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V. An Analysis and Interpretation

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In the preceding four papers of this series, we have presented evidence from four different hospital newborn services, which substantiates several hypotheses: (1) the presence of one particular strain of *Staphylococcus aureus* may interfere with the subsequent acquisition of another staphylococcal strain; (2) this phenomenon can be utilized to protect an infant against infection and disease caused by the so-called epidemic phage types; and (3) certain staphylococcal types are more pathogenic to infants than others.[1-4]

These studies were carried out in hospitals which cared for different population groups and engaged in a variety of nursery practices. The epidemiology of infectious disease in general is complex and perhaps even undefinable when several mutually independent factors operate simultaneously. It is thus

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TABLE 1.—*Protective Effect of Strain 502A on Subsequent Nasal Colonization With Other Types of Staphylococcus Aureus: Summary of Data from Three Epidemics*

Infants	Colonization with 80/81 or Staphylococci Other Than 502A: During Hospital Stay *	Colonization with 80/81 or Staphylococci Other Than 502A: At Follow-Up * †
Inoculated "Takes" ‡	4/76 (5)	4/69 (6)
Control	45/111 (41)	56/104 (54)

* Infants colonized/total number of infants in category (%).
† Follow-up at 2-4 weeks after birth.
‡ "Take" indicates presence of strain 502A detected at 24 hours after inoculation. Two infants colonized with two strains on day 1 of life and nine no-"take" infants excluded.

essential that observations in this field be subjected to repeated tests under different conditions before they can be considered to have general validity and applicability. Certainly, many related factors are responsible for the transmission of staphylococci in the newborn infant. The epidemiology of staphylococcal colonization and disease in the newborn may be influenced by nursery design, density of infant population, hospital obstetrical and nursery practices, the biologic and epidemiologic properties of the various strains of staphylococci simultaneously present in the nursery, the presence of viruses, various host factors, and no doubt a number of other variables of which little is known. It is the difficulty of isolating and investigating each factor in the epidemiologic equation separately that accounts for the disagreement among investigators. Some particular facet which may have been of crucial importance in one situation many prove impossible to reproduce under different circumstances.

It is of interest, therefore, that our results were reproducible within relatively narrow limits, that each of three outbreaks studied yielded statistically valid results confirming the hypotheses previously stated. Certain data from the individual epidemics are summarized in Tables 1 and 2 to illustrate several factors to be discussed in the present communication, which presents a general review of the applicability of the interference phenomenon as a control measure and the problems which still have to be resolved.

An obvious solution to the problem of staphylococcal infection and disease among newborn infants would consist of preventing colonization of the infant by coagulase-positive staphylococci. This goal has been avidly pursued by a variety of means, all designed either to reduce the number of organisms reaching the baby or to render local host tissues unavailable as colonization sites. Thus, nurseries have been equipped with various devices to sterilize the air, and to reduce the contamination by fomites. "Aseptic technique" has been stressed, antimicrobial nasal preparations have been used in mothers,[5] infants,[6] and attendants,[7] cord stumps have been treated with antiseptic dyes,[8] and the entire infant has been washed repeatedly with detergents containing a substance inhibiting bacterial growth.[9] In observations that included both controls and follow-up studies, none of these approaches used collectively or individually has been completely effective, and in fact little more was ac-

TABLE 2.—*The Relationship of Staphylococcus Type in Nose to Development of Disease: Summary of Data From Three Epidemics* *

Location of Epidemic	80/81	80/81 + Other Coag.-Pos. Staph	Other Coag.-Pos. Staph Only	No Coag.-Pos. Staph	502A †
Ohio	2/4	3/4	3/22	0/3	3/18
Georgia	10/14	1/5	1/4	2/11	3/54
Louisiana	10/12	0/6	2/9	2/5	3/24
Total	22/30 (73)	4/15 (26)	6/35 (17)	4/19 (21)	9/96 (9)

* Number of lesions in infants and household members/total number of infants colonized (%). Colonization indicates isolation of more than one colony of coagulase-positive staphylococci at any time during the hospital stay or during first follow-up.
† Strain 502A isolated four times: three cases of conjunctivitis w/502A isolated; one case of impetigo w/502A and 80/81 isolated; four abscesses w/80/81 isolated; one maternal mastitis not cultured.

complished than a reduction in the rate of colonization to levels which might be more acceptable to the investigator while still falling short of offering the newborn a reliable and reproducible defense against the acquisition of potentially pathogenic phage types of staphylococci.

