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NASAL REJECTION OF EXPERIMENTALLY INOCULATED *STAPHYLOCOCCUS AUREUS*: EVIDENCE FOR AN IMMUNE REACTION IN MAN[1]

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Resistance to nasal colonization blocks a critical link in person-to-person spread of *Staphylococcus aureus* (1). Mucociliary sweeping within the nasopharynx together with organisms already resident there act to exclude ambient staphylococci. The resident nasal flora, by weight of numbers, undoubtedly prevent implantation of some outside bacteria. Numerical reduction of resident microflora by tetracycline treatment appears to be a major factor in promoting spread of antibiotic-resistant *S. aureus* (1–3). In addition to their population effect, some staphylococci, streptococci, lactobacilli and pseudomonas can elaborate antibiotic substances that inhibit growth of *S. aureus in vitro* (4); this may occur within the nose as well. Lastly, species-specific interference by strains of *S. aureus* already colonizing the nose may prevent establishment of new strains in some other unidentified fashion (5, 6). Species-specific interference by prior colonization, however, cannot explain the absence of carriage of any *S. aureus* in some individuals (7, 8). Little is known of the host's role in blocking implantation of staphylococci other than through the mechanical action of mucociliary cleansing (9). Nasal secretions contain lysozyme but this bacteriolytic enzyme seems to have no important effect upon *S. aureus* (10). Whether there is specific host resistance to staphylococcal colonization is unknown.

The present study was undertaken to determine the intranasal survival of experimentally inoculated staphylococci in untreated subjects. The mucociliary system rapidly eliminates inert foreign particles: the transit time from the farthest point in the nasopharynx to the oropharynx is estimated to be 20 min or less (11), a cleansing layer of mucus floods all parts of the nasopharynx at least every hour (9) and the intranasal survival of non-invasive bacteria and bacteriophage is a matter of minutes or a few hours at most (9, 12). Thus, intranasal persistence of inoculated bacteria for 5 days may be taken as evidence of implantation and, to a limited extent, incorporation into the body of the host's resident microflora. The minimal inoculum establishing a strain of *S. aureus* within the nose for 5 days is one measure of host susceptibility to that strain; another is the duration of intranasal survival of the inoculated strain on initial and subsequent administration of the same number of organisms. Evidence will be offered that the host, apart from his resident nasal bacteria, determines the fate of intranasally inoculated *S. aureus* and that the nasal rejection of an experimentally administered strain has the characteristics of an immune reaction.

MATERIALS AND METHODS

Subjects were healthy volunteers, aged 6 to 43 years; informed consent of each volunteer or his parent was obtained before inoculation. Persons receiving antibiotics, corticosteroids or insulin were excluded as were those who were pregnant or suffered such nasal abnormality as acute rhinitis (including "colds" and allergic rhinitis), or symptomatic nasal ulcers. Persons with past histories of rhinitis or sinusitis were not excluded.

Test bacteria. S. aureus HG, initially isolated from an asymptomatic carrier, had been previously employed in human inoculation studies without incident (1). The strain produces coagulase and fibrinolysin, ferments mannitol, and lyses sheep, rabbit and human erythrocytes. It is highly resistant to penicillin G and grows freely on agar containing tetracycline or streptomycin in concentration of 5 μg/ml. It produces no

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growth-inhibitory substances for other staphylococci or diphtheroids. Its phage pattern at both routine test dilution (RTD) and 100 times RTD is 52/52A/80/81.

S. aureus 29/79 is a descendant of *S. aureus* HG which changed its phage pattern to 29/52/52A/79/80/81 at RTD and 100 RTD after serial subculture from Trypticase soy (TS) to Brain-Heart infusion agar. This phage pattern has been maintained on subsequent subcultures on TS agar and after inoculation into volunteers. The two strains are otherwise indistinguishable in all respects including serotype abcphkm 263-1, as determined by Dr. Jay Cohen of the U.S.P.H.S. Communicable Disease Center.

Staphylococcus epidermis R, recovered from an asymptomatic carrier, has rose pigmented colonies. The strain produces fibrinolysin and catalase, ferments glucose anaerobically, and hydrolyzes starch and liquified gelatin. It does not produce coagulase or ferment mannitol. It grows readily on agar containing 5 μg/ml tetracycline but is inhibited by low concentrations of streptomycin and penicillin G. Its identity as *S. epidermidis* was confirmed by Dr. James Evans of North Carolina State University.

