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MULTIPLE INFECTIONS AMONG NEWBORNS RESULTING FROM COLONIZATION WITH *STAPHYLOCOCCUS AUREUS* 502A

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ABSTRACT

Blair, E. B., and Tull, A. H.: Multiple infections among newborns resulting from colonization with *Staphylococcus aureus* 502A. Am. J. Clin. Path., *52:* 42–49, 1969. *Staphylococcus aureus* 502A was used to colonize newborns in the nursery of a hospital experiencing staphylococcal cross-infection problems. Within 1 to 4 days after implantation, 34% of 50 infants developed pustules. The daily transfer through broth of cultures used to implant infants resulted in population changes which revealed the instability and heterogeneity of the original 502A culture. Although the lack of concordance between colony morphology, phage type, and serotype prevented precise grouping of the strains isolated, there was little doubt that strains from implantation sites and lesions on infants came from the original inocula. The higher incidence of lesions observed in these procedures could possibly be attributed to the increase in numbers of mutants occurring during transfers through broth of cultures used for colonizing infants, coupled with the implanting of heavy concentrations of cells.

Numerous reports attest to the efficacy of *Staphylococcus aureus* 502A in aborting outbreaks of staphylococcal disease among newborns through the phenomenon of "bacterial interference." Implantation of the nasal mucosa and umbilicus of newborn infants with a staphylococcal strain "not known to be associated with illness" has been shown to curb outbreaks of disease by interfering with the natural colonization of infants by epidemic strains.[11] That the 502A strain might possess pathologic potential was indicated by Light and associates,[8] who noted that "in rare instances (less than 5%) tiny vesiculo-pustular skin lesions occurred and the 502A organism was isolated." Drutz and colleagues[4] reported that multiple abscesses developed in a patient with chronic furunculosis after implantation with the 502A strain. In later colonization procedures, Light and co-workers[9] observed a 14% incidence of staphylococcal lesions among infants who were colonized with heavy concentrations of 502A cells. Neither of these groups, however, felt that use of the 502A strain was contraindicated.

This report presents in more detail observations on an outbreak of disease, mentioned by Blair and associates,[1] which occurred among infants in the nursery of a civilian community hospital subsequent to their being artificially colonized with the 502A strain. Bacterial interference procedures had been initiated in efforts to quell an outbreak of disease caused by Phage Type 3A/3B/3C/55/71. Shortly after implantations were begun, a high incidence of minor vesiculopustular lesions occurring on the infants caused the colonizations to be terminated, and the infants were treated with antibiotics. Isolates from the infants and from the original implantation cultures were sent to this laboratory, U. S. Army Medical Research and Nutrition Laboratory (USAMRNL), for examination and bacteriophage typing.

MATERIALS AND METHODS

The 502A cultures were handled by the hospital laboratory (as described to E. B. B.) in the following manner: a lyophilized culture, *Ly 1*, of the 502A strain, obtained from Dr. H. R. Shinefield, was reconstituted in the hospital laboratory with 5 ml. of sterile, distilled water, and inoculated to brain

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heart infusion broth (BHIB). BHIB cultures to be used for implanting infants were transferred daily; a new BHIB was inoculated with 0.1 ml. of the culture from the previous day. Each new culture was streaked to blood agar for purity and was tested for resistance to tetracycline by the disk method. Within 1 hr. of birth the anterior nares and umbilicus of the newborn were touched with swabs which had been dipped into an 18- to 24-hr. broth culture of the 502A strain.

Bacteriophage typing was performed by the Microbiology Division, USAMRNL, on isolates from infants and cultures used for implantation. Typing procedures utilized a modified Tarr–Lidwell phage applicator.[7] Lysates prepared in this laboratory from phages and propagating strains obtained from the National Communicable Disease Center (NCDC), Atlanta, Georgia, included Phages 29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D, 81, and 187. Each lysate used throughout the studies was from the same propagation and was one passage removed from the NCDC strains. Routine test dilutions of the 22 phages were placed on the surface of predried, sterile trypticase soy agar plates (Baltimore Biological Laboratory, Baltimore, Md.).* After the liquid from the phage suspensions had absorbed, the plates were flooded with 1 to 2 ml. of a Difco Laboratories (Detroit, Mich.) heart infusion broth (HIB) culture of the coagulase-positive Staphylococcus to be typed; plates were incubated overnight at 30 C. Strains were identified using both major reactions (+++, confluent or semiconfluent; ++, more than 50 plaques), and minor reactions (+, 20 to 50 plaques; ±, less than 20 plaques). The term Staphylococcus in this report always means *S. aureus*.