Some of our early observations indicated that the most likely explanation for the frequent failures noted with these preventive approaches was the fact that small numbers of staphylococci were sufficient to establish initial colonization in a newborn infant [1] and that therefore only maintaining and rearing a baby in a germ-free environment would prevent the ultimate colonization of a fair proportion of babies.

It is probably technically but not necessarily economically or socially feasible to rear an infant in a germ-free environment during his few days in the hospital, but is such a step desirable? There are reasons to suppose that this approach might be harmful as well as unrealistic. As long ago as 1885 Pasteur speculated that it would probably become impossible for animals to survive if they were deprived of their bacterial flora.[10] With the advent of antiobiotic agents sufficiently potent to be able to eradicate the normal bacterial flora, it has become obvious that the suppression of these normal inhabitants may be associated with serious consequences to the host. For example, in instances where elimination of resident bacteria has been accomplished by antibiotics,[11] or hexachlorophene washes,[9] colonization with Gram-negative organisms has been noted to occur during the hospital stay; bacteria which may represent a greater potential hazard to the host than hospital strains of coagulase-positive staphylococci. In addition, the toxicity of the antibiotics themselves may produce devastating effects on the newborn.[12,13] Aside from the technical and economic difficulties of such a plan, sending an infant home with bacteriologically sterile mucous membranes is not a satisfactory arrangement; studies indicate that such babies readily acquire staphylococci and other organisms prevalent in the household.[11] Although these bacteria may be of a nonepidemic variety, recent investigations have shown that up to 40% of family populations may carry penicillin-resistant staphylococci.[14] Although the property of penicillin resistance itself is not necessarily associated with pathogenicity or virulence, disease produced by these types of staphylococci limits the scope of effective antibiotic therapy and represents a potential hazard to the newborn.

At this point, it would seem important to emphasize that colonization of the newborn with *Staphylococcus aureus* is a normal event, that this will always take place sooner or later, and that harmful effects from such a situation can occur only if the organism in question is one that is potentially pathogenic to the newborn. Very little disease is attributable to the vast majority of staphylococcal strains encountered in infants during their stay in the nursery or during the follow-up period. Most nursery epidemics have been caused by strains of staphylococci lysed by the phages of the 52/52A/80/81 complex [15] and a very few others.[16,17] Since only these types cause a high proportion of all staphylococcal disease in newborns, the problem can be solved by preventing colonization of the infant with these specific types rather than with all staphylococci. This may be accomplished in two different ways: (1) by utilizing various suppressive or eradicative measures which operate more or less equally against all staphylococci as well as against other normal flora, and which, as pointed out, may thus lead to undesirable consequences or (2) by employing the more biologic approach of utilizing the phenomenon of bacterial interference to inhibit colonization with the pathogenic strains without robbing the baby of his normal flora.

Before artificial colonization can be seriously considered as the ideal approach, adequate information about the organism employed and its biological stability must be available. Unfortunately, no laboratory test exists which measures potential pathogenicity of staphylococci. In the laboratory, strains of

Staphylococcus aureus which cause epidemic disease cannot be differentiated from those strains which have been observed to have little virulence for man. Because of this fact, a hypothesis has been proposed which states that the important disease determinant is inherent not in the host or in the strain of the staphylococcus, but is represented by a third factor. This is a nebulous concept, since this third factor is not defined or presently definable. In the epidemics investigated by us, great care was taken not to alter any nursery routines during the artificial colonization of randomly selected infants. Therefore, any third factor, be it infectious or not, which might have been responsible for or contributed to the virulence of staphylococci would have been equally disseminated among artificially colonized or noncolonized infants. The statistical difference in the rate of lesions encountered among infants colonized with strain 502A and the type 52/52A/80/81 complex does not support a third factor hypothesis (Table 2).

Disease-Causing Potential of Staphylococcal Strain 502A

As mentioned above, strain pathogenicity can be evaluated only on an epidemiologic basis. It seems worthwhile, therefore, to summarize the epidemiologic data bearing on strain 502A which we have accumulated over the past three years.

We have observed over 500 infants colonized deliberately or spontaneously with strain 502A. Almost all of these were normal, full-term infants. Therefore, questions related to the effect of such colonization on debilitated and premature infants have not been answered.