Stock cultures of the three test staphylococci were kept at 4°C on agar slants with yearly subculture; *S. aureus* HG was maintained for 2½ years, *S. aureus* 29/79 for 1½ years and *S. epidermidis* R for 1 year.

Inoculation and bacteriologic studies of volunteers. Two days before inoculation, two consecutive overnight subcultures of a stock culture were made in TS broth. Shortly before inoculation serial tenfold dilutions of the second subculture were made in iced TS broth. The same wire loop calibrated to deliver 0.01 ml was used for each subject; a loopful of the appropriate dilution was placed just inside each nostril. Duplicate 0.1-ml amounts of the serial dilutions were spread on plates which were incubated at 37°C; the size of the bacterial inoculum administered to each subject was calculated from surface colony counts.

Repeat inoculation with a tenfold increase in number of organisms to establish a minimal inoculum persisting 5 days was not done until the volunteer had been free of the test bacteria for 4 weeks. Cultures were made weekly for at least 3 weeks before inoculation, and 1, 5, 7 days, and then weekly for 4 weeks, after the last recovery of the test bacteria in order to determine the initial duration of implantation of the minimal inoculum. On reinoculation of this same number of organisms cultures were taken only weekly.

Cultures were made as follows: Two broth-moistened swabs were twirled inside both nostrils and mechanically shaken for 20 min in iced TS broth containing 7.5% NaCl. Two to three serial tenfold dilutions in broth were then made, and 0.1 ml of each dilution and the swab itself were spread on the surface of a tetracycline-fibrinogen, a streptomycin-fibrinogen and two plain fibrinogen plates. All plates contained 1% bovine fibrinogen, 0.25% fresh human plasma and 0.1% soy bean trypsin inhibitor. In tetracycline or streptomycin agar the amount of each antibiotic was 5 μg/ml.

At least two coagulase-positive colonies per plate, easily identified by characteristic halos of increased density after 48 hr incubation at 37°C, were picked for phage typing, which was done by standard methods (13). If the test organism's phage pattern was not found, up to 10 more coagulase-positive colonies were phage typed. *S. aureus* strains HG and 29/79 were identified by their growth on antibiotic agar and characteristic phage patterns at RTD and 100 RTD. These two staphylococci were never used together in the same volunteers. *S. epidermidis* R was identified by its roseate colonies and its growth on tetracycline but not streptomycin agar.

The number of naturally acquired *S. aureus* or *S. epidermidis*[2] per swab present prior to inoculation was calculated from the geometric mean of colony counts on plain fibrinogen agar of coagulase-positive or coagulase-negative staphylococci from duplicate 10^{-1} broth samples. The qualitative distribution of other genera of bacteria on nasal swabs was determined by identification of isolated colonies growing on sheep blood and MacConkey agar. The presence of growth inhibitory substances elaborated by volunteers' resident microflora was sought by spotting a loopful of bacteria upon a freshly streaked plate of *S. aureus* HG and examining for zones of inhibition after 24 and 48 hr incubation at 37°C (14).

Serum bacteriostatic substances. Serum was separated and stored at −20°C the same day it

[2] For the purpose of this study *S. epidermidis* includes all coagulase-negative micrococci and staphylococci.

TABLE I

Distribution of minimal inocula of Staphylococcus aureus HG persisting 5 days by test series, sex and natural carriage of S. aureus at time of inoculation

Minimal Inocula	Subjects	Test Series[a] A	Test Series[a] B	Sex M	Sex F	Natural[b] Carriage +	Natural[b] Carriage 0
10^4	17 (43%)	6 (33%)	11 (52%)	9 (41%)	8 (47%)	6 (43%)	11 (44%)
10^5	14 (36%)	7 (39%)	7 (33%)	8 (26%)	6 (35%)	5 (35%)	9 (36%)
10^6	8 (21%)	5 (28%)	3 (14%)	5 (23%)	3 (18%)	3 (21%)	5 (20%)
Totals	39	18	21	22	17	14	25

[a] See text for explanation of differences between Series A and B.

