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A LONGITUDINAL STUDY OF NEISSERIA MENINGITIDIS  
CARRIERS IN SUBMARINE CREWS

by

LT Russell J. Reit, MC, USN

and

LT Michael D. Smith, MC, USNR

Bureau of Medicine and Surgery, Navy Department  
Research Work Unit MF022.03.03-9025.30

Released by:  
Gerald J. Duffner, CAPT MC USN  
COMMANDING OFFICER  
Naval Submarine Medical Center

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Transmitted by:



CDR Donald R. Feeley, MC, USN  
Director, School of Submarine Medicine

Reviewed and Approved by:



Charles F. Gell, M.D., D.Sc.  
SCIENTIFIC DIRECTOR  
SubMedResLab

Approved and Released by:



Gerald J. Duffner, CAPT MC USN  
COMMANDING OFFICER  
Submarine Medical Center

## SUMMARY PAGE

### THE PROBLEM

To report studies of carriers of the causative organism of meningitis, which were carried out over a period of more than a year aboard a nuclear-powered submarine—on both its Blue and its Gold crew, during both the time on patrol and the time when living ashore between patrols.

### FINDINGS

In this study of 342 men, it was found that the overall carrier rate was 26 percent, with four percent chronic carriers, 28 percent always negative, and 68 percent intermittent carriers. Group B was the predominant serogroup for this organism. The closed environment did not appear to influence the carrier rate, nor create a selective advantage for any one serogroup.

### APPLICATION

The results of this study and the subsequent phases which are planned will add considerably to the body of medical knowledge concerning this organism and should assist in the devising of preventive measures.

### ADMINISTRATIVE INFORMATION

This study was completed by the Medical Officers of the Blue and Gold crews of the polaris submarine, USS MARIANO G. VALLEJO (SSBN-658) and was presented by them in thesis form to the Board of Examiners for Qualification as a Submarine Medical Officer, in partial fulfillment of the requirements for that qualification. Their thesis was selected for publication as a Submarine Medical Research Laboratory Report in order to make the material available in the Technical Library at the Submarine Medical Center, and as study material in the School of Submarine Medicine. The report has been designated as No. 532 under date of 7 June 1968; it is Report No. 30 on Work Unit MF 022.03.03-9025—Assessment of Factors Related to Submarine Habitability, Escape and Rescue, and New Equipment.

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## ABSTRACT

A total of 342 men from both the Blue and Gold crews of the Polaris submarine USS MARIANO G. VALLEJO (SSBN-658), were surveyed periodically over a 13-month period for the causative organism of meningitis (*Neisseria meningitidis*). It was found that the overall carrier rate was 26 percent, with four percent chronic carriers, 28 percent always negative, and 68 percent intermittent carriers. Of all isolates, 21 percent were resistant to sulfadiazine. Group B was the predominant serogroup.

The closed environment did not appear to influence the carrier rate or create a selective advantage for any one serogroup. It may influence the development of resistant organisms positively.

It is concluded that *N. meningitidis* carrier rate in seasoned military personnel is 26 percent. The three factors of crowding, recruit seasoning, and the presence of chronic carriers are discussed.

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# A LONGITUDINAL STUDY OF NEISSERIA MENINGITIDIS CARRIERS IN SUBMARINE CREWS

## INTRODUCTION

Studies of meningococcal meningitis and the causative organism, *Neisseria meningitidis*, have been numerous during epidemics and sporadic between them.<sup>1</sup> Consequently, the literature on *N. meningitidis* is abundant but difficult to integrate, and several questions concerning its antigenicity, pathogenicity, and epidemiology remain unanswered.<sup>2</sup> The discovery of sulfonamide-resistant meningococci in 1963<sup>3</sup> and the outbreak of several meningococcal epidemics at military recruit stations together yielded a renewed interest in research pertaining to this organism. Several extensive epidemiological studies on recruit populations<sup>4,5,6</sup> and civilian groups<sup>7,8,9</sup> have since been published; however, their findings were not definitive. It has been found that five percent of all cases in the United States occur in seasoned military personnel and their dependents,<sup>10,11</sup> but only limited information is available on the carrier rate and epidemiology of *N. meningitidis* in these two groups.<sup>12</sup> Thus, further investigation in this population would seem productive.

In December of 1966, a two-year prospective longitudinal study of the *N. meningitidis* carrier rate of the crew members aboard the Polaris submarine, USS MARIANO G. VALLEJO (SSBN-658), was begun. The submarine population presented a unique opportunity for epidemiological study as the crew members rotated between the open environment of the community while on off-crew status and the closed of the submarine while on patrol.

There are four investigative aspects to the total study. The first pertains to the *N. meningitidis* carrier states of the crew members prior to and just after their patrol. Further data including serogrouping and sulfonamide sensitivity testing were obtained at those times. Miniaturized bacteriological study techniques and equipment, previously developed aboard other Polaris submarines,<sup>13,14,15</sup> were utilized in an attempt to monitor the meningococcus carrier rate during patrol.