Among the total of 524 colonized infants, only 5 lesions were associated with the presence of strain 502A. Four lesions were noted in infants colonized in the three epidemics (Table 2) and one in the preliminary studies. Three lesions consisted of conjunctivitis, a disease which was observed just as frequently in infants uncolonized by staphylococci. The remaining two lesions were impetiginous, and culture yielded not only strain 502A but also type 80/81 staphylococci.

A number of events which might increase the possibility of staphylococcal disease have subsequently occurred in some of the strain 502A colonized infants. For example, four infants have undergone cardiac surgery, and three infants required operations for inguinal hernia or pyloric stenosis. One colonized infant had a repair of a tracheo-esophageal fistula. The operative procedures were uneventful, and staphylococcal complications did not occur.

A number of other infants colonized with strain 502A developed viral respiratory tract disease subsequently, and six of these babies were hospitalized with bronchiolitis or bronchitis. In no case was the respiratory illness related to the staphylococcus, and in none of the babies did these illnesses progress to staphylococcal pneumonia. Many babies colonized with strain 502A were intubated in an attempt to determine the patency of the nares or of the esophagus, and despite such instrumentation and resultant trauma to the nasal mucosa, no staphylococcal disease was observed in these patients.

It appears likely, therefore, that the pathogenicity of strain 502A is sufficiently low so that disease does not occur even after a variety of insults to the host's defense mechanisms.

Of utmost importance is the question whether the desirable characteristics of the 502A strain are stable, or whether significant changes in this organism can occur with time.

TABLE 3.—*Persistence of Nasal Colonization With Strain 502A Following Systemic Antibiotic Therapy*

Antimicrobial Agent Used	No. of Infants		
	Strain Lost	Strain Retained	Total
Penicillin	16	3	19
Tetracycline	2	7	9
Penicillin + other agent *	5	1	6
Total	23	11	34

* Other agents included streptomycin, kanamycin, or chloramphenicol.

Transduction of staphylococci has been demonstrated to occur in vitro, with important biologic changes taking place. For example, non-penicillinase-producing organisms were transduced and the recipient staphylococcal strains were found to produce penicillinase.[18] No evidence for in vivo transduction or lysogenization has been reported to date. In 10 infants colonized both with strain 502A and another coagulase-positive staphylococcus during our nursery observations, no evidence for transduction has been detected. Neither do antimicrobial agents produce a measurable change in the strain 502A. For a variety of reasons unrelated to staphylococcal disease, 34 infants colonized with this organism were treated with various antibiotics. Nineteen infants received penicillin alone, 9 were treated with tetracycline alone, and 6 were given a combination of antimicrobial agents which included penicillin and streptomycin and/or kanamycin and/or chloramphenicol. Most babies receiving penicillin alone or in combination with another antimicrobial agent lost the 502A strain, but tetracycline-treated babies generally retained the organism (Table 3). When strain 502A was cultured from the infants following the course of treatment, antimicrobial sensitivity, penicillinase production, as well as agglutination and phage patterns were unchanged.

The above data suggest that strain 502A when in close contact with other coagulase-positive staphylococci in vivo and when exposed to antimicrobial agents in the human host does not undergo measurable immunologic, biologic, or genetic changes.

The preceding data must not be interpreted as indicating that disease due to strain 502A will never occur; in fact it would appear likely that illness will be found on occasion which may prove to be very severe in an unusual baby. This prediction is based on the premise, confirmed repeatedly by the use of vaccines employing live but attenuated agents, that a few individuals, for reasons more or less poorly understood, possess defense mechanisms operating at a level insufficient to protect against disease from even highly attenuated microbes. Staphylococci, no doubt, are no exception; we have observed an occasional infant with overwhelming and unrelenting disease caused by untypable, nonepidemic, penicillin-sensitive staphylococci which failed to respond to massive appropriate antimicrobial therapy; these infants showed an abnormal and unusual response to the presence of the microbe.

It is because of these considerations that we would not advocate the "routine" use of strain 502A or similar types in nonepidemic situations, much as the use of routine prophylactic antimicrobial therapy in newborns is not recommended. We feel that our data permit the conclusion that *Staphylococcus aureus* strain 502A, if used properly, results in the prompt termination of a nursery outbreak of infection and disease due to virulent types of staphylococci with little potential danger to infants or the staff. Never should the use of this organism be substituted for good nursery technique, adequate facilities and supervision, or strict surveillance of infants during their stay in the hospital and after discharge. Perhaps in the future when more data about the staphylococcus and about "the host" are available, a different approach might be used. Perhaps then a solution to the staphylococcal problem in the newborn may be to furnish the infant with a carefully selected respiratory flora within a short period of time following birth. Perhaps a similar procedure may prove equally effective with other troublesome bacteria, for example the pathogenic serotypes of *Escherichia coli*.

