

## II. The Ohio Epidemic

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*Definition of Terms.*—A glossary of terms used in this publication is included in a previous paper.<sup>1</sup>

The present communication describes a study carried out in the nurseries of the

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Cincinnati General Hospital and represents our first controlled investigation utilizing artificial nasal and umbilical colonization with strain 502A. These series of observations provided evidence which further substantiated the concept of interference between coagulase positive staphylococcal strains of bacteria and indicated that this phenomenon could be used on a practical scale to terminate an epidemic of staphylococcal disease.

Impetigo and furuncles in recently discharged newborn infants and their family contacts had been reported intermittently to one of us (J.S.), the Director of the nursery of the Cincinnati General Hospital. This particular nursery had been under continuous close observation for over two years, and a previous outbreak of staphylococcal disease had been intensively investigated.<sup>2</sup> A system of weekly discharge cultures had been instituted at that time in order to furnish current data of staphylococcal strains present in the unit. During October and November of 1961, these routine discharge cultures indicated a steadily increasing colonization rate of infants with two variants of the 52/52A/80/81-phage complex of staphylococci, henceforth referred to as type 80/81. By late November, nearly 40% of infants housed in the nursery became colonized by these strains prior to the time of their discharge, usually at five days of age (Fig 1).

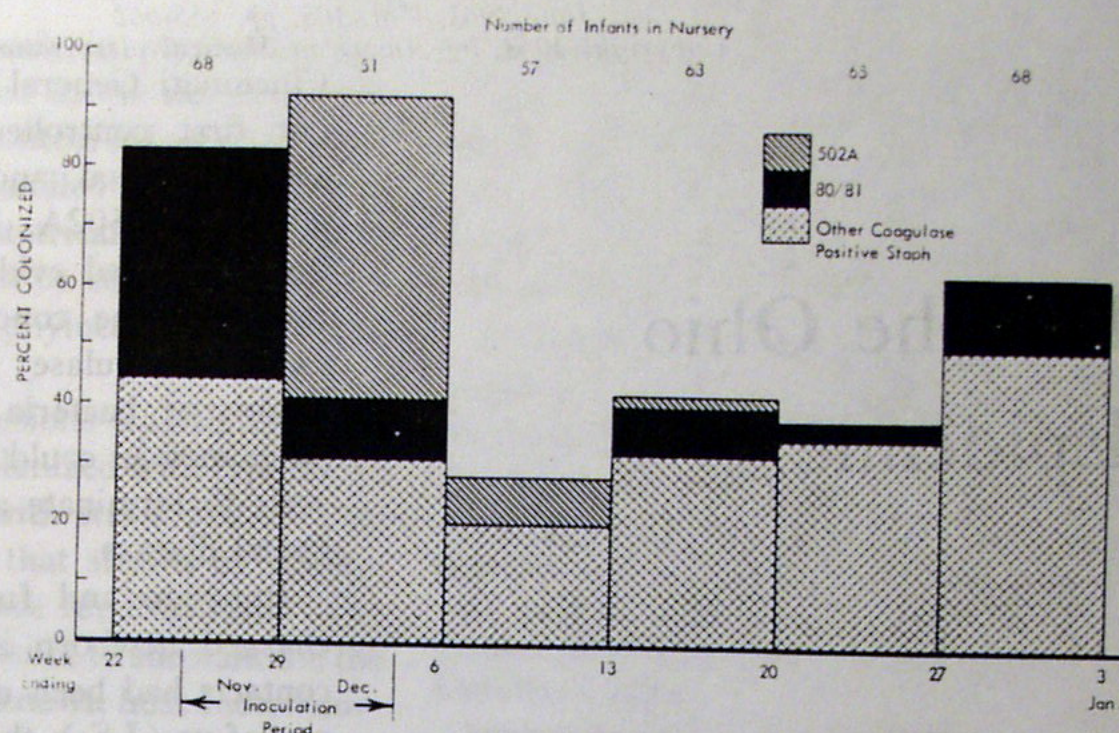
### Materials and Methods

1. *The Obstetrical and Nursery Unit.*—Approximately 4,300 infants are born annually at the Cincinnati General Hospital. Premature babies, weighing less than 2,200 gm, are assigned to a special unit in a separate building, while all full-term babies are housed in a suite consisting of three nurseries sharing common equipment and connected by open doors (Fig 2). Total nursery space is 1,200 sq ft or 12,000 cu ft. The average daily census is 40-45 infants.