These techniques and their use constitute a second area of interest. The third is related to immunoassay methods currently undergoing development.<sup>16</sup> Serum specimens were collected and preserved for future analysis of group-specific meningococcal antibody titers which will utilize these methods. A correlation will be made at that time between the carrier state and immunity status of individuals over the two-year period. In the final component of the study, the families of a dozen crew members will be surveyed to evaluate the interrelationships between the carrier statuses of seasoned personnel and their families.

The primary purpose of this preliminary report is to summarize the carrier studies conducted from December 1966 through December 1967 on the Blue and Gold crews of the VALLEJO which constitute the first part of the investigation outlined above.

## METHODS AND MATERIALS

### Population

The population consisted of both Blue and Gold crews of the Polaris submarine, USS MARIANO G. VALLEJO (SSBN-658). There were approximately 135 men on each crew. All were considered seasoned personnel, as each had had at least six months active duty prior to reporting to the submarine. Individuals were transferred from the submarine intermittently; however, approximately 200 of the original 270 men were with the submarine the entire 13 months of the study. Replacements were incorporated into the study when they reported for duty. Men transferred from the submarine were not followed.

### Crews' Operational Schedules

The VALLEJO, as do other Polaris vessels, has two complete crews called the Blue and the Gold which alternate duty every three months. The on-crew spends three consecutive months aboard the submarine. One month is spent in port moored alongside a tender in Guam in preparation for patrol and the next two months on patrol continuously

submerged. The off-crew during the same three-month period undergoes refresher training at the home port in Hawaii. During this time the off-crew lives with their families or in barracks. Refer to Table I for a graphic description of the typical crew rotation.

even in view of the irregular schedule. Table II indicates the *N. meningitidis* nasopharyngeal carrier surveys and serum collections done for the thirteen-month period. The Blue crew was surveyed a total of six times with three serum collections; the Gold crew seven

TABLE I. TYPICAL ROTATION AND LOCATION OF CREWS

	OFF-CREW	ON-CREW
FIRST MONTH	Hawaii—Leave Live with family or in barracks Exposure to other people in community	Guam—Refit Preparation of submarine for patrol Live on submarine Exposed to other personnel in Guam Anchored alongside submarine tender
SECOND MONTH	Hawaii—Refresher training (Same as first month)	Patrol—Live on submarine Continuously submerged Closed environment No exposure to people other than crew
THIRD MONTH	Hawaii—Preparation to leave for Guam (Same as first month)	Patrol—(Same as second month)

\*Blue and Gold crews alternate every three months.

\*\*Both crews are in contact for a 5-day changeover period in Guam. However, the off-going crew lives on the submarine while the on-coming crew lives on the Submarine Tender.

### N. Meningitidis Carrier Surveys And Serum Collections

As the VALLEJO was a newly constructed submarine it conducted pre-deployment operations in 1966 and the first six months of 1967. The location and timing of the surveys varied during these months from the typical operational schedule and is shown in Table II. The results of these surveys can thus be analyzed in respect to environmental factors

times with three serum collections. In addition both crews were surveyed at biweekly intervals during their first patrol for a total of three for the Blue crew and four for the Gold.

TABLE II. N. MENINGITIDIS CARRIER SURVEYS AND SERUM COLLECTIONS  
DECEMBER 1966 THROUGH DECEMBER 1967

DATE	BLUE	GOLD	LOCATION	REMARKS
Dec '66	*NP **S	NP, S	Mare Island, California	Submarine recently constructed. Crews working together. No prolonged submerged periods. Personnel had lived at home or barracks in Mare Island area for several months.
Apr '67	—	NP	Hawaii	Gold crew had arrived in Hawaii 2 weeks earlier following a 2-week submerged transit from the eastern coast of the United States.
Jul '67	NP	NP	Hawaii	Gold crew had arrived in Hawaii 1 week earlier following a 2-week submerged transit from the western coast of the United States. Blue crew had arrived in Hawaii 1 month earlier with their families from Mare Island, California.
Aug— Sep '67	NPx3	—	Patrol	Blue crew surveyed every 2 weeks while on patrol for a total of 3 surveys. Closed environment.
Oct '67	NP, S	—	Guam	Blue crew surveyed and sera collected shortly after returning from patrol.
Sep '67	—	NPx3 Sx1	Hawaii	Gold crew surveyed each of the last 3 weeks of off-crew period in Hawaii and sera collected once. Crew had been living in community for 3 months.
Nov— Dec '67	—	NPx4	Patrol	Gold crew surveyed every 2 weeks while on patrol for a total of 4 surveys. Closed environment.
Dec '67	—	NP, S	Guam	Gold crew surveyed and sera collected shortly after returning from patrol.
Dec '67	NPx3	—	Hawaii	Blue crew surveyed each of last 3 weeks of off-crew period in Hawaii and sera collected once. Crew had been living in community for 3 months.

\*NP—Nasopharyngeal survey for *N. meningitidis* carriers.

\*\*S —Serum collection (5 cc.)

In general the survey protocol, since September 1967, was as follows: (1) Hawaii—Crew surveyed for *N. meningitidis* once a week during the last three weeks of the off-crew cycle and a serum specimen collected the next to the last week. (Personnel had been in Hawaii for three months at the time of these surveys.) (2) Patrol—Crew surveyed biweekly preserving isolates for definitive identification upon return from patrol. (Crew were confined to closed environment of submarine.) In addition crew surveyed

the day before return from patrol. These isolates processed as those obtained ashore. (3) Guam—Crew surveyed and sera collected shortly after returning from patrol.