The lyophil 502A culture (*Ly 1*) used for implantation cultures and a second vial (*Ly 2*), which was received at the same time as *Ly 1*, were sent to USAMRNL and examined as follows. (a) Twelve days after reconstitution, the contents of *Ly 1* were examined by inoculation to 5% sheep blood agar (SBA), salt mannitol plasma agar (SMPA),[1] and HIB. After incubation for 18 hr. at 35 C., the HIB culture was streaked to SBA and SMPA. (b) Vial *Ly 2* was opened and reconstituted with 1.0 ml. of sterile, distilled water, then immediately inoculated to the media described in (a). (c) A tube of HIB was inoculated with 0.1 ml. of *Ly 2* and eight serial transfers were made by daily transferring 1 loopful from the mixed contents of the culture from the previous day. At the time of each broth transfer, 1 loopful of the mixed contents was streaked to SBA and SMPA; the percentage of colony forms appearing on these plates was estimated from counting 100 to 200 random colonies. Each broth and representative colonies from agar platings of these broths were phage typed. Serotyping and additional phage typing on isolates from *Ly 1*, *Ly 2*, and infants were kindly performed by Dr. P. B. Smith, NCDC.

* The use of commercial trade names is for the purpose of identification only and does not constitute an endorsement by the Department of Defense.

RESULTS

Within 15 days after colonization procedures were initiated, 115 cultures of *S. aureus* 502A from 50 colonized infants were examined by bacteriophage typing. Over the succeeding 3 months, 17 additional isolates with lytic patterns similar to the 502A strain were recovered from these babies. Table 1 shows the source and phage types of isolates from the colonized infants. Major reactions with our typing phages revealed three lytic patterns to be present among isolates from infants, lesions, and implantation cultures. Minor reactions, often with less than five plaques, were consistently present in varying patterns with Phages 29, 52, 52A, 79, 80, 6, 42E, 47, 53, 54, 75, 77, 83A, 42D, and 81. Although in most instances only a single colony per specimen was received for typing, the three strains were found to be distributed among the colonization sites (nose and umbilicus), lesions, stools, and implantation cultures. Types 7+ and 7/77+ were recovered from the few nasopharyngeal cultures taken. Type 7+ was the predominating strain and was recovered from all sites, whereas Types 29/7/81+ and 7/77+ appeared much less

TABLE 1

SOURCE AND PHAGE TYPE OF ISOLATES FROM COLONIZED INFANTS

Site	Total No. of Infants	Total No. of Cultures	Isolates with Major Reactions*		
			7+	29/7/81 +	7/77+
Nose	42	52	44 (37)	4 (4)	4 (4)
Umbilicus	40	43	34 (33)	4 (4)	5 (5)
Nasopharynx	9	9	8 (8)	0	1 (1)
Stool	7	8	5 (4)	2 (2)	1 (1)
Gastric	1	1	1 (1)	0	0
Pustules	17	19	15 (14)	3 (3)	1 (1)
Total	50	132	107 (44)	13 (12)	12 (9)

* Numbers in parentheses represent numbers of infants involved.

TABLE 2

RELATION OF CULTURE HISTORY OF THE 502A INOCULUM TO TIME OF APPEARANCE OF PUSTULES IN INFANTS

Culture Transfer No.	No. of Infants Implanted	No. of Infants with Pustules	Day* Lesions were Cultured
1	1	0	
2	2	0	
3	4	1	1
4	6	4	2, 3, 4, 4
5	1	1	2
6	8	4	1, 1, 3, 4
7	4	1	1
8	8	0	
9	4	2	1, 3
10	4	3	3, 2, 3
11	7	1	3
12	1	0	

* After implantation.

frequently. Taking all sites into account, Types 7+ and 29/7/81+ were both recovered from eight infants, Types 7+ and 7/77+ were found in cultures from five infants, and all three strains were isolated from two infants. Stool specimens collected from seven infants also contained the 502A strain; four specimens were taken about 24 hr. after birth, one within 48 hr., and two within 72 hr. Pustules from which only the 502A strain was isolated appeared on 17 of 50 infants within 4 days of their being colonized. Again, all three phage types were found, and Types 7+ and 29/7/81+ were each isolated at different times from pustules on the same infant.