Full-term newborns are routinely divided into three groups depending on maternal status: infants of healthy mothers are assigned to the K Nursery, and infants of mothers with complications are housed in the J Nursery. All infants from room J and K are taken to their mothers for feedings during the day, with about 60% to 70% of them



Fig 1.—Discharge nasal colonization of infants before, during, and following artificial colonization with 502A.



being breast fed. Babies who for a variety of reasons are not taken to their mothers are assigned to the C Nursery.

Each baby is housed and cared for separately in his own bassinet which is stocked with an individual linen supply. Routine practice consists of dry skin

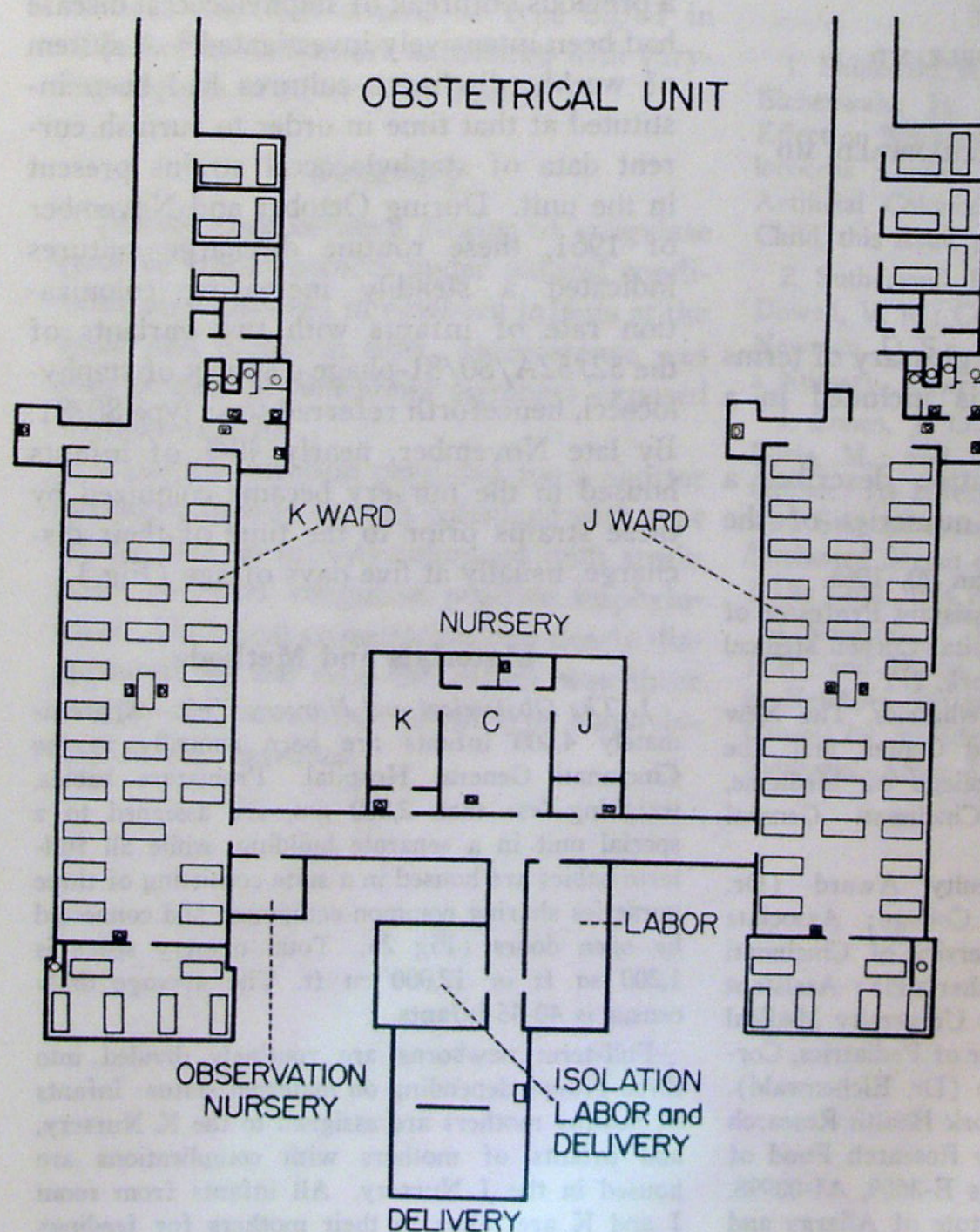


Fig 2.—A simplified floor plan of the obstetrical unit. J, K, and C, nurseries share utility room, linen room, refrigerator, and scale. Average daily maternal census is 50-60. Infant census is 40-45.



care after initial removal of blood and meconium with water; cords are not dressed, but alcohol is applied daily with a cotton-tipped applicator stick. The latter practice was discontinued for the period of the study, which was the only change made in the nursery routine. The cleanliness of the nursery was judged to be adequate; the floors are washed at regular intervals using a wet mop technique; equipment is cleaned with either soap and water, hexachlorophene or commercial antiseptic solutions.

Nursery personnel and physicians entering the nursery area wear fresh gowns and wash their hands with a hexachlorophene detergent (PhisoHex) for two minutes. Hands are also washed each time an infant has been handled, but gowns are not changed unless soiled.

The nursery and obstetrical units are staffed by 32 nurses and aides who are sometimes assigned to other areas within the suite. Nose and throat cultures were performed on all staff members at the beginning of the study; four nasal carriers of staphylococcus type 80/81 were found, 12 attendants harbored coagulase positive staphylococci of various other types. All staphylococcal carriers were permitted to remain on duty, and they were not informed of their culture status.