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REFERENCES

1. Shinefield, H. R.; Ribble, J. C.; Boris, M.; and Eichenwald, H. F.: Bacterial Interference: Its Effect on Nursery Infection With Staphylococcus Aureus; I. Preliminary Observations on Artificial Colonization of Newborns, Amer J Dis Child, this issue, p 646.

2. Shinefield, H. R.; Sutherland, J. M.; Ribble, J. C.; and Eichenwald, H. F.: Bacterial Interference: Its Effect on Nursery-Acquired Infection With Staphylococcus Aureus; II. The Ohio Epidemic, Amer J Dis Child, this issue, p 655.

3. Shinefield, H. R.; Boris, M.; Ribble, J. C.; Cale, E. F.; and Eichenwald, H. F.: Bacterial Interference: Its Effect on Nursery-Acquired Infection With Staphylococcus Aureus; III. The Georgia Epidemic, Amer J Dis Child, this issue, p 663.

4. Boris, M.; Shinefield, H. R.; Ribble, J. C.; Eichenwald, H. F.; Hauser, G. H.; and Caraway, C.: Bacterial Interference: Its Effect on Nursery-Acquired Infection With Staphylococcus Aureus; IV. The Louisiana Epidemic, Amer J Dis Child, this issue, p 674.

5. Spink, M. S.: Staphylococcal Sepsis in Mothers and Newborn Babies, J Hyg (London) 60:105, 1962.

6. Klein, J. O., and Rogers, E. F.: Use of a Nasal Antibiotic Cream During a Nursery Outbreak of Staphylococcal Disease, New Engl J Med 260:1012, 1959.

7. Cooper, M. L.; Keller, H. M.; Partin, J. S.; and Wegman, J. C.: Nasopharyngeal Carriers of Staphylococcus Aureus, Amer J Dis Child 104:53, 1962.

8. Jellard, J.: Umbilical Cord as Reservoir of Infection in a Maternity Hospital, Brit Med J 1: 925, 1957.

9. Simon, H. J.; Yaffe, S. J.; and Gluck, L.: Effective Control of Staphylococci in a Nursery, New Engl J Med 265:1171, 1961.

10. Pasteur, L.: Observations relatives à la note de M. Duclaux, CR Acad Sci 100:68, 1885.

11. Sutherland, J. M.; Hotz, R. M.; Rytell, F. E.; Dowell, V. R.; Cochran, M. L.; Short, M. L.; and Newman, D. S.: Environmental Antimicrobiosis in a Nursery, Amer J Dis Child 102:793, 1961.

12. Sutherland, J. M.: Fatal Cardiovascular Collapse of Infants Receiving Large Amounts of Chloramphenicol, AMA J Dis Child 97:761, 1959.

13. Sutherland, J. M., and Keller, W. H.: Novobiocin and Neonatal Hyperbilirubinemia, AMA J Dis Child 101:447, 1961.

14. Anderson, K. F.; Coulter, J. R.; and Keynes, D. R.: Staphylococcal Nasal Carriage in Mothers, Babies and Staff in a Maternity Hospital, J Hyg (London) 59:15, 1961.

15. Shaffer, T. E.; Sylvester, R. F.; Baldwin, J. N.; and Rheins, M. S.: Staphylococcal Infections in Newborn Infants: II. Report of 19 Epidemics Caused by an Identical Strain of Staphylococcus Pyogenes, Amer J Public Health 47:990, 1957.

16. Howells, C. H., and Jones, H. E.: Two Outbreaks of Neonatal Skin Sepsis Caused by Staphylococcus Aureus Phage Type 71, Arch Dis Child 36:214, 1961.

17. Gillespie, W. A.; Pope, R. C.; and Simpson, K.: Pemphigus Neonatorum Caused by Staphylococcus Aureus Type 71, Brit Med J 1:1044, 1957.

18. Blair, J. E., and Carr, M.: Lysogeny in Staphylococci, J Bact 82:984, 1961.

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