Table 2 relates the history of the cultures used to colonize each of the 50 infants to the length of time elapsing between implantation and the appearance of pustules. The first infant to develop lesions had been implanted with the 502A strain on the 3rd day after colonization procedures were begun. Of the 17 infants evidencing a pathologic response to implantation, five developed lesions 24 hr. after birth, and pustules appeared on three, six, and three infants within 2, 3, and 4 days, respectively. The absence of lesion cultures from infants colonized on Day 8 could not be explained, as later implantations resulted in pustules on six of 16 infants. One baby diagnosed as having impetigo and pustules 12 days after birth was carrying the 502A strain in his nose; another diagnosed as having "staph infection" 24 days after implantation carried the 502A strain in the nose and throat; it was not determined whether these strains were responsible for the lesions. Staphylococci isolated from the nose, umbilicus, or nasopharynx of three healthy babies born approximately 3 months later were similar, by phage type, to the 502A strain, indicating that cross-colonization might still have been occurring in the nursery.

Four morphologically different *S. aureus* colonial forms with phage patterns 7+, 29/7/81+, and 7/77+ were found in subcultures from *Ly 1* (from which implantation cultures were made) on SMPA and SBA

TABLE 3

MORPHOLOGY, PHAGE TYPES, AND SEROTYPES OF ISOLATES FROM IMPLANTATION CULTURES AND INFANTS' SPECIMENS

Source	Morphology	Phage Type*	Serotype
Ly 1	Predominant colony (orange)	7:29/52/52A/80/6/42E/47/53/54/81	bc_1 ; $(b)c_1$; abc_1 ; $(a)bc_1$; $a(bc_1n)$; $(a)bc_1(n)$
		29/7/81:52/52A/80/6/42E/47/53/54	bc_1
	Yellow colony	7:29/52/6/42E/47/53/54/77/83A/42D/81	abc_1 ; $(a)bc_1$; abc_1n
	Sectored (transparent)	7/77:29/6/42E/53/54/83A/42D/81	Not done
	Sectored (opaque)	7:29/6/42E/53/54/77/83A/42D/81	Not done
Ly 2	Predominant colony	7:29/52/52A/80/6/42E/47/53/54/83A/42D/81	$b(c_p)$; c_1 ; $c_1(i)$
	White colony	UC18/7:53/77/83A/42D	bc_pn
	Sectored (transparent)	7:6/42E/47/53/54/77/83A/42D/81	$c_1(i)$; c_1
	Sectored (opaque)	UC18/7:6/42E/47/53/54/77/83A/42D	$b(c_1m)$
	Strong hemolysis	7:6/47/53/42D/83A	b
	Zone	UC18/7/77:29/52/52A/80/6/42E/47/53/54/83A/42D/81	c_1 ; bc_pn
Infants		7:29/52/52A/80/6/42E/47/53/54/83A/42D/81	bc_1 ; $(b)c_1$; c_1 ; abc_1n
		29/7/81:52/52A/80/6/42E/47/53/54/83A	$(b)c_1$
		UC18/7/77:29/52/52A/80/6/42E/47/53/54/83A/42D/81	bc_1 ; c_1

* USAMRNL phages except for UC18. Minor reactions are composites from several representative colonies.

(Table 3). Predominating was a weakly hemolytic, opaque, orange colony, the majority of which reacted strongly with Phage 7 and weakly with several Group I and Group III phages. Occasional colonies which were morphologically indistinguishable from those in predominance typed 29/7/81+. Also present in very low numbers were three other colonial forms: (1), nonhemolytic yellow colonies typing 7+, and (2) and (3), sectored colonies, the transparent portion usually typing 7/77+ and the opaque portion typing 7+ and reacting only weakly or negatively with Phage 77.