**2. Bacteriologic Methods.**—Bacteriologic cultures from the nares and umbilicus were obtained daily from all infants throughout the period of study and processed in the manner described in a previous publication.<sup>1</sup> The total number of cultures processed was over 800 during the hospital study and over 1,200 in the follow-up; 5,870 colonies were identified by the combined phage and antibiotogram procedure, an average of more than 50 colonies per infant.

The *Staphylococcus aureus* employed for artificial colonization was a coagulase positive, penicillin-sensitive organism designated as strain 502A and described in detail in companion communications.<sup>1,2</sup>

**3. Technique of Colonization and Selection of Infants.**—During the initial phase of the study, infants were assigned at random to four groups depending on the anatomic site to be colonized: (1) the nose, (2) the umbilicus, (3) both nose and umbilicus, and (4) no artificial colonization (control).

On the third day, this procedure proved too cumbersome, and the number of groups was reduced to two by colonizing one group of randomly selected babies both in the nose and on the umbilicus, and leaving another group of infants as controls.

A microburette\* was employed to dispense 0.0005 cc containing 600 to 2,000 organisms. The exact number of viable bacteria received by an infant was determined by culturing an aliquot of each inoculum, utilizing standard dilution and plate counting techniques.

\* Manufactured by Calab, Berkeley, Calif.

**4. Follow-Up.**—Follow-up visits were made by a public health nurse trained in epidemiology, who visited each infant's home at regular four to five week intervals following the baby's discharge from the hospital. On the occasion of each visit, nasal and umbilical cultures were taken from the baby, and nose cultures were obtained from mothers and other household members. All lesions observed were cultured. Specimens were streaked immediately on mannitol salt agar plates which were processed in the same manner as cultures taken in the hospital. By means of a standard form, information concerning illnesses and social data was also obtained at the time of the visit.

**5. Results.**—A. Incidence of Successful Inoculations: A total of 27 infants was deliberately colonized with strain 502A; the umbilicus only was inoculated in 4, the nose only in 10, and both sites in 13. By criteria previously defined, 18 infants (83%) were successfully colonized in the nose and 15 (83%) on the umbilicus (Table 1).