The typical survey and serum collection schedule to be followed throughout 1968 is illustrated in Table III. During this year each crew will make two patrols. Thus, by December 1968, carrier data will be available covering a two-year period with each crew having made three complete patrol-off-crew cycles.

TABLE III. SURVEY AND SERUM COLLECTION SCHEDULE FOR 1968

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
<b>Blue Crew</b>												
Duty	Refit	Patrol	Patrol	Crew Turn-over	Training	Training	Refit	Patrol	Patrol	Crew Turn-over	Training	Training
Location	Guam	—	—	Guam (Hi.)	Hi.	Hi.	Guam	—	—	Guam (Hi.)	Hi.	Hi.
NP*		AA	AC	B		BBB		AA	AC	B		BBB
S**				B		B				B		B
<b>Gold Crew</b>												
Duty	Leave	Training	Training	Refit	Patrol	Patrol	Crew Turn-over	Training	Training	Refit	Patrol	Patrol
Location	Hi.	Hi.	Hi.	Guam	—	—	Guam (Hi.)	Hi.	Hi.	Guam	—	—
NP			BBB		AA	AC	B		BBB		AA	AC
S			B				B		B			

\*NP—Nasopharyngeal survey for *N. meningitidis* carriers.

\*\*S —Serum collection (5 cc.).

A —Performed on patrol by medical officer.

B —Performed ashore by medical officer and PMU6.

C —Performed on patrol by medical officer on day before patrol's termination.

### Methods Used On Patrol

A. Preparation of culture media: A VCN (BBL)<sup>17</sup> inhibited Mueller-Hinton (Difco) media was used. To 100 ml. of Mueller-Hinton<sup>18</sup> media was added 1.0 ml. of VCN inhibitor. The solution was agitated and then poured into 15 space-saving culture plates. These were stored at room temperature and used within 24 hours.

B. Nasopharyngeal swab and culture techniques: The nasopharyngeal swabs were prepared by breaking a cotton tipped applicator about one inch from the end, taping it at a 30° angle and autoclaving it for 15 minutes. The nasopharynx was vigorously swabbed. This was facilitated by asking the subject to breathe through the mouth, thus elevating the soft palate and depressing the tongue. The swab was rolled over one quadrant of a VCN-Mueller-Hinton culture plate. Five streaks were made through the inoculated area flaming and cooling the loop be-

tween each streaking. The plates were incubated in an inverted position in a candle jar at 36°C for 18-24 hours.

C. Candle jar: The incubator aboard the submarine measures 18x24x18 inches and has an inner second glass door. The plates were placed in the incubator along with some moist gauze sponges and a lighted candle. The inner door was closed and taped creating a sealed compartment.

D. Transfer and preservation of isolates: Suspect *N. meningitidis* colonies were identified according to gross colony morphology. Three colonies were inoculated into one dram vial chocolate agar mini-slants. Care was taken to touch only the top of the selected colony and to flame the the loop between inoculations. The three inoculated mini-slants were incubated at 36°C with their caps loose in the candle jar for 18-24 hours. Their caps were then tightened. Two were

placed in a Revco field portable deep freeze at  $-50^{\circ}\text{C}$  and the remaining one in the ship's deep-freeze at  $-5^{\circ}\text{C}$ .

E. Shipping: Upon return from patrol all mini-slants were packed in dry ice and shipped to the Naval Biological Laboratory (NBL), Oakland, California, for positive identification, serogrouping and sulfonamide sensitivity testing.

#### Methods Used Ashore

A. Preparation of culture media: Primary isolation of meningococci was accomplished on a selective medium prepared from Mueller-Hinton agar (Difco); to each liter of agar was added 10 ml. of rehydrated antibiotic (Antimicrobial Vials, Difco) containing 10,000 mcgm. of Ristocetin and 25,000 units of Polymixin B. The final concentration of antibiotic in the plated medium was 10 mcgm Ristocetin and 25 units of Polymixin B per ml. The antibiotic solution was added after the basal medium had been autoclaved and cooled to  $50^{\circ}\text{C}$ .

B. Nasopharyngeal swab and culture techniques.

C. Candle jar: Methods for both B and C were essentially the same as those employed while on patrol.

D. Isolation and identification of suspected cultures: Preventive Medicine Unit No. 6 (PMU6) processed the specimens obtained ashore and those from the survey done the last day of patrol. NBL processed the specimens obtained from the first three surveys done on patrol. The methods described below were used by both laboratories.

Suspect cultures were plated on chocolate agar containing 2.6 percent Blood Agar Base (Difco), 1.0 percent Todd-Hewitt Broth (Difco), 1.0 percent Hemoglobin (BBL), and 1.0 percent Bacto Agar (Difco). These plates were then incubated in a candle jar for 24 hours at  $36^{\circ}\text{C}$ .