Agar and broth cultures from Vial *Ly 2* revealed six morphologic types in the lyophil population. Phage Type 7+ again represented the orange-pigmented predominating strain, but none of the colonies selected typed 29/7/81+. The initial plating revealed rare sectored colonies and transparent colonies. The transparent colonies usually typed 7+ and the opaque sectors typed UC18/7+. (UC18 susceptibility of these strains was determined by NCDC; however, the remainder of the lytic patterns shown was determined using USAMRNL phages.) Also seen were rare, nonpigmented colonies which were later observed to increase rapidly in numbers as *Ly 2* cultures were serially transferred through HIB. These colonies produced variable major reactions with Phages 7, 77, and UC18. Broth cultures brought forth two additional colony types: weakly pigmented colonies, usually typing UC18/7+, which were surrounded by dark zones in blood agar similar to those described by Elek and Levy[5] for β-hemolysin producing staphylococci; and weakly pigmented colonies, typing 7+, surrounded by large zones of clear hemolysis. Although the differences between lytic patterns were fewer than recommended for differentiating strains, they were consistent and certainly

TABLE 4

Percentage of Colony Forms* Occurring During Eight Serial Transfers of 502A Through HIB

Transfer No.	Occurrence of Colonies				
	Orange	White	Sectored	Hemolytic	Zone
	%				
0	99	<1	<1		
1	99	1			
2	96	3			1
3	90	10			<1
4	85	15		<1	
5	83	15		2	
6	78	20	2		
7	67	30	3		
8	50	47	3	<1	<1

* Estimated by counting 100 to 200 colonies on SBA.

furnished evidence for the instability or nonhomogeneity of the original culture.

To simulate the conditions under which the implantation culture *Ly 1* was handled, an inoculum from *Ly 2* was serially passed through eight transfers in HIB. In spite of the rather crude method used to measure population changes, Table 4 shows the strikingly rapid emergence of a nonpigmented variant from less than 1% to about 47% of the population. Also indicating the heterogeneity of the original culture were the increases in numbers of sectored or transparent colonies (Fig. 1) to approximately 3% of the population, as well as the occurrence on SBA of colonies surrounded by dark zones or strong hemolysis.

Thus, with our typing phages, lytic patterns 7+, 29/7/S1+, and 7/77+ characterized the isolates from *Ly 1* and from infants. Type 29/7/S1+ was not found in *Ly 2*. Three of 10 infants' cultures (pustule, stool, umbilicus) sent to NCDC reacted with Phage UC18, but none of the *Ly 1* strains, possibly because of unfortunate selection, were reactive with this phage. However, the recovery of this variant from implantation sites could be accounted for after several isolates reacting strongly with UC18 were found in subcultures from the *Ly 2* vial.

Serotyping of selected strains from *Ly 1*, *Ly 2*, and infants' cultures also revealed variations of serotype within morphologic and phage groups, as shown in Table 3. With few exceptions, all strains possessed the characteristic c_1 antigen of the 502A strain. The number of serotypes appearing opposite the morphologic groups more accurately reflects the number of strains selected for typing than concordance of serotype with morphology or phage type.

DISCUSSION

Despite the relative avirulence of the 502A strain, its implantation in newborn infants is apparently not completely without complications. In contrast with other reports on bacterial interference studies using the 502A strain, pustules occurring on 34% of 50 colonized infants within 4 days after implantation indicated a greater virulence for the cultures used in these procedures. Recovery of only the 502A strain from lesions left little doubt as to the etiology of the infections.

With the USAMRNL set of typing phages isolates from infants could be placed into three lytic patterns: 7+, 29/7/S1+ and 7/77+. This differentiation of strains was based on repeated observations that each type appeared on replicate plates during the same series of phage testing, and duplicate typing from the same colony gave identical major reactions. All types were found among isolates from infants, Vial *Ly 1*, and subcultures used for implantation. The less frequent recovery of Types 29/7/S1+ and 7/77+ from lesions and other sites reflected their low numbers in the cultures used for implantation, but revealed that "takes" had occurred with variants. Had additional colonies been selected from the specimen cultures, it is quite likely that multiple strains would have been found in each site. It was noteworthy that the serial broth transfers, which consisted of various proportions of mutants, were quite similar in phage type and consistently produced minor reactions with Group I phages. These broths were not reactive with Phages UC18 and 77, the latter reactions possibly being masked; however, individual isolates revealed UC18/77 reactive strains to be present. Phage