B. Nasal Colonization with 502A: None of the 18 infants with successful "takes" of strain 502A in the nose became colonized with type 80/81 during the hospital stay (Table 2), although two were colonized with coagulase positive staphylococci other than type 80/81 or strain 502A. This represents a total colonization rate with coagulase positive staphylococci not strain 502A of 11%. In the control group of 28 infants, 3 became spontaneously colonized with type 80/81 and 10 with coagulase positive staphylococci other than type 80/81 or strain 502A. Thus, a total of 13 of the 28 control infants became colonized with a coagulase positive staphylococcus other than strain 502A. Differences in the rate of colonization between inoculated "take" and control infants are significant at a level of  $P=0.013$ .† At age four weeks, two infants in the inoculated "take" group were found to be colonized with staphylococci other than the 502A strain, while in the control group 16 of 25 babies were colonized with coagulase positive staphylococci other than

† Statistical analyses were performed using  $\chi^2$  with Yates correction term. If numbers totaled less than 50 or contained a zero Fisher's exact test was used.

TABLE 1.—Site and Number of Takes\* in Infants Inoculated with Strain 502A

Site	Controls, No.	Number † Inoculated	Take (%)	No Take
Nose	28	23	18 (83)	5
Umbilicus	33	18	15 (83)	3

\* Take—presence of strain 502A detected 24 hours after inoculation.

† Some infants inoculated only on nasal mucosa or umbilicus; others at both sites.



TABLE 2.—Nasal Colonization Rates With Coagulase Positive Staphylococci, Other Than 502A, in Control and Inoculated Take Infants During Hospital Stay and Initial Follow-Up

Infants	80/81		Coagulase Positive Staphylococci † Not 80/81 or 502A		Total Coagulase Positive not 502A	
	H ‡	F ‡	H ‡	F ‡	H ‡	F ‡
Inoculated takes §	0/18 * ‡ (0%)	1/14 (7.1%)	2/18 ‡ (11.1%)	1/14 (7.1%)	2/18 ‡ (11.1%)	2/14 (14.2%)
Control	3/28 (10.7%)	6/25 (24.0%)	10/28 (35.7%)	10/25 (40.0%)	13/28 (46.4%)	16/25 (64.0%)
					P = 0.013	P = 0.003

\* Infants colonized/total number of infants.

† Second strain colonization = &gt;one colony of the second strain isolated during H or F.

‡ H, hospital stay; F, follow-up at one month.

§ Take, presence of strain 502A detected 24 hours after inoculation.

| Five inoculated infants without "takes" excluded from analysis of data.

TABLE 3.—Umbilical Colonization Rates With Coagulase Positive Staphylococci, Other Than 502A, in Control and Inoculated Take Infants During Hospital Stay and Initial Follow-Up

Infants	80/81		Coagulase Positive Staphylococci † Not 80/81 or 502A		Total Coagulase Positive not 502A	
	H ‡	F ‡	H ‡	F ‡	H ‡	F ‡
Inoculated takes §	0/15 * ‡ (0%)	0/13 (0%)	0/15 ‡ (0%)	1/13 (7.6%)	0/15 ‡ (0%)	1/13 (7.6%)
Control	11/33 (33.3%)	4/29 (13.7%)	15/33 (45.4%)	14/29 (48.2%)	26/33 (78.7%)	18/29 (62.1%)
					P = <0.0001	P = <0.01

\* Infants colonized/total number of infants.

† Second strain colonization = one colony of the second strain isolated during H or F.

‡ H, hospital stay; F, follow-up at one month.

§ Take = presence of strain 502A detected 24 hours after inoculation.

| Three inoculated infants without takes excluded from analysis of data.

strain 502A. This difference is significant at a level of  $P=0.003$ .

C. Umbilical Colonization: A total of 15 infants was successfully colonized at the umbilical site with strain 502A. None of these infants later became colonized at this site by staphylococcus type 80/81 or by other coagulase positive organisms during the nursery stay. At one month of age, one infant was colonized with a coagulase positive staphylococcal strain other than 80/81 or 502A (Table 3). Of the 33 control infants, 11 were found infected with type 80/81 and 15 were colonized with a coagulase positive staphylococcus other than type 80/81 during the hospital stay. The differences between artificially colonized infants and the control group are significant at a level of  $P=<0.001$ .

D. Isolation of Two or More Strains of Staphylococci from the Same Infant: Two infants in both the inoculated "take" and control groups harbored two or more strains on the nasal mucosa during their hospital stay (Table 4). At the umbilical site, two or more staphylococcal strains were isolated from 10 of 33 control infants, while only strain 502A was found in the 15 infants whose umbilical site had been successfully colonized. This difference between the control and umbilical "take" infants is significant at  $P=0.014$ .