[b] Naturally-acquired *S. aureus* carried for 3 weeks or more prior to and during *S. aureus* HG implantation; noncarriers had no naturally-acquired *S. aureus* at implantation. + = carriers, 0 = noncarriers.

Random distribution	Chi square	*P*
Series A and B	1.69	$0.5 > P > 0.3$
Males and females	0.22	$0.9 > P > 0.8$
Carriers and noncarriers	0.08	$P > 0.9$

was obtained; an aliquot was heated to 56°C for 30 min prior to storage. Testing for bacteriostatic activity was carried out within 1 week of storage as follows: Serial twofold dilutions were made in TS broth starting with an initial dilution of 1/8; 0.6 ml of each dilution was added to 0.7 ml of TS broth containing 0.3% Tween; to this mixture was added 0.1 ml of a dilution of an overnight culture of the test bacteria containing approximately 100 organisms. Tubes were then mechanically shaken in a 37°C water bath for 4 hr. Pour plates were made from each tube and after 48 hr incubation at 37°C colony counts were compared to counts from three similarly prepared controls containing saline instead of serum. The endpoint was the highest serum dilution showing tenfold reduction in counts as compared with the mean of the saline controls.

RESULTS

Susceptibility to S. aureus HG—determination of minimal inoculum persisting 5 days. In preliminary tests the minimal intranasal inoculum persisting 5 days[3] was found to be 10^4 organisms.[4] This was confirmed in the first 18 volunteers of the present study; they were inoculated as a group (series A) with serial tenfold increases in dose, starting at 10^1 organisms, until 5-day implantation was observed. Doses of 10^1 to 10^3 organisms at times survived for 1 day, occasionally with a tenfold intranasal increase, but did not last 5 days. Inoculations in the remaining 21 subjects (series B) were begun with 10^4 organisms after the minimal inocula for series A subjects had been established. Distribution of minimal inocula of 10^4, 10^5 and 10^6 organisms was roughly similar in the two groups. Distribution was also similar among male and female subjects, and among natural carriers of *S. aureus* and noncarriers (Table I). In natural carriers *S. aureus* had been consistently recovered for 3 weeks prior to and during implantation of *S. aureus* HG; the noses of non-carriers had been found free of other strains of *S. aureus* during implantation.

To determine if the amount of naturally acquired *S. aureus* in carriers or the amount of *S. epidermidis* in non-carriers influenced susceptibility to the minimal inoculum of *S. aureus* HG, 13 carriers were ranked by the geometric mean of their *S. aureus* counts for 3 weeks or more just prior to implantation, and 12 noncarriers were ranked by the mean of their *S. epidermidis* counts over a similar period. Each minimal inoculum was represented by 3 to 5 carriers and noncarriers. The rank of a carrier by his naturally-acquired *S. aureus* bore no relation to the size of his minimal inoculum of *S. aureus* HG (Spearman's rank correlation test (15); $P > 0.2$), nor did the rank of a noncarrier by his *S. epidermidis* bear any such relation ($P > 0.2$). It is noteworthy that *S. aureus* HG was rarely recovered from

[3] Hereafter referred to as the minimal inoculum.

[4] Number of organisms equals number of colony forming units.

natural carriers on non-selective agar, even with repeated cultures. Had selective antibiotic-fibrinogen media not been employed, entirely different results would have been observed (Table II).

A variety of bacteria were recovered on sheep blood and MacConkey agar from 18 subjects during or shortly after *S. aureus* HG implantation. Two to three colonies of each genus from each of these individuals were tested for growth inhibitory substances to *S. aureus* HG that might have been induced by contact with the inoculated strain. None were found. Bacteria other than staphylococci were detected more frequently in subjects resistant to *S. aureus* HG. Two or more non-staphylococcal genera were recovered in 2 of 6 susceptible to a minimal inoculum of 10^4 organisms, 3 of 7 susceptible to 10^5 and 4 of 5 susceptible to 10^6.