Confirmation parameters were gram stain, oxidase activity, and carbohydrate fermentation (dextrose, maltose and sucrose). Fermentation studies were conducted in tubes containing Cysteine-Tryptic Agar (Difco), and 1.0 percent carbohydrate under serobic conditions at  $36^{\circ}\text{C}$  for 24 hours.

E. Serogrouping: Meningococcal serogroups were determined by the slide agglutination method. Only 18-24 hour culture grown on the medium described in D were used for grouping. The antisera used were groups A, B, C, D, and Poly (Contains A, B, C, D) (Difco). Especially prepared groups E and F furnished by N. A. Vedres<sup>16</sup> were also used.

Confirmed and grouped, meningococcal isolates were preserved by lyophilization or freezing at  $-70^{\circ}\text{C}$  in Trypticase-Soy Broth (Difco) containing 30 percent alpha-gamma-horse serum.

F. Sulfonamide sensitivity testing: Sensitivity studies were conducted on Mueller-Hinton agar culture containing the following concentrations of sulfadiazine (Sodium Sulfadiazine, Vitarine Co.): 0.1 mg percent, 0.5 mg percent, 1.0 mg percent, 5.0 mg percent.

Growth, as described above in D was suspended in sterile vials containing 1.5 ml saline. A 3 mm loopful was drawn out and streaked across a plate from the periphery to the center for a distance of approximately 1.5 cm. After 18-24 hours incubation under candle jar at  $36^{\circ}\text{C}$ , the plates were interpreted. Sensitivity was designated as the lowest concentration of sulfadiazine which resulted in total inhibition of growth.

G. Obtaining serum specimens: Ten cc of venous blood was taken via venopuncture, spun down and the sera frozen.

## RESULTS

### Ashore Surveys

Tables IV and V present the results of the 13 surveys completed ashore. Table VI shows that the six Blue crew surveys included a total of 181 men who were cultured 634 times out of a possible 827 giving a 77 percent culture rate. Corresponding values for the Gold crew were 161 men cultured 853 times out of 956 possible for an 89 percent culture rate. Thus 342 men were cultured 1,487 times out of 1,783 possible for an overall culture rate of 83 percent and an overall carrier rate of 26 percent.

TABLE IV. ISOLATES COLLECTED FROM BLUE CREW DURING  
1966-67 SURVEYS

Date	Location	No. of crew sampled	Total Positive	Percent of total	B	E	C	A	SA	X	NG	Unspecified
Dec 66	Mare Island, California	133* (139)	49** (12)	37%	27 (4)	15 (7)	2	1 (1)	3			1
Jul 67	Hawaii (pre-patrol)	113 (141)	30 (6)	27%	6 (1)		3 (2)		6	4 (1)	2 (1)	Poly 2 (1) Other 7
Oct 67	Guam (post-patrol)	116 (141)	31 (6)	27%	8 (2)		10 (1)	6 (1)	4 (1)		3 (1)	
Dec 6, 67	Hawaii	110 (136)	34 (2)	31%	14 (1)		8 (1)	1	3	8		
Dec 13, 67	Hawaii	94 (135)	18 (7)	19%	4 (2)		8 (4)		1	5 (1)		
Dec 20, 67	Hawaii (pre-patrol)	68 (135)	20 (4)	29%	4 (1)		3 (1)		2 (1)	10 (1)	1	
Totals		634 (827)	182 (37)		63 (11)	15 (7)	34 (9)	8 (2)	19 (2)	27 (3)	6 (2)	10 (1)

KEY: \* —No. in ( ) indicates no. of crew at time of survey in the first column only.

\*\* —No. in ( ) indicates no. of resistant organisms in all other columns.

B, C, A —Agglutinated with group specific antisera A, B, or C (Difco).

E, F —Agglutinated with group specific antisera E or F (N. A. Vedros).<sup>16</sup>

SA —Agglutinated with saline.

X —Agglutinated with more than one group specific antisera.

Poly —Agglutinated with antisera containing all major antibodies A, B, C, D (Difco).

NG —Did not agglutinate with any group specific or poly antisera.

TABLE V. ISOLATES COLLECTED FROM GOLD CREW DURING  
1966-67 SURVEYS

Date	Location	No. sampled	Total positive	Percent of total	B	E	C	A	SA	X	NG	Unspec.
Dec 66	Mare Island, California	128* (141)	42** (10)	33%	25 (6)	12 (4)	2		2			1
Apr 67	Hawaii	125 (140)	38 (6)	30%	16 (4)	1 (1)	5		10	4 (1)	2	
Jul 67	Hawaii	120 (132)	31 (1)	27%	15 (1)		1		8			7
Sept 5, 67	Hawaii	110 (136)	20	18%	11		1		4	3	1	
Sept 13, 67	Hawaii	112 (137)	11 (4)	10%	4		2 (2)		1	3 (2)	1	
Sept 18, 67	Hawaii (pre-patrol)	124 (136)	26 (5)	21%	16 (2)		4 (3)	2	4			
Dec 67	Guam (post-patrol)	134 (134)	32 (17)	25%	13 (10)		4 (3)	1 (1)	12 (2)		1 (1)	1
Totals		853 (956)	200 (43)		100 (23)	13 (5)	19 (8)	3 (1)	41 (2)	10 (3)	5 (1)	9

KEY: Refer to Table IV key.