E. Lesions: During the follow-up period, 10 infants or their household contacts developed 11 lesions from which staphylococci could be recovered

TABLE 4.—Isolation of Two\* or More Strains of Coagulase Positive Staphylococci From a Single Infant During Hospital Stay

	Nose	
	Total Number of Infants	Number with 2 or more Strains (%)
Inoculated Take †	18	2 (13.3)
Control	28	2 (7.1)
	Umbilicus	
	Total Number of Infants	Number with 2 or more Strains, (%)
Inoculated Take	15	0, (0)
Control	33	10, (30.3)
		P = 0.014

\* Second strain colonization = one colony of the second strain isolated during hospital stay.

† Take = presence of inoculated 502A strain detected at 24 hours after inoculation.



TABLE 5.—Relationship of Type of *Staphylococcus* Carried in the Infant's Nose to Development of Lesions in Infant and Household Contacts

Lesions †	Nasal Colonization Status *				
	80/81	80/81 + Others Coag. Pos. Staph ‡	502A	Other Coag. Pos. Staph	Non Coag. Pos. Staph
Infants					
Impetigo	2	1	1 §	2	0
Conjunctivitis	0	1	1	(1) †	0
Abscess	0	0	0	0	0
Household					
Abscess	0	1	1 ‖	0	0
Total Lesions/Total No. Colonized	2/4	3/4	3/18	3/22	0/3

\* Isolation of more than one colony of a coagulase positive staphylococci at any time during the nursery stay or during first or second follow-up.

† Coagulase positive staphylococci recovered from all lesions except one ( ).

‡ Three infants colonized with both type 80/81 and 77. One infant colonized with both type 80/81 and 502A. All lesions yielded type 80/81.

§ Lesion yielded type 80/81; Infant only inoculated on nasal mucosa—umbilical culture at discharge was type 80/81.

‖ Type 80/81 isolation from abscess of sib who carried type 80/81 nasally.

(Table 5). Five lesions occurred in the eight infants whose nose had become colonized either with type 80/81 or with this organism plus another staphylococcus. The cultures from these five lesions grew *Staphylococcus aureus* type 80/81. In 18 infants who were nasally colonized with strain 502A, three lesions developed in the babies or in their household contacts. One infant in the group had been inoculated with strain 502A on the nasal mucosa but not on the umbilicus. At discharge, culture from the nose yielded strain 502A but from the umbilicus type 80/81 was grown. The organism isolated from the infant's skin lesion was *Staphylococcus aureus* type 80/81.

Staphylococci type 80/81 were isolated from an abscess in a sibling of a "take" infant. This sibling was found also to be a type 80/81 nasal carrier, while the index infant was colonized with strain 502A and never acquired the 80/81 organism. No

skin lesions due to 502A were found in infants nasally colonized with 502A; however, one baby infected by strain 502A was noted to have a mild, purulent conjunctivitis, and the marker strain was isolated from this site.

The difference between the incidence of lesions in infants who carried *Staphylococcus aureus* type 80/81 and strain 502A in their nose is significant at  $P \approx 0.05$ .

Two skin lesions and one case of conjunctivitis were found in infants noted to be colonized at discharge with staphylococci other than strains 502A or type 80/81. The staphylococcus recovered from one of these lesions was similar to the strain isolated from a household contact, while the other was similar to an organism isolated from the nasal mucosa of the infant at the time of discharge. No staphylococcus was recovered from the case of conjunctivitis.