Susceptibility to S. aureus HG—duration of implantation of minimal inocula on initial and repeat administration. The duration of intranasal survival of the minimal inoculum of *S. aureus* HG ranged from 5 days in 4 subjects to 70 weeks in 2. In the 39 subjects systematically tested, the minimal inocula survived 3 weeks or more in 12 (6 of series A, 6 of series B), 2 weeks or less in 25 (11 of series A, 14 of series B) and, because of antibiotic treatment within the first few weeks, an unknown time in 3 (1 of series A, 2 of series B). The longest duration of intranasal implantation occurred in those susceptible to the smallest minimal inoculum. Nine of the 12 persons in whom *S. aureus* HG persisted for 3 weeks or more were susceptible to a minimal inoculum of 10^4 organisms (Table III).

To determine if the duration of implantation of a given number of *S. aureus* was constant in an individual, volunteers were reinoculated with the previously determined minimal inocula 4 weeks or more after the strain spontaneously disappeared from their noses. Repeat inoculations gave similar results in almost all. The duration of reinoculation was 2 weeks or less in 6 of 7 persons initially carrying *S. aureus* HG 4 to 15 weeks, as well as in all 14 subjects initially implanted for 2 weeks or less. In subject #8, the first and second inoculations were recovered for 4 and 5 weeks, respectively; a third administration of his minimal inoculum, however, was found to persist only 2 weeks.

Double minimal inocula were administered to

TABLE II

Recovery of Staphylococcus aureus HG (phage type 52/52A/80/81) from natural carriers of other strains: efficacy of selective and non-selective agar

Subject	Date	Strain of *S. aureus* Recovered	
		Non-selective fibrinogen agar	Selective antibiotic-fibrinogen agar
19	3/12/64	7/54	52/52A/80/81
	10/14/64	47/53/54	52/52A/80/81
	10/21/64	47/53/54	52/52A/80/81
24	2/17/64	Non-typeable	52/52A/80/81
	10/19/64	Non-typeable	52/52A/80/81
	3/26/65	Non-typeable	52/52A/80/81
	4/2/65	Non-typeable	52/52A/80/81
37	3/19/65	81/42E	52/52A/80/81
	4/15/65	81/42E	52/52A/80/81
	4/29/65	52/52A/80/81	52/52A/80/81
	5/6/65	81	52/52A/80/81
	5/13/65	81/42E	52/52A/80/81
	5/21/65	81/42E	52/52A/80/81
39	3/5/65	29/52/52A/79/80/81	52/52A/80/81
	3/12/65	29/52/52A/79/80/81	52/52A/80/81
	3/19/65	29/52/52A/79/80/81	52/52A/80/81
	4/2/65	29/52/52A/79/80/81	52/52A/80/81
	5/6/65	29/52/52A/79/80/81	52/52A/80/81
42	6/22/64	3B/3C/55	52/52A/80/81
	8/26/64	3B/3C/55/71	52/52A/80/81

TABLE III

Duration of intranasal implantation of first administration of minimal inocula of Staphylococcus aureus HG

S. aureus HG Minimal Inoculum Size								
10^4			10^5			10^6		
Subject	Test Series	Duration	Subject	Test Series	Duration	Subject	Test Series	Duration
		weeks			*weeks*			*weeks*
15	A	70[a]						
20	A	70[a]	21	A	44[b]			
2	A	15						
38	B	11						
35	B	8						
3	A	6	49	B	6			
43	B	5						
56	B	4				8	A	4
E3	B	3						
29	B	2	11	B	2	14	A	2
48	B	2	36	B	2	28	B	1
7	A	1	18	A	2	6	A	<1
10	A	1	5	A	1	19	A	<1
26	B	1	13	A	1	11	A	<1
42	B	1	22	A	1			
58	B	1	24	A	1			
E2	B	1	31	B	1			
			37	B	1			
			E1	B	1			
			40	B	<1[c]			

Comparison of duration of 2 weeks or less with 3 weeks or more at 10^4, 10^5 and 10^6 minimal inocula: Chi square = 6.04; $0.05 > p > 0.02$

[a] *S. aureus* HG still present at last culture.

[b] Antibiotic therapy started at 44 weeks.

[c] <1 = at least 5 days but less than 1 week.

14 subjects who had previously rejected their minimal inocula after 2 weeks or less on one or two occasions. Again the duration of reimplantation was brief. A maximum of 3 weeks was observed in 5 persons and this was found at the 10^6 double inoculum in 4 (Table IV).

