

This is a comprehensive review of the occurrence of serogroups and resistance to sulfonamides of meningococci among civilians in the United States for the period 1964-1970. The data are correlated with clinical sources of isolation and the geographical area from which the isolates were obtained, with emphasis on changing trends in serogroup prevalence and/or sulfadiazine susceptibility.

# Prevalence of Serogroups and Sulfonamide Resistance of Meningococci from the Civilian Population in the United States, 1964-1970

## Introduction

Branham reviewed the serological relationship among meningococci in 1953,<sup>1</sup> and in 1958 she suggested reference neotype strains for groups A, B, C, and D.<sup>2</sup> More recently several groups of workers<sup>3-8</sup> have described additional serogroups. Some of these new groups appear to be identical, although the designations used by different workers are not the same. Devine and Hagerman<sup>9</sup> summarized the nomenclature used by various investigators and discussed the relationships of a nongroupable strain of *N. meningitidis* (RAS-10) to the serological groups.

Feldman<sup>10</sup> reviewed the history of sulfonamide-resistant meningococci. As he noted, even though resistant strains had been reported earlier, a great deal of interest in them was not aroused until 1963 when Millar et al.<sup>11</sup> reported that attempts to reduce the meningococcal carrier rates significantly with sulfadiazine prophylaxis failed at the San Diego Naval Training Center.

This laboratory began surveillance of sulfadiazine susceptibility of *Neisseria meningitidis* strains in 1964.

Serogrouping and sulfadiazine susceptibility data on strains of meningococci isolated from the civilian population from 1964 through 1970 are presented. These data are correlated with the clinical source of isolation and the geographical area from which the isolates were submitted, with particular emphasis on changing trends in serogroup prevalence and/or sulfadiazine susceptibility.

## Materials and Methods

**Strains**—The clinical sources of meningococcal strains and the geographical areas from which they came are in Table I. Most of these strains had been identified by the state health department laboratories which submitted them to this laboratory for bacteriological confirmation, serogrouping, and antimicrobial susceptibility tests or only for the latter. The categories of clinical source are "blood or

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spinal fluid," including strains from blood, spinal fluid, brain and heart; "pharynx," including strains from pharyngeal or nasopharyngeal areas; "sputum"; "other-known," including 22 strains from the eyes, 17 from bronchial washings or lungs, 12 from genito-urinary areas, 7 from skin or petechia, 9 from joints, 6 from the rectum or feces, and 1 from the ear; and "other-not known," including strains submitted without information on the clinical source.

**Identification and Serogrouping**—Approximately half of these strains received from 1964 through 1970 were studied biochemically and serologically as previously described.<sup>12</sup> Most of the remaining strains were checked by a Gram stain, oxidase reaction, and serological grouping with *N. meningitidis* antisera. The antisera used for groups A, B, C, D, and Slaterus' X, Y, and Z were prepared by the Biological Reagents Section, Center for Disease Control (CDC). Antiserum to Slaterus' Z' strain and ungroupable strain A4317 were prepared as previously described.<sup>6</sup> Those strains which reacted specifically in one antiserum were listed as within the group represented by that serum; strains which reacted in either Z or Z' antisera were listed as group Z; if a strain reacted in two or more antisera, it was put in the cross-reaction category; if there was spontaneous agglutination in saline, the strain was placed in the autoagglutination category; if there was no reaction in any of the antisera used, the strain was listed as smooth.

**Antimicrobial testing**—Tests from 1964 through August 1970 were performed by a plate dilution method.<sup>12,13</sup> Inoculum was prepared by suspending 18-hr. growth from a Heart Infusion Agar slant containing 5% rabbit blood in distilled water or Mueller-Hinton Broth. The inoculum, approximately equal in density to a McFarland tube-standard No. 5, was streaked with a 1 mm loop onto plates con-

taining a series of antimicrobial concentrations in Mueller-Hinton Agar (MHA). A modified procedure was used to test strains received after August 1970. This procedure employed inoculum from a 4- to 5-hr. Mueller-Hinton Broth, adjusted to a density equal to 0.5 of a McFarland tube-standard No. 1. The organisms were inoculated onto the antimicrobial plates by a Steers-Foltz replicator (Thornsberry and Baker, personal communication).