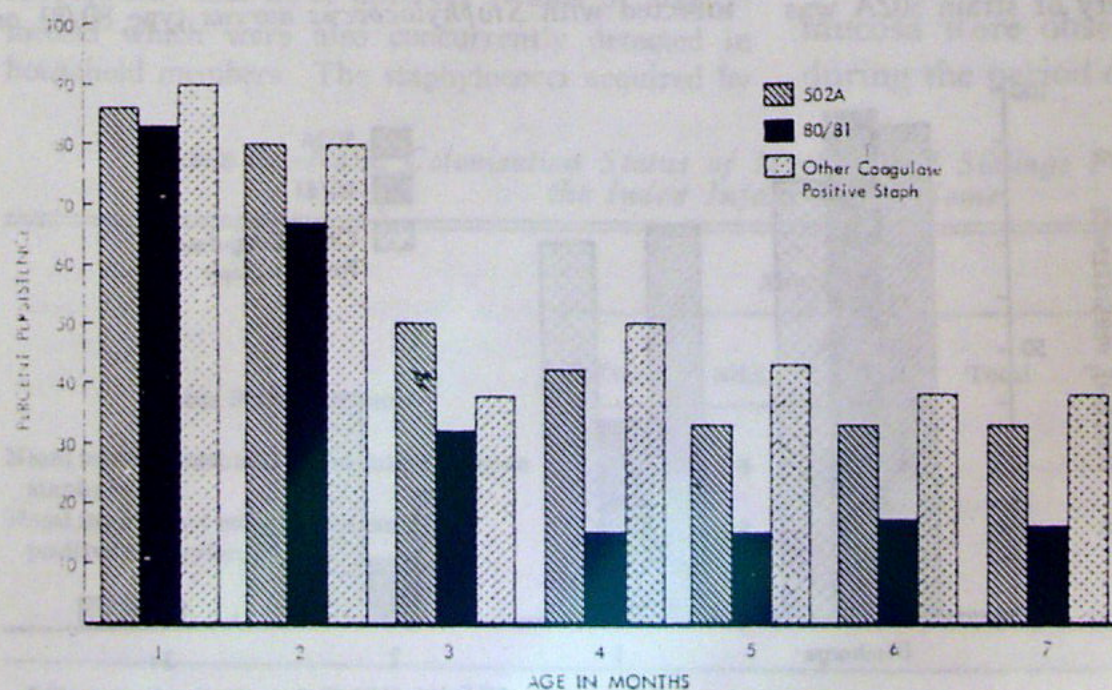


Fig 3.—Comparison of persistence of nasal colonization of the different groups of coagulase positive staphylococci.



TABLE 6.—Persistence\* of Nasal Colonization of 48 Staphylococcal Strains in 46 Infants Followed From 1 to 7 Months

Group	Discharge Colonization Status	Age, Mo						
		1	2	3	4	5	6	7
502A strain								
Inoculated takes	18/18 (100)	12/14 (86)	12/15 (80)	6/12 (50)	5/12 (42)	4/12 (33)	4/12 (33)	4/12 (33)
80/81 †								
Strain	24/25 (96)	20/24 (83)	18/24 (75)	7/22 (32)	3/20 (15)	3/20 (15)	3/18 (17)	3/19 (17)
Other coagulase								
Positive staphylococci	10/10 (100)	9/10 (90)	8/10 (80)	3/8 (38)	4/8 (50)	3/7 (43)	3/8 (38)	3/8 (38)

\* Number with persistence/number colonized initially with strain (%).

† Includes 20 infants spontaneously colonized one week before inoculations begun.

F. Persistence of Colonization at the Nasal Site: Data on the persistence of the initial staphylococcal flora have been obtained from 46 infants carrying 48 strains of staphylococci in the nose (Fig 3, Table 6). It was possible to follow 39 of these babies throughout the seven-month follow-up period.

The infants followed for at least two months could be divided into five groups depending on colonization status at discharge: (1) 12 infants were colonized only with strain 502A, (2) 23 infants were colonized only with type 80/81, including 20 infants spontaneously infected with type 80/81 one week before the inoculation study in the nursery was undertaken and three infants colonized with type 80/81 during the week of inoculation, (3) one infant was colonized with strain 502A and type 80/81, (4) one infant was colonized with both strain 502A and type 77, and (5) nine infants were infected with coagulase positive staphylococci other than 80/81 or 502A.

At four months of age, five of 12 infants originally colonized with strain 502A and still under observation were found to carry the same organism. The following month, recovery of strain 502A was

possible in four of the 12 babies and this rate did not change over the next two months.

It is of interest that staphylococcus type 80/81 disappeared more rapidly so that by age four months, the carrier rate declined to 15% in the 20 babies followed, a rate which remained reasonably constant for the last three months of the follow-up period.