In association with or shortly after repeat inoculations of all but one reinoculated volunteer, the stock cultures of *S. aureus* HG were administered to susceptible subjects to test the *in vivo* viability of the organisms; inocula of 10^4 to 10^6 organisms showed no loss of vigor in these persons and persisted 4 to 18 weeks in their noses. The amount of *in vitro* growth after overnight incubation also remained constant throughout the study and was 10^9 organisms per ml at each inoculation.

To determine if the rapid rejection of *S. aureus* HG on reinoculation was a general or specific phenomenon, *S. aureus* 29/79—a descendant of *S. aureus* HG—was inoculated into 14 subjects who had twice rejected *S. aureus* HG after 2 weeks or less. The inoculum size of *S. aureus* 29/79 was the same as the minimal inoculum determined for the HG strain. In 6 subjects *S. aureus* 29/79 survived 4 to 12 weeks. This longer duration was not dose related but was found with inocula of 10^4, 10^5 and 10^6 (Table V).

As another test of specificity, *S. aureus* HG and *S. epidermidis* R, were administered together to 7 volunteers; the two strains were easily distinguished from each other. In three, subjects 2, 8 and 38, *S. aureus* HG had been found to persist

TABLE IV

Comparison of implantation of Staphylococcus aureus HG on initial, repeat and double dose inoculation

Subject	Minimal Inoculum	Duration of Nasal Implantation		
		First inoculation	Second inoculation	Double dose[a] inoculation
		weeks	*weeks*	*weeks*
2	10^4	15	<1[b]	2
38	10^4	11	1	
35	10^4	8	<1	
3	10^4	6	<1	3
43	10^4	5	1	
56	10^4	4	<1	
7	10^4	1	2	<1
10	10^4	1	<1	<1
26	10^4	1	1	<1
29	10^4	2		1
42	10^4	1	<1	
48	10^4	2	<1	
5	10^5	1	1	2
24	10^5	1	2	
31	10^5	1		<1
36	10^5	2	<1	<1
37	10^5	1	1	
40	10^5	1	1	1
6	10^6	1	1	3
11	10^6	<1	1	3
14	10^6	2	<1	3
19	10^6	<1	2	3
8	10^6	4	5	

[a] Double minimal inoculum administered.

[b] <1 = less than 1 week.

for 5 to 15 weeks after the first inoculation. In the others, subjects 6, 10, 14 and 19, the HG strain had been recovered for no more than 2 weeks after initial administration of the minimal inoculum. On subsequent inoculations, this staphylococcus had been rejected after 2 weeks by all. *S. aureus* HG was placed in the left nostril, *S. epidermidis* R into the right; the inoculum per nostril was the same as the minimal inoculum of *S. aureus* HG. Specificity was again evident—*S. epidermidis* R was found to persist intranasally for 3 to 14 weeks in 5 subjects, whereas *S. aureus* HG was rejected after 2 weeks by all (Table VI).

These 7 volunteers were then reinoculated with a freshly reisolated strain of *S. aureus* HG to be certain that the organism had not lost any colonizing potential as a result of prolonged storage. Reisolation was made from subject 15 who had continually carried *S. aureus* HG for 70 weeks, from the time it was first implanted for 5 days in any subject of this study. The strain was stored for 12 days without subculturing, then two serial overnight subcultures were made immediately prior to inoculation. The reisolated *S. aureus* HG was promptly rejected after 2 weeks by all 7 subjects (Table VI); in a new volunteer inoculated at the same time a 10^4 inoculum was implanted for 4 weeks.

TABLE V

Survival of Staphylococcus aureus 29/79 in volunteers twice rejecting minimal inoculum of S. aureus HG in 2 weeks

Subject	Staph 29/79 Inoculum[a]	Duration of Nasal Implantation
		weeks
7	10^4	<1[b]
10	10^4	1
26	10^4	1
42	10^4	4
48	10^4	8
5	10^5	5
24	10^5	<1
36	10^5	<1
37	10^5	9
40	10^5	7
6	10^6	2
11	10^6	12
14	10^6	2
19	10^6	<1

[a] Same as minimal inoculum for *S. aureus* HG.
[b] <1 = less than 1 week.