Analysis of only those subjects positive at least once for *N. meningitidis* altered Table VI figures considerably as shown in Table VII. For example the 155 subjects proven to be carriers at least once were cultured 715 times out of a possible 822 for a culture rate of 87 percent. The total 382 positive isolates from this group gave a 53 percent carrier rate.

TABLE VI. OVERALL CULTURE RATE AND CARRIER RATES

	Blue Crew	Gold Crew	Total
Total subjects cultured	181	161	342
Total cultures done	634	853	1,487
Total cultures possible	827	9 <sup>6</sup>	1,783
Percent culture rate	77%	89%	85%
Total isolates	182	200	382
Percent positive	28%	24%	26%

TABLE VII. POSITIVE SUBJECTS' RATE AND CARRIER RATES

	Blue Crew	Gold Crew	Total
Total subjects cultured	181	161	342
Total subjects always negative	101	86	187
Total subjects positive	80	75	155
Total cultures done	317	398	715
Total possible cultures	380	442	822
Percent culture rate	88%	88%	87%
Total isolates	182	200	382
Percent positive	57%	50%	53%

The cooperation of crew members was voluntary. This plus the absence of some men due to leave and training resulted in a culture rate less than 100 percent.

Group B was the predominant serogroup, except in December 1967, in Hawaii, when the Blue crew recorded only four group B's and eight group C's (Table IV). Group E

was prominent in the December 1966 survey at Mare Island but has not been isolated since then.

In this study, as in most involving serogrouping of *N. meningitidis*, difficulties were encountered.<sup>10</sup> Table VIII shows that a total of 127 isolates out of 382 or 31 percent, were not serogroupable.

Resistant isolates were found in all serogroups including group A. There was little difference between crews in the total number of resistant organisms isolated during the year. Table IX shows that 21 percent of all isolates were resistant. Resistance was defined as any growth in 1.0 mg percent or greater sulfadiazine.

TABLE VIII. UNGROUPABLE ISOLATES

	Blue Crew	Gold Crew	Total
Total isolates ungroupable	62	65	127
Total isolates	182	200	382
Percent ungroupable	34%	33%	31%

TABLE IX. RESISTANT ISOLATES

	Blue Crew	Gold Crew	Total
Total resistant isolates	37	43	80
Total isolates	182	200	382
Percent resistant	20%	21%	21%

Individual Carriers

Tables X and XI summarize the culture histories for all 155 subjects positive at least once for *N. meningitidis*. Of all subject cultured four or more times only 13 were carriers every time (illustrated in Table XII). During the seven Gold crew surveys only two subjects were positive all seven times and for the six surveys of the Blue crew only two were positive for all surveys. The two Gold crew subjects were always positive for group B, except once, when a saline agglutinable meningococcus was present. The two Blue crew subjects displayed no consistency in serogroups. The remaining nine subjects also failed to show any consistency. Overall, serogroup B was the predominant organism.

TABLE X. INDIVIDUAL CARRIER HISTORY - BLUE CREW

Dec 66	Jul 67	Oct 67	Dec 67	Dec 67	Dec 67	Jul 67	Oct 67	Dec 67	Dec 67	Dec 67
1	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—
5	—	—	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	—	—
7	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—	—	—
13	—	—	—	—	—	—	—	—	—	—
14	—	—	—	—	—	—	—	—	—	—
15	—	—	—	—	—	—	—	—	—	—
16	—	—	—	—	—	—	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—
20	—	—	—	—	—	—	—	—	—	—
21	—	—	—	—	—	—	—	—	—	—
22	—	—	—	—	—	—	—	—	—	—
23	—	—	—	—	—	—	—	—	—	—
24	—	—	—	—	—	—	—	—	—	—
25	—	—	—	—	—	—	—	—	—	—
26	—	—	—	—	—	—	—	—	—	—
27	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—
29	—	—	—	—	—	—	—	—	—	—
30	—	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—

KEY: Refer to Table IV key  
 T - Transferred from Valdez.  
 (Mark) - Not assayed in Valdez at time of survey  
 NC - Not cultured.  
 — - No data  
 (R) - Resistant isolate.  
 T - Unperfected isolate.

TABLE XI. INDIVIDUAL CARRIER HISTORY - GOLD CREW

Dec 66	Apr 67	Jul 67	Sept 67	Sept 67	Dec 67	Dec 67	Apr 67	Jul 67	Sept 67	Sept 67	Sept 67	Dec 67
1	—	—	—	—	—	—	—	—	—	—	—	—
2	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—
4	—	—	—	—	—	—	—	—	—	—	—	—
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17	—	—	—	—	—	—	—	—	—	—	—	—
18	—	—	—	—	—	—	—	—	—	—	—	—
19	—	—	—	—	—	—	—	—	—	—	—	—
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25	—	—	—	—	—	—	—	—	—	—	—	—
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27	—	—	—	—	—	—	—	—	—	—	—	—
28	—	—	—	—	—	—	—	—	—	—	—	—
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30	—	—	—	—	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	—	—	—	—	—
32	—	—	—	—	—	—	—	—	—	—	—	—
33	—	—	—	—	—	—	—	—	—	—	—	—
34	—	—	—	—	—	—	—	—	—	—	—	—
35	—	—	—	—	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	—	—	—	—	—
37	—	—	—	—	—	—	—	—	—	—	—	—
38	—	—	—	—	—	—	—	—	—	—	—	—
39	—	—	—	—	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—	—	—