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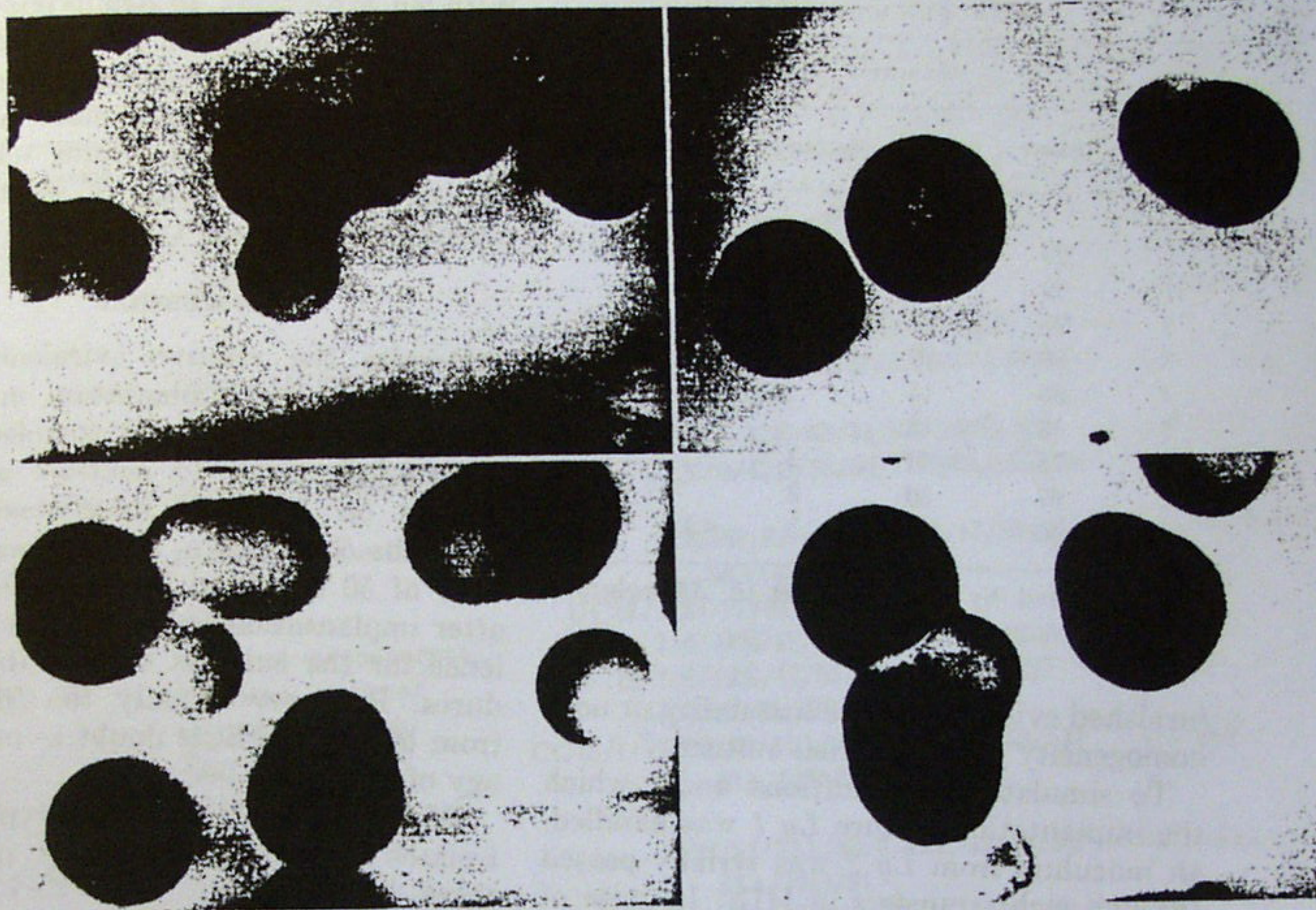

FIG. 1. Variant 502A colonies from lyophil vial 2 on sheep blood agar

UC18 proved helpful in revealing strain differences: susceptible cultures usually reacted strongly with Phage 77 and were morphologically distinct from the predominating colony type.