TABLE VI

Specificity of rejection of Staphylococcus aureus HG, Staphylococcus epidermidis R and reisolated S. aureus HG

Subject	Minimal Inoculum	Weeks of Nasal Implantation				
		Simultaneous Inoculation				
		Staph HG[a]		Staph HG[a]	Staph R	Reisolated[b] Staph HG
		First	Second	Third		
2	10^4	15	<1[c]	2	3	<1
10	10^4	1	<1	<1	4	<1
38	10^4	11	<1	<1	1	<1
6	10^5	<1	1	1	14	2
8	10^5	4	2[d]	2[e]	14	1
14	10^5	2	2	<1	8	1
19	10^5	<1	2	2	1	<1

[a] *S. aureus* HG—stored for 2½ years with yearly subcultures and 2 subcultures just before inoculation.
[b] Reisolated *S. aureus* HG stored for 12 days with 2 subcultures just before inoculation.
[c] <1 = less than 1 week.
[d] Third administration of minimal inoculum for subject 8, first and second inoculations persisted 4 and 5 weeks respectively.
[e] Fourth administration.

Serologic studies. Serum bacteriostatic activity against *S. aureus* HG was the same in heated and unheated serum specimens. Titers of 1/8 to 1/128 were present in 12 subjects first tested after they had rejected the minimal inoculum. In two others (subjects 15 and 20) titers of 1/16 and 1/64 were found while the HG strain continued to be recovered from them for 2 to 3 months. In six subjects tested immediately prior to simultaneous inoculation of *S. aureus* HG and *S. epidermidis* R, serum bacteriostatic titers were 1/32 or higher to the HG strain and less than 1/8 to the R strain. It may be noteworthy that, despite differences in titer, two persons (subjects 19 and 38) rejected both organisms after 2 weeks or less (Table VI). There was no increase in titer beyond one dilution to either organism 1 to 2 months after inoculation.

DISCUSSION

The two measures of susceptibility to *S. aureus* HG, the minimal number of organisms necessary

for 5-day implantation and the full duration of intranasal persistence of this inoculum, were initially related. The longest implantation on first administration of the minimal inoculum occurred in those volunteers susceptible to the smallest number of organisms (Table III). Volunteers of series B, inoculated subsequent to those in series A, were equally represented among subjects implanted with *S. aureus* HG for 3 weeks or more. On readministration of the minimal inocula, however, a short duration of *S. aureus* HG implantation of 2 weeks or less occurred in almost all subjects, and reinoculation of a double minimal inoculum resulted in implantation for no more than 3 weeks (Table IV). Since this staphylococcus continued to implant for 4 weeks or more in other individuals and since a reisolated *S. aureus* HG persisted in the reinoculated persons no longer than the stock culture, it must be presumed that rejection of *S. aureus* HG after 2 weeks upon its readministration was due to changes induced in the volunteers and not in the bacteria.

Individuals who rejected *S. aureus* HG after 2 weeks continued to do so on the second, third and fourth (reisolated strain) challenges. Moreover, this rejection was specific: *S. aureus* 29/79 and *S. epidermidis* R_4 survived 3 to 14 weeks in the noses of subjects who now eliminated the same number of *S. aureus* HG after 2 weeks (Table V and VI). Antigenically, *S. epidermidis* R and *S. aureus* HG are obviously different. *S. aureus* 29/79, though similar to its parent HG strain in many ways including serotype, may be presumed to have acquired antigenic differences as a result of subculture. New antigens appear and others disappear in a strain of *S. aureus* as a consequence of subculturing (16). Thus, resistance to intranasal implantation of *S. aureus* HG was characterized by induction, anamnestic response and specificity—the cardinal signs of an immune reaction.