KEY: Refer to Table X Key

TABLE XII. SUBJECTS CONSISTENTLY POSITIVE FOR 4 OR MORE CULTURES

							Times Positive
<b>Gold Crew</b>							
Subject 3	B	B	B	B	B	B	SA 7
Subject 13	B	SA	B	B	B	B	B 7
Subject 15	B	B	B	B	NC	NC	B(R) 5
Subject 27	B	NC	SA	X	NC	SA	SA 5
Subject 71			B	B	B	B	B(R) 5
<b>Blue Crew</b>							
Subject 21		B	B	B	B	X	X 6
Subject 55		B	SA	C	C	X	X 6
Subject 9		B(R)	B(R)	C	X	SA	NC 5
Subject 20		E(R)	SA	C	NC	C	X 5
Subject 26			B(R)	NG(R)	X	C	C 5
Subject 48		B	X	A	SA	C	NC 5
Subject 38		E(R)	NC	C	SA	X	NG 4
Subject 51		B	C	C	C	NC	NC 4

From the total 59 isolates eight resistant strains were present for an average of 14 percent.

**Comparison of Pre and Post Patrol Surveys**

The comparison of pre- and post-patrol surveys are taken from Tables IV and V. The Blue crew had 30 isolates, including six resistant strains, and a 27 percent carrier rate at pre-patrol survey in July 1967; it had 31 isolates with six resistant strains and a 27 percent carrier rate at post patrol in October 1967. Corresponding values for the Gold crew were 26 isolates, including five resistant strains and a 21 percent carrier rate at pre-patrol in September 1967; 32 isolates, 17 resistant and a 25 percent carrier rate at post-patrol in December 1967.

TABLE XIII. ISOLATES FROM BLUE CREW DURING FIRST PATROL

Date	Location & time	No. cultured	Total Positive	Percent Positive	B	E	C	A	SA	X	NG	Unspec
Aug 67	On patrol 2nd week	128 of 135	23	18%	11(4)	5(3)	1		2(1)			4(1)
Sept 67	On patrol 4th week	110 of 135	24	21%	10(2)	3(3)	2		6(1)			3(1)

**Results of Surveys During Patrol**

Technical problems precluded conducting definitive surveys during either the Blue or Gold crews' first patrols. A logistic difficulty resulting in the absence of the Revco field, portable deep-freeze during the Blue crew patrol prevented storing isolates at -50°C as required in the protocol. Three surveys were done during patrol, however, and preserved in the ship's deep-freeze at -5°C. The results of the first two are shown in Table XIII. The third survey isolates were

grossly contaminated. All three surveys done during the Gold crew's first patrol were contaminated.

**DISCUSSIONS AND CONCLUSIONS**

This study was unique in several ways when compared to other epidemiological research on *N. meningitidis*. Some of these unique aspects served to eliminate or minimize commonly encountered variables. (1) Two different crews were surveyed separately, except for one joint survey, 13 times following exposure to similar environmental conditions; thus each acted as the other's control. (2) Surveys were done randomly over a year's time thus minimizing seasonal variation of carrier rates. (3) Surveys were done at three widely separated geographical areas, thereby eliminating the variation due to location.

Based upon the results of this study the average carrier rate of seasoned military personnel is 26 percent. Mueller and Aycock reported a similar figure in 1944 from a study done at two Army installations.<sup>20</sup> The only recent study, PMU6, in 1965, reported an average carrier rate of 36 percent in seasoned personnel stationed at five military installations throughout the Pacific.<sup>12</sup>

In this report, 155 of the 342 subjects surveyed were positive at least once. The cumu-

lative carrier rate was 45 percent. This figure is consistent with Silverthorne's 41 percent reported in studies done on civilians in Toronto, Canada.<sup>24</sup> The 155 subjects of the VALLEJO study had an average carrier rate of 53 percent, i.e., over one-half of these subjects were positive at any one survey.

Analysis of the data pertaining to subjects who had four or more cultures reveals some interesting facts. Of this group 28 percent were never carriers of *N. meningitidis*. Only four percent were positive every time.

The remaining 68 percent were intermittent carriers. Approximately 100 subjects were not surveyed four times; however, it is probable they would have fallen into these three groups in the same proportions. (The criterion of four surveys was selected arbitrarily.) Therefore, in seasoned personnel one can expect to find 4 percent chronic carriers, 68 percent intermittent carriers, and 28 percent always negative.

The civilian carrier rate, although difficult to ascertain, is variously reported from 2-38 percent,<sup>7,9,21,22</sup> and is generally estimated at 2-8 percent.<sup>19</sup> Military recruits have a much higher carrier rate during their first weeks of training with values ranging up to 60-90 percent.<sup>4,5,6,23</sup> The recruit carrier rate decreases after about three months presumably to the 26 percent level reported here. The carrier pattern is low in civilians, high in recruits, and somewhere in between in seasoned military personnel.