The persistence rate in babies nasally colonized with coagulase positive staphylococci other than strain 502A or type 80/81 was similar to that observed in infants colonized with strain 502A.

The difference in persistence rate between the group colonized with type 80/81 and the infants colonized with other strains of staphylococci is significant at  $P < 0.05$ .

G. The Persistence of Colonization at the Umbilical Site: Umbilical colonization rates in infants who carried strain 502A fell from 93% at discharge to 71% and 21% at the one and two month follow-up period respectively. No recovery of strain 502A from the umbilicus was possible after two months of age (Fig 4, Table 7).

At one month of age, nine of 24 (37%) infants infected with *Staphylococcus aureus* type 80/81 on

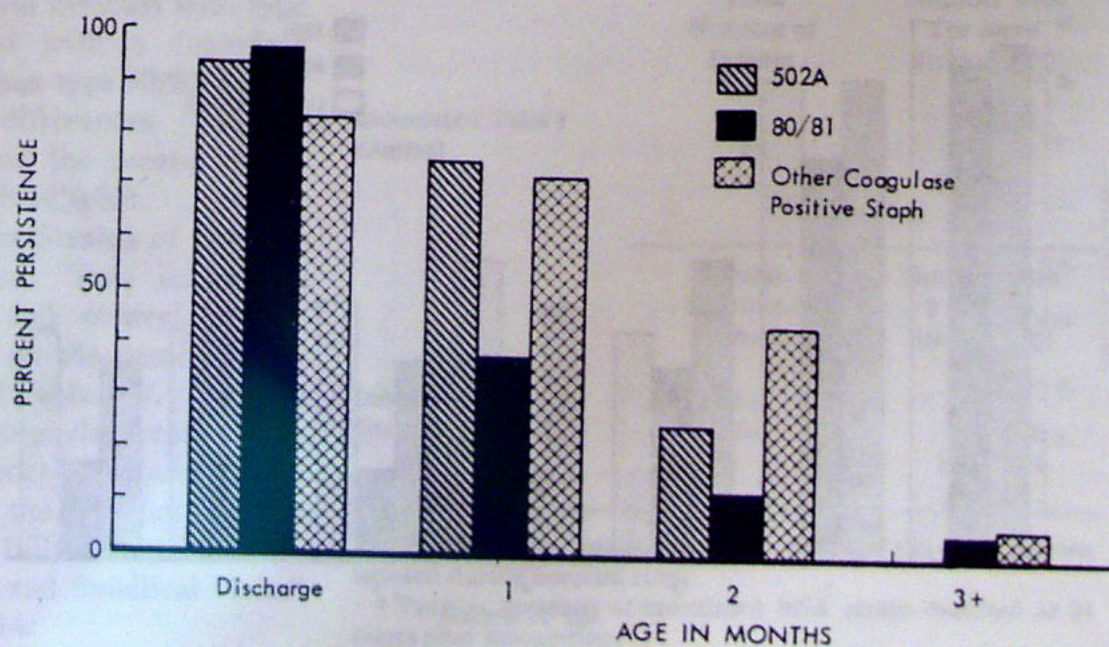


Fig 4.—Comparison of persistence of umbilical colonization of the different groups of coagulase positive staphylococci.



TABLE 7.—Persistence\* of Umbilical Colonization of 56 Staphylococcal Strains† from 46 Index Infants

Group	Discharge	Age, Mo		
		1	2	3
80/81 Strain	23/24	9/24 (37)	3/24 (13)	1/22 (4)
502A Strain	13/14	10/14 (71)	3/14 (21)	0/14 (0)
Coagulase positive other than 502A or 80/81	15/18	13/18 (72)	8/18 (44)	1/18 (6)

\* Number with persistence/total number in group (%).

† Ten infants with two strains.

the umbilicus were found to carry the organism on this site, a rate which declined to 13% by two months. After this time, organisms of this type were isolated from the umbilical site only on a single occasion.

H. Household Cross Infection: Seventeen mothers and 41 siblings were found to be colonized with coagulase positive staphylococci before their exposure to index infants infected with either strain 502A or type 80/81 (Table 8). One sibling in this group acquired a staphylococcus similar to the strain of the index infant. Of the 106 mothers and siblings not colonized with coagulase positive staphylococci prior to their exposure to infected index infants, 12 subsequently became colonized with strains similar to those carried by the infants, 8 with strain 502A and 4 with type 80/81. The difference between colonized and noncolonized household members in their rate of acquisition of staphylococci is significant at  $P=0.03$ .

