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 heart to be transferred with 0.1 day. E. agar for 1 hr. of the which broth.
Bacterial isolate implantation: modified Lysate phage from Center Phage 71, 6, 81; and the standard NCD 22 phage (Baltimore), more phage were orato.
broth were (+, -) from (+, -) plaque repor.

implications (L.)

as L.

aminoreco.

purine.

acid.
FROM "S. 502A"

KING, laboratory, borns resulting Path., 52: 42-
newborns in the ion problems. oped pustules. sulted in
in genea of the colony morphol-
trains isolated, ons on infants served in these
ers of mutants nizing infants, ak of disease, men-
sociates, which oc-
the nursery of a tal subsequent to colonized with the interference proce-
dein efforts to quell
caused by Phage
Shortly after in-
a high incidence of estions occurring on
onizations to be
ants were treated
from the infants
mplantation cultures
ory, U. S. Army
trition Laboratory
mination and bac-

3 METHODS
ere handled by the
scribed to E. B. B.:
: a lyophilized cul-
train, obtained from
as reconstituted in
with 5 ml. of sterile,
soculated to brain

heart infusion broth (BHIB). BHIB cultures
be used for implanting infants were trans-
ferred 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.
brroth culture of the 502A strain.

Bacteriophage typing was performed by the Microbiology Division, USAMRML, on isolates from infants and cultures used for
implantation. Typing procedures utilized a
modified Tarr–Lidwell phage applicator. 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, 50, 3A, 3B, 5C, 55,
71, 6, 7, 42E, 47, 53, 54, 75, 77, 83A, 42D,
51, 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 pre-
dried, sterile trypticase soy agar plates
(Baltimore Biological Laboratory, Balti-
more, Md.).* After the liquid from the
phage suspensions had absorbed, the plates
were flooded with 1 to 2 ml. of a Difeo
Laboratories (Detroit, Mich.) heart infusion
broth (HIB) culture of the cagulase-positive
Staphylococcus to be typed; plates were
incubated overnight at 30 C. Strains
were identified using both major reactions
(++, confluent or semi-confluent; +++,
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 USAMRML and ex-
amined 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), 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 esti-
mated from counting 100 to 200 random
colonies. Each broth and representative
colony from agar platings of these broths
were phage typed. Serotyping and addi-
tional phage typing on isolates from Ly 1,
Ly 2, and infants were kindly performed by
Dr. P. B. Smith, NCDC.

RESULTS
Within 15 days after colonization pro-
cedures were initiated, 115 cultures of S.
aureus 502A from 50 colonized infants were
examined by bacteriophage typing. Over
the succeeding 3 months, 17 additional iso-
lates 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 implant-
atation cultures. Minor reactions, often with
less than five plaques, were consistently
present in varying patterns with Phages 29,
52, 52A, 79, 50, 6, 42E, 47, 53, 54, 75, 77,
83A, 42D, and 51. Although in most in-
stances 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

<table>
<thead>
<tr>
<th>Site</th>
<th>Total No. of Infants</th>
<th>Total No. of Cultures</th>
<th>Isolates with Major Reactions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>7+</td>
</tr>
<tr>
<td>Nose</td>
<td>42</td>
<td>52</td>
<td>44 (37)</td>
</tr>
<tr>
<td>Umbilicus</td>
<td>40</td>
<td>43</td>
<td>34 (33)</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>9</td>
<td>9</td>
<td>8 (8)</td>
</tr>
<tr>
<td>Stool</td>
<td>7</td>
<td>8</td>
<td>5 (4)</td>
</tr>
<tr>
<td>Gastric</td>
<td>1</td>
<td>1</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Pustules</td>
<td>17</td>
<td>19</td>
<td>15 (14)</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>132</td>
<td>107 (44)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses represent numbers of infants involved.

TABLE 2

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

<table>
<thead>
<tr>
<th>Culture Transfer No.</th>
<th>No. of Infants Implanted</th>
<th>No. of Infants with Pustules</th>
<th>Day* Lesions were Cultured</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2, 3, 4, 4</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1, 1, 3, 4</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1, 1, 3, 4</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* 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 infans 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 5 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 SMDM and SBA...
### Table 3
Morphology, Phage Types, and Serotypes of Isolates from Implantation Cultures and Infants’ Specimens