Knowing the precise causes of this pattern is not within current capabilities and this will continue to be so until sophisticated immunoassay methods are developed. Then correlations between carrier states and group specific antibody titers to the four serogroups of *N. meningitidis* can be made.<sup>16</sup> However, it is interesting to analyze the pattern utilizing the results of this study in conjunction with others previously reported.

Greenfield has shown that there exist pockets of high infectivity, called tribes, in the civilian population which have carrier rates of 44 percent.<sup>7</sup> People in contact with tribe members, called first contacts, have a lower carrier rate (20 percent) than the tribe, but higher than the general population (3 percent). Second contacts, those who associate with the first contacts and not the tribe members, have carrier rates similar to the general population. Greenfield did not conduct longitudinal studies on these tribe members so the exact nature of the individual carrier state over a period of time is not known. The cause of the tribe's high carrier rate as compared to that of the overall population is unknown; however, data from this study suggests the existence of chronic carriers as the source of the higher tribal carrier rate.

It is probable that most people who have never lived in a crowded environment, or who have not lived in more than one locale, have never developed an immunity to all strains of *N. meningitidis*. It is thought that the mixing of such susceptibles with carriers drawn from many locales in a crowded environment harboring several strains results in the high carrier rates in recruits.<sup>25</sup> Feldman supports this thesis by stating the cumulative carrier rate in recruits as 100 percent. A few recruits appear to have inadequate immunological mechanisms and acquire meningococcal meningitis. However, the majority acquire immunity to the various strains of the organism and the recruit carrier rate drops presumably to that of the seasoned personnel (26 percent).

Thus three factors appear to influence the carrier rate in seasoned personnel. These are: (1) relatively crowded environment, (2) recruit seasoning process, and (3) presence of chronic carriers.

The influence of the first is illustrated in Bristow's report 3-12 percent carrier rate in the permanent personnel at the San Diego Naval recruit training program camp in 1963, during a meningococcal epidemic.<sup>5</sup> This group's environment was not crowded, as it was similar to that of the civilian, and is presumably the basis of the low carrier rate. It is possible that the number of chronic carriers increases during recruit camp as a result of a subject's exposure to several strains. Another possibility is that the intermittent carriers among seasoned personnel are repeatedly reinfected in the crowded environment and only appear to be chronic carriers. The evidence in this study tends to support this latter view in that its chronic carriers did not harbor the same strain during each survey.

The seasoning process is also important. Without this concept there is no explanation for the decrease in recruit carrier rate during training camp. Furthermore, there is little environmental difference between the civilian "tribe" (carrier rate of 44 percent) and seasoned personnel (carrier rate 26 percent). It

appears that the seasoning process is an influence contributing to the lower carrier rate in seasoned personnel.

Support of the hypothesis that carrier rates (civilian, recruit, seasoned personnel) are determined by the presence of chronic carriers is found in the results of the two surveys completed on patrol, and the four pre- and post-patrol surveys. In all six surveys, the carrier rate was about 26 percent. During the two months of patrol there was no opportunity for the introduction of new strains of *N. meningitidis* into the environment. Had all individuals had the capacity to develop immunity to the strains, the expected carrier rate at end of patrol might have been zero. The consistent 20 percent figure can only mean the continued presence of chronic and intermittent carriers.

Other conclusions can be made from this study. Group B is the predominant serogroup among seasoned personnel. This is consistent with other studies which show group B to be predominant during interepidemic periods.<sup>2,19,20</sup> No serogroup appears to have a selective advantage in the closed environment, inasmuch as the percentage of serogroups was the same pre-and post-patrol. Finally, the number of ungroupable organisms is fairly constant at about 30 percent. This is consistent with past reports.<sup>10</sup>

No reason is known for the isolation of serogroups E and F only during the survey in December of 1966 at Mare Island. It has been shown recently that other serogroups of *N. meningitidis* may exist.<sup>9</sup> This is undergoing further study.<sup>16</sup>

Of the isolated organisms 21 percent were resistant to 1.0 mg percent sulfadiazine. This is lower than the 33 percent figure for civilian isolates reported by Feldman.<sup>25</sup> More significantly no serogroup had a consistent disproportionate number of resistant isolates. However, over 50 percent of the Gold crew's isolates were resistant after patrol. This was the highest value of resistant organisms throughout the year and indicates a possible selectivity for resistant strains in the closed environment. The Blue crew post-patrol survey does not coincide with the Gold crew sur-

vey. A firmer hypothesis must await data from further study.

Three resistant serogroup A isolates were discovered. Prior to this study resistant group A strains had been reported only in Africa.<sup>26</sup> Further studies are being conducted on these isolates.

Individual intermittent and chronic carriers displayed a certain consistency in serogroups when the isolate was groupable. However, most isolates from individuals were intermittently ungroupable as they were either cross-reactors, not groupable with any antisera, or saline agglutinable. Of more significance is the fact that resistant organisms did not persist in any individual carriers.