I. Recolonization of Index Infants: Of the eight infants who lost strain 502A and the 16 who lost type 80/81 following discharge, 20 did not reacquire any strain of coagulase positive staphylococci. Two of the remaining four infants each acquired and subsequently lost three different strains of staphylococci which were also concurrently detected in household members. The staphylococci acquired by

the remaining two infants were not typable and persisted for a two-month period.

J. Staphylococcal Colonization in the Nursery After Cessation of Artificial Colonization: After cessation of artificial colonization a fall in the rate of colonization of newborns with type 80/81 occurred (Fig 1). During the week after artificial colonization 21% of the infants were colonized with coagulase positive staphylococci, none of which were type 80/81. Over the next three-week period, the rate of colonization with all strains of coagulase positive staphylococci increased and type 80/81 colonization reached 11%.

After artificial colonization attempts were stopped, spontaneous colonization with type 502A was limited to two infants infected over a two-week period.

### Comment

These data offer direct evidence to support the concept that interference between strains of coagulase positive staphylococci takes place in newborn infants under routine nursery conditions. Differences in colonization rates at both umbilical stump and nasal mucosa were observed in a nursery where, during the period of the study, no significant

TABLE 8.—Nasal Colonization Status of Mothers and Siblings Prior to Exposure to the Index Infant in the Home

Status Before Exposure	Mothers			Siblings				
	Total	502A	Acquired 80/81	Total	Total	502A	Acquired 80/81	Total
Nasal mucosa colonized w/coagulase positive staphylococci	17 *	0	0	0	41 *	0	1	1
Nasal mucosa not colonized w/coagulase positive staphylococci	25	3	0	3	81	5	4	9
Total	42	3	0	3	122	5	5	10

\* Two mothers and six siblings colonized with type 80/81 prior to exposure to index infant.



changes had been made in personnel, procedures, techniques, or in the nursery itself. The inoculated and control infants had equal opportunity to be handled by members of the nursery and obstetrical staffs, 50% of whom were known to be colonized with coagulase positive staphylococci other than strain 502A.

The incidence of lesions was significantly different in infants colonized with type 80/81 and those colonized with strain 502A. This supports the concept that strains of staphylococci vary in virulence for the infant. The data do not afford evidence to substantiate the hypothesis that an unknown host factor during an epidemic induces increased pathogenicity of a strain of staphylococcus.

All lesions occurring during this epidemic were relatively minor, supporting previous observations that strains of type 80/81 in different epidemics are associated with varying degrees of severity of disease.<sup>4</sup>

### Summary

Interference between strains of coagulase positive staphylococci under natural conditions was observed in newborn infants at the nasal and umbilical sites. Interference was also evident in household members exposed to index infants.

Nasal colonization persisted for a shorter period of time in infants colonized with type 80/81 than in infants colonized with strain 502A or other coagulase positive staphylococci. Umbilical colonization had nearly disappeared by the time the infant was three months old, no matter which type staphylococcus was involved.

The incidence of disease was significantly greater in infants colonized with staphylococcus type 80/81 than in infants colonized with strain 502A. Only one mild case of conjunctivitis was associated with the presence of strain 502A.

Following termination of the artificial colonization study, the 80/81 type disappeared completely from infants in the nursery for one week and then gradually returned.

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### REFERENCES

1. Shinefield, H. R.; Ribble, J. C.; Boris, M.; and Eichenwald, H. F.: Bacterial Interference: Its Effect on Nursery-Acquired Infection with Staphylococcus Aureus; I. Preliminary Observations on Artificial Colonization of Newborn, *Amer J Dis Child*, this issue, p 646.
2. 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.
3. Cohen, J. O.; Smith, P. B.; Shotts, E. B.; Boris, M.; and Updyke, E. L.: Bacterial Interference: Its Effect on Nursery-Acquired Infection with Staphylococcus Aureus; VI. Detection of An Implanted Strain of Staphylococcus Aureus, *Amer J Dis Child*, this issue, p 689.
4. 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.