The heat stable serum substance inhibiting growth of *S. aureus* HG is likely to be similar to serum factors associated with protection of animals against experimental subcutaneous *S. aureus* infection (17, 18). Subjects 15 and 20 with serum bacteriostatic activity to *S. aureus* HG in titer of 1/16 or more continued to carry this organism for two to three months, whereas subjects 19 and 38, with levels of less than 1/8 to *S. epidermidis* R and 1/32 or greater to *S. aureus* HG rejected both organisms in 2 weeks or less. This suggests that nasal rejection of staphylococci is not dependent on a particular level of serum bacteriostatic activity, although additional studies need to be done to clarify this. Nasal immunity has no clear relation to serum antibodies. Intranasal immunization of rabbits with *Diplococcus pneumoniae* results in resistance to nasal infection and even some systemic immunity without induction of serum antibodies (19, 20). Systemic immunization of rabbits against *D. pneumoniae* and *Pasteurella leptiseptica* induces serum antibodies to both bacteria, but provides nasal immunity only against *D. pneumoniae* (21). Nasal secretions do contain immunoglobulins in γ_1A- and γ2-globulin fractions (22, 23), and the congenital lack of γ_1A-globulin in persons with ataxia telangectasia is associated with recurrent infections in the nasal sinuses as well as in the lower respiratory tract (24). Adequate local concentration of γ_1A-globulin may be a determinant of nasal immunity (25).

Neither the presence of naturally-acquired *S. aureus* (Table I) nor the amounts of these strains or of strains of *S. epidermidis* in noncarriers influenced susceptibility to *S. aureus* HG. The use of both tetracycline and streptomycin-fibrinogen agar permitted easy recovery of *S. aureus* HG from natural carriers of other strains; the antibiotic-resistant coagulase-positive colonies could be identified at a glance. By contrast, in nonselective agar the inoculated strain was generally lost in the midst of the carrier's own *S. aureus* (Table II). This difference in technique would account for the difference between results observed in this study and those showing prevention of *S. aureus* implantation due to species-specific interference (5, 6). Other selective antibiotics are probably equally useful as long as there is no inoculum size effect as with penicillin G (26), or interaction with other bacteriostatic substances in the medium as with novobiocin in 7.5% NaCl agar (27).

Notwithstanding the lack of evidence for species-specific interference by resident strains of *S. aureus*, it is likely that the nonspecific population effect of various genera comprising the host's resident microflora did prevent implantation of small numbers of intranasally multiplying *S. aureus* HG, inocula of 10^1 to 10^3

organisms. A more varied microflora in the noses of non-carriers was recently reported (28) and a similar trend was noted among those less susceptible to implantation of *S. aureus* HG in the present study. This nonspecific population inhibition of colonization could be easily suppressed by antibiotic treatment.

No relation between susceptibility to *S. aureus* HG and sex of the volunteers was found (Table I). The greater susceptibility of male infants to staphylococcal infection and disease (29) does not appear to be reflected in experimental staphylococcal nasal colonization in adults and children.

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SUMMARY

A study of susceptibility to experimental staphylococcal nasal colonization in 39 volunteers was carried out by determining both the minimal number of *S. aureus* organisms required to establish 5-day intranasal implantation and the total duration of survival of this inoculum *in vivo*. These two measures of susceptibility were initially related: the longer durations of colonization, 3 to 70 weeks, were observed predominantly in those persons susceptible to the smallest 5-day inoculum, 10^4 organisms. Once the host spontaneously eliminated the inoculated *S. aureus* strain, however, its intranasal survival on reinoculation of the same number of organisms was almost always uniformly brief—2 weeks or less—while other susceptible subjects continued to be colonized for 4 weeks or more. Rapid rejection of a fixed inoculum of the *S. aureus* was found to persist for 4 challenges. Specificity of nasal rejection of inoculated staphylococci was evident. Subjects repeatedly rejecting the reinoculated staphylococcus after 2 weeks or less were experimentally colonized for 3 to 14 weeks with the same inoculum of 2 different staphylococci. The natural development, persistence and specificity of rapid rejection of an intranasally inoculated strain of *S. aureus* are interpreted as evidence of an immune reaction.

There was no evidence of specific bacterial interference to the experimentally inoculated *S. aureus* strain by *S. aureus* strains naturally colonizing the volunteers. However, a nonspecific inhibitory effect of the total population of the host's resident microflora is considered likely to have prevented implantation of small numbers of staphylococci, 10^1 to 10^3 organisms, not surviving 5 days. The elaboration of substances inhibiting staphylococcal growth was not detected in samples of resident nasal microflora of 18 volunteers. Bacteriostatic activity of serum from 14 subjects in titers of 1/8 to 1/128 was found against one staphylococcal strain but its relation to resistance to nasal colonization is not clear.

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