<table>
<thead>
<tr>
<th>Source</th>
<th>Morphology</th>
<th>Phage Type*</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ly 1</td>
<td>Predominant colony (orange)</td>
<td>7:29/52/52A/80/6/42E/47/53/54/81</td>
<td>abc1, b(c)1, abc2, a(bc)2, abc3, a(bc)m, abc4, a(bc)n</td>
</tr>
<tr>
<td></td>
<td>Yellow colony</td>
<td>29/7/81:52A/80/6/42E/47/53/54</td>
<td>bc1, ab1, a(bc)1, abc1</td>
</tr>
<tr>
<td></td>
<td>Sectored (transparent)</td>
<td>7:29/52/6/42E/47/53/54/77/83A/42D/81</td>
<td>Not done</td>
</tr>
<tr>
<td></td>
<td>Sectored (opaque)</td>
<td>7:29/52/6/42E/47/53/54/77/83A/42D/81</td>
<td>Not done</td>
</tr>
<tr>
<td>Ly 2</td>
<td>Predominant colony</td>
<td>7:29/52/52A/80/6/42E/47/53/54/83A/42D/81</td>
<td>b(c)2, c1, c(c)2</td>
</tr>
<tr>
<td></td>
<td>White colony</td>
<td>UC18/7:53/7/80A/24D</td>
<td>bc1, b(c)3</td>
</tr>
<tr>
<td></td>
<td>Sectored (transparent)</td>
<td>7:6/42E/47/53/54/77/83A/42D/81</td>
<td>c1(t)1, c1</td>
</tr>
<tr>
<td></td>
<td>Sectored (opaque)</td>
<td>UC18/7:6/42E/47/53/54/77/83A/42D</td>
<td>b(c1)m</td>
</tr>
<tr>
<td></td>
<td>Strong hemolysis Zone</td>
<td>7:6/47/53/42D/83A</td>
<td>b</td>
</tr>
<tr>
<td>Infants</td>
<td>7:29/52/52A/80/6/42E/47/53/54/83A/42D/81</td>
<td>bc1, b(c)1, c1, abc1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>29/7/51:52A/80/6/42E/47/53/74/83A</td>
<td>b(c)2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>UC18/7:77/20/52/52A/80/6/42E/47/53/54/83A</td>
<td>b(c)1, c1</td>
<td></td>
</tr>
</tbody>
</table>

*USAMRIID 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 were morphologically indistinguishable from those in predominance typed 29/7. All were present in very low numbers as three other colonial forms: (1) alphahemolytic yellow colonies typing 7+, and (2) and (3), sectored colonies, the transient portion usually typing 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+ except those in subculture on SMAP and SBA. 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 USAMRIID 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 rections 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 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

<table>
<thead>
<tr>
<th>Transfer No.</th>
<th>Occurrence of Colonies</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Orange</td>
<td>White</td>
<td>Sectored</td>
<td>Hemo-</td>
<td>lytic</td>
</tr>
<tr>
<td>0</td>
<td>99</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>&lt;1</td>
</tr>
<tr>
<td>4</td>
<td>85</td>
<td>15</td>
<td>3</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>5</td>
<td>83</td>
<td>15</td>
<td>3</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>20</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>30</td>
<td>&lt;1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>47</td>
<td>3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Estimated by counting 100 to 200 colonies on SBA.

furnished evidence for the instability or non-

homogeneity 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 nonpig-
m ented 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. 11) to approxi-
mately 37% of the population, as well as the
occurrence of SBA of colonies surrounded
by dark zones or strong hemolysis.

Thus, with our typing phages, lytic pat-
terns 7+, 29.7, S1+, and 7/77+ charac-
terized the isolates from Ly 1 and from in-
fections. 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 α1 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 with-
out 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 vir-
ulence for the cultures used in these proce-
dures. Recovery of only the 502A strain
from lesions left little doubt as to the etiol-
ology of the infections.

With the USAMRL 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 sub-
cultures 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 "stages" 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 propor-
tions of mutants, were quite similar in phage
type and consistently produced minor re-
actions with Group 1 phages. These broths
were not reactive with Phage UC18 and 77,
the latter reactions possibly being masked;
however, individual isolates revealed UC18/77
reactive strains to be present. Phage

UC18 produced a difference at morpho-

licating colonies.

The use of typing phages limited to 7+

and S1+ was encountered in this series of

Infants. The serotype ω-1 antigen, and

502A, all morphophases having a speci-
cifie ide type, reported by certain
as also revealed

in Table 3. The 502A strain,
possessed the

was selected for

in these pro-

urea cell

rs occurring on

within 4 days

a greater viru-

on in Table 3.

ins possessed the

the 502A strain,
promising opposite

more accurately

studies which

in these pro-

etiological

phages

be placed into

29/7/81+ and

of strains was

that each plate during the

and duplicate

gave identical

were found among

Lg and sub-

concordance between phage type, serotype, or

culture 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

phages, which could indicate differences in the

strain used in these procedures. The

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

precision identification. Cohen and associ-

ates reported that variations in the phage type

certain 502A strains that they were study-

UC18 proved helpful in revealing strain

differences: susceptible cultures usually

acted strongly with Phage 77 and were

morphologically distinct from the predomin-

ating colony type.

The use of bacteriophage typing and sero-
typing to identify variants such as were

encountered in this situation had definite

limitations. There was not sufficient concor-
dance between phage type, serotype, or

culture 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

phages, which could indicate differences in the

strain used in these procedures. The

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

precision identification. Cohen and associ-

ates reported that variations in the phage type

certain 502A strains that they were study-

using made identification difficult, but they

were able to identify them serologically

using the characteristic 

antigen as a

marker. Fine and colleagues found that

strains of staphylococci from 502A-im-

planted sites exhibited variable differences

in colonial morphology, antibiogram, sero-
type, 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 

caracterized the 502A cultures,

while the majority of colonial variants were

either 

or 

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

characterized two of four lesion isolates

which typed 7+, whereas the remaining

two isolates serotyped 

and 

phage-
Blair and Tull, Burns and Holtman,5 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 Levy4 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.

REFERENCES

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