#### SUMMARY

A total of 342 men from both the Blue and Gold crews of the Polaris submarine, USS MARIANO G. VALLEJO (SSBN-658), were surveyed periodically over a 13-month period for *N. meningitidis*. The overall carrier rate was 26 percent with four percent chronic carriers, 23 percent always negative and 68 percent intermittent carriers. Of all isolates, 21 percent were resistant to sulfadiazine. Group B was the predominant serogroup.

The closed environment does not appear to influence the carrier rate or create a selective advantage for any one serogroup. It may influence the development of resistant organisms positively.

It is concluded that the *N. meningitidis* carrier rate in seasoned military personnel is 26 percent. The three factors of crowding, recruit seasoning, and presence of chronic carriers are discussed.

#### REFERENCES

1. Branham, S. E., A defense of Epimetheus. *J. Amer. Med. Women's Assoc.*, 15:571, 1960.
2. Vedros, N. A., Hunter, D. H., and J. H. Rust, Studies on immunity in meningococcal meningitis. *Mil. Med.*, 1413-17, 1966.
3. Millar, J. W., Siess, E. E., Feldman, H. A., Silverman, C., and P. Frank, In vivo and in vitro resistance to sulfadiazine in strains of *Neisseria meningitidis*. *J.A.M.A.* 186:139-141, 1963.
4. Farrell, D. G. and E. V. Dahl, Preliminary communication: nasopharyngeal carriers of *Neisseria meningitidis*: Studies among air force recruits. *J.A.M.A.*, 198:1189-92, 1966.

5. Bristow, W. M., Van Peenen, P. F. D., and R. Volk, Epidemic meningitis in naval recruits. *Am. J. Pub. Health.* 55:1039-45, 1965.
6. Gauld, J. R., Nitz, R. E., Hunter, D. H., Rust, J. H., and R. L. Gauld, Epidemiology of meningococcal meningitis at Fort Ord. *Am. J. Epid.*, 85:56-72, 1965.
7. Greenfield, S. and H. A. Feldman, Familial carriers and meningococcal meningitis. *New Eng. J. Med.*, 277:497-502, 1967.
8. Reed, D., Brody, J., Huntley, B., and T. Overfield, Epidemic in Eskimo village due to Group-B meningococcus, Part I: Epidemiology. *J.A.M.A.*, 196:383-387, 1966.
9. Slaterus, K. W., Ruys, C. A., and I. G. Sieberg, Types of meningococci isolated from carriers and patients in non-epidemic period in Netherlands. *Antonie van Leeuwenhoek*, 29:265-71, 1963.
10. Morbidity and Mortality, Atlanta, Ga., US Dept. of HEW, Communicable Disease Center, Vol. 16, 1967.
11. Pelczar, M. J., Jr., et al., Round table on biology of the meningococcus and meningococcal meningitis. Washington, D.C., Navy Department, Office of Naval Research, May 6, 1964, p. 18.
12. Preventive Medicine Activities (RCSMED 6200-1), Semi-annual Period ending December 31, 1965, Honolulu, Hawaii, US Navy Preventive Medicine Unit No. 6, January 14, 1966, Appendix 1, pp. 1-12.
13. Watkins, H. M. S., et al., 38th, 39th and 40th Technical Progress Reports. Naval Biological Laboratory, Oakland, California, 1965-1966.
14. Watkins, H. M. S., Personal communication. Naval Biological Laboratory, Oakland, California, 1967.
15. Marlor, R., Personal communication. Medical Officer, Submarine Squadron I, Pearl Harbor, Hawaii, 1967.
16. Vedros, N. A., Personal communication. Naval Biological Laboratory, Oakland, California, 1967.
17. Thayer, J. D. and J. E. Martin, Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Publ. H. Rep.*, 81:559-62, 1966.
18. Difco Laboratories, Bacto Mueller Hinton Medium, 0252-01, Detroit, Michigan.
19. Maxcy, K. F., The relationship of meningococcus carriers to the incidence of cerebrospinal fever. *Am. J. Med. Sci.*, 193:438-445, 1937.
20. Aycock, W. L. and J. H. Mueller, Meningococcus carrier rates and meningitis incidence. *Bact. Rev.*, 14:115-60, 1950.
21. Silverthorne, N., Presence of meningococcus in nasopharynx of normal individuals and bactericidal property of blood against meningococcus. *J. Pediat.*, 9:328-330, 1936.
22. Rake, G., Studies on meningococcal infection, VI, Carrier Problem. *J. Exper. Med.*, 59:553-576, 1934.
23. Singer, R. C., Sulfonamide-resistant meningococcal disease. *Med. Cl. N. A.*, 51:719-27, 1967.
24. Silverthorne, N., Fitzgerald, J. G., and D. T. Fraser, Studies on meningococcus and meningococcus infection. *J. Pediat.*, 15:491-502, 1939.
25. Feldman, H. C., Recent developments in therapy and control of meningococcal infections. *Dis.-a-Mon.*, February, 1966, pp. 5-31.
26. Feldman, H. A., Sulfonamide-resistant meningococci. *Ann. Rev. Med.*, 18:495-506, 1967.

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