The use of bacteriophage typing and serotyping to identify variants such as were encountered in this situation had definite limitations. There was not sufficient concordance between phage type, serotype, or colony morphology to permit a consistent grouping of strains from infants or colonization cultures. The 502A strain, to the best of our knowledge, has not been reported to be susceptible to Phage UC18 or the Group I phages, which could indicate differences in the strain used in these procedures. The c_1 antigen was present in almost all strains and furnished evidence of the relation to 502A, although antigenic variation between morphologically similar colonies and isolates having the same phage type prevented precise identification. Cohen and associates[3] reported that variations in the phage type of certain 502A strains that they were studying made identification difficult, but they were able to identify them serologically using the characteristic c_1 antigen as a marker. Fine and colleagues[6] found that strains of staphylococci from 502A-implanted sites exhibited variable differences in colonial morphology, antibiogram, serotype, and phage type, although most were variants of the 502A strain. Most of the mutants were similar to 502A in phage type (Phage 7 with minor Group III reactions). Serotype bc_1 characterized the 502A cultures, while the majority of colonial variants were either abc_1mn or bc_1n. The observations of Fine and associates indicated that the 502A strain could independently vary in one or more of the characters of morphology, phage type, serotype, or antibiogram.

These data did not allow identification of the strain(s) responsible for the lesions. For example, each phage type was recovered from at least one lesion culture; Serotype bc_1 characterized two of four lesion isolates which typed 7+, whereas the remaining two isolates serotyped c_1 and $(b)c_1$ and phage-

typed UC18/7/77+ and 7+, respectively. Unexplained also was the sporadic nature of the infections. Pustules appeared on infants who were implanted with 502A cultures that had been transferred three or more times through BHIB. During the 10 days following the first appearance of lesions, 50% or more of the infants colonized on each of 5 days developed pustules, whereas on days 8 and 11 only one of 15 infants developed lesions. An interesting sidelight was the rapidity of passage of the 502A strain through the gastrointestinal tract of seven infants.

The higher infection rate of 34% in these colonization procedures, as compared with the 14% lesion rate found by Light and colleagues,[9] could possibly have been due to the additive influences of increased numbers of more virulent mutants in implantation cultures which had been transferred through broth, and to the use of heavy concentrations of cells for implantation. Light and co-workers[8, 9] reported less than 5% occurrence of lesions among infants implanted with diluted broth cultures which had been transferred one time in broth, but they found that implantation swabs containing heavy concentrations of cells raised the lesion rate from 3.5 to 14%. Drutz and associates[4] used swabs which had been dipped into a 1:200 dilution of an 18-hr. broth culture, one transfer from the stock 502A culture, for implanting a chronic furunculosis patient, who subsequently developed multiple abscesses. Shinefield's group[11] used highly diluted cultures and carefully controlled the number of cells implanted. If the cultures used by Light and colleagues had contained variants such as were found in this study, then the increased infection rate among their infants following implantation of heavy concentrations of cells could possibly be attributed to the greater number of variants implanted.

S. aureus cultures have been found to mutate readily with respect to many biologic characters. Parisi[10] noted appreciable variation in a parent strain of 80/81 and four of its chromogenic variants with regard to pigmentation, coagulase, hyaluronidase, proteinase, and egg yolk opacity, although all strains were equally virulent for mice. Burns and Holtman,[2] using a special medium demonstrated pigmented, biochemically active, coagulase-positive clones within colonies of white, coagulase-negative staphylococci which had been repeatedly recovered from antemortem blood cultures and from heart valve lesions of a patient with bacterial endocarditis. The studies by Elek and Levy[5] on variation in staphylococcal hemolysin production led those authors to infer that "strains with a low incidence of virulent colonies might immunize a population, while the emergence of strains from virulent clones might lead to an epidemic, if the toxic variant were to remain stable." The possibility of such an occurrence existed in these procedures. At any rate, the demonstrated instability and nonhomogeneity of this particular 502A culture precludes its use in further implantations and should cause intensive examination of any 502A strain prior to its being used as a bacterial interference agent.

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