interfered with the subsequent colonization of the lesions with a staphylococcus phage type 80/81. The interference depended upon the multiplication of the protecting strain in that nine or more hours had to elapse between the inoculation of the protecting strain and the application of the challenge organism. Administration of large numbers of heat-killed staphylococci did not result in interference. In the lesions where interference was demonstrated, there was essentially no multiplication of the challenge 80/81 strain. The mechanism of interference in these experiments is not evident. The interfering 502A strain did not produce an inhibitor of bacterial growth. Interference could not be transferred with serum or homogenates of burned skin from animals infected with 502A. Exhaustion of nutrients by growth of the protecting strain in lesions was not directly examined.

An interesting finding of these studies was the ability of the staphylococci grown in vivo to resist the interference induced by the 502A strain. In vivo grown bacteria were more capable than those grown in vitro of superinfecting lesions colonized by 502A.

Table 4.—Content of Amino Acids in Normal Allantoic Fluid and in Growth-Suppressing Filtrates

Amino Acid	Concentration Micromoles per Milliliter Normal Allantoic Fluid	Filtrate of Allantoic Fluid in Which Coagulase-Negative Staph had Grown for 48 hr	
		1*	2†
Alanine	0.079	0.016	not done
Aspartic acid	0.027	0.023	0.011
Cysteine	none detected	none detected	none detected
Citrulline	0.012	0.029	0.012
Glycine	0.145	0.041	0.018
Glutamic acid	0.055	0.031	0.078
Isoleucine	0.048	trace‡	trace‡
Leucine	0.071	trace‡	trace‡
Phenylalanine	0.055	0.004	0.010
Proline	0.206	none	not done
OH-proline	not done	0.055	0.021
Serine	0.307	0.003	0.011
Threonine	0.153	not done	0.016
Valine	0.072	none detected	0.025
Methionine	0.056	not done	not done
Tyrosine	0.133	0.038	0.029

* 1 = Coagulase-negative staphylococci grown in vitro.
† 2 = Coagulase-negative staphylococci grown in ovo.
‡ Less than 0.05μmol/ml.

Staphylococcal Interference In Vitro

Studies in Broth.—The animal studies indicated that frequently one staphylococcal strain interferes with the growth of another. The mechanism of the suppression of growth was examined further in vitro.

Several experiments were carried out with the two staphylococcal strains used in the chick embryo experiments.[18] The coagulase-negative strain was able to suppress the growth of the coagulase-positive strain when the two bacterial organisms were grown together in trypticase soy broth. Bacteria-free filtrate prepared from broth cultures of coagulase-negative staphylococci was less able to support the growth of coagulase-positive staphylococci than was fresh broth. Inhibition of growth could be abolished by the addition of small amounts of niacinamide. Boiling of the filtrates also abolished the inhibition of growth. It was hypothesized that the coagulase-negative staphylococcus growing in broth produced an inhibitor of growth of the coagulase-positive strain, and that this inhibitor was inactivated by heating or by the addition of niacinamide.

Studies in Allantoic Fluid.—The following studies were carried out in allantoic fluid in vitro.[19] The coagulase-positive strain growing alone in allantoic fluid reached a concentration of greater than 10^8/ml after 24 hours of incubation. In contrast the coagulase-positive strain growing in mixed culture with coagulase-negative staphylococci grew to a concentration of 10^4/ml.

Similar suppression of bacterial growth occurred in bacteria-free filtrates of allantoic fluid in which coagulase-negative staphylococci had grown for 48 hours before removal of

bacteria by filtration. In the sterile filtrated, growth of the coagulase-positive strain was suppressed to 10^4 to 10^6/ml compared with growth to 10^9 bacteria/ml in fresh normal allantoic fluid.

To test for the presence of an inhibitor of bacterial growth 4 ml of inhibiting filtrate was injected into the allantoic cavity of chick embryos before challenge with virulent coagulase-positive staphylococci. No inhibition of bacterial growth or decreased mortality of the embryos was observed. In another experiment 100 ml of filtrate was concentrated by freeze drying and added to normal allantoic fluid or broth inoculated with coagulase-positive staphylococci. No antibacterial activity was noted. Thus, a potent inhibitor of bacterial growth was not present in inhibitory fluid filtrates rendering it likely that the suppression of growth resulted from depletion of nutrient substances necessary for growth of the coagulase-positive staphylococci.

Inhibitory filtrates of allantoic fluid contained a large amount of niacinamide. Addition of niacinamide and other vitamins did not alter their capacity to support growth. This indicated that vitamin deficiency was not responsible for suppression of bacterial growth in filtrates of allantoic fluid in which coagulase-negative staphylococci had grown. Thus, there was a different mechanism for suppression of growth in allantoic fluid from that present in broth.

The content of amino acids in normal allantoic fluid and in growth-suppressing filtrates of allantoic fluid was determined by chromatography by George Frimpter, MD. The findings are tabulated in Table 4 and indicate that the concentrations of many amino acids was decreased as a result of bacterial growth. The deficiency of amino acids was an important factor in suppression of growth in vitro inasmuch as addition of a combination of amino acids resulted in restoration of the ability of the filtrates to support growth of the coagulase-positive staphylococcus.

Recently bacterial interference has been described between other species of organisms and at sites other than the nasal mucosa.[20-22] Future investigations may lead to a better understanding of the phenomenon and eventually to better control of disease through the ability to manipulate the bacteriologic environment.

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