## Results

Table 2 shows a summary of all the isolates from civilians studied from 1964 through 1970. The isolates are listed by clinical source, serogroup, and susceptibility to sul-

fadiazine. Group B isolates were the most numerous in each clinical source category. Group C isolates were next, except in the sputum category. Of the cultures examined, Group B strains were predominant with 58.2%, Group C was next with 25%, Group Y accounted for 3.8%, and the per cents of other serogroups ranged from <0.1 to 1.3. The serogroup with the largest per cent resistant to 1 mg/100 ml sulfadiazine was Group C (62.3%); Group B (39.5%) was next. The per cents of other serogroups resistant ranged from 0 to 25. The per cent of resistant strains from blood and spinal fluid (45.8%) was greater than the per cent from pharynx (25.8%), sputum (6.2%), or other-known sources (33.8%). Of all the strains studied, 41.1% were resistant to 1 mg/100 ml of sulfadiazine.

**Table 1—Geographical and Clinical Sources of Isolates from the Civilian Population 1964-1970**

Geographical division	Geographical area of U.S.	Blood or spinal fluid	Clinical Source			Total isolates
			Pharynx	Other known and sputum	Other not known	
Eastern	Northeast	158	70	20	22	270
	Middle Atlantic	143	21	10	40	214
	South Atlantic	452	152	44	60	708
North Central	East North Central	183	15	23	47	268
	West North Central	120	52	7	7	186
South Central	East South Central	111	17	9	8	145
	West South Central	270	43	8	39	360
Western	Mountain	49	22	15	30	116
	Pacific	774	97	51	79	1001
Total isolates		2260	489	187	332	3268

**Table 2—Summary of Civilian Isolates from 1964-1970 by Clinical Source, Serogroup, and Susceptibility to Sulfadiazine**

Clinical Source	Number of isolates/serogroup												Total
	A	B	C	D	X	Y	Z	A4317	Cross	Auto agglut.	Smooth	Not tested	
Blood or spinal fluid	15	1426	631	0	3	76	3	11	16	6	11	62	2260
Pharynx	1	229	76	2	10	24	15	2	26	65	14	25	489
Sputum	0	44	10	0	10	12	18	0	4	11	2	2	113
Others—known	0	37	17	0	1	3	5	0	1	6	0	4	74
Others—not known	0	166	84	0	0	9	0	3	7	7	2	54	332
Total isolates	16	1902	818	2	24	124	41	16	54	95	29	147	3268
Per cent	.5	58.2	25.0	<.1	.7	3.8	1.3	.5	1.6	2.9	.8	4.5	
Clinical source	Number of isolates resistant to 1 mg/100 ml sulfadiazine												Total
	A	B	C	D	X	Y	Z	A4317	Cross	Auto agglut.	Smooth	Not tested	
Blood or spinal fluid	0	595	402	0	0	9	1	2	6	1	1	18	1035
Pharynx	0	60	48	0	1	6	0	1	1	4	2	3	126
Sputum	0	4	1	0	0	1	0	0	0	1	0	0	7
Others—known	0	14	8	0	0	0	2	0	0	0	0	1	25
Others—not known	0	79	51	0	0	0	0	1	2	1	0	17	151
Total resistant isolates	0	752	510	0	1	16	3	4	9	7	3	39	1344
Per cent resistant	0	39.5	62.3	0	4.2	12.9	7.3	25.0	16.7	7.4	10.3	26.5	41.1

The per cent of cultures that could not be grouped because of cross-reactions or autoagglutination was greater for the pharynx (18.6%), sputum (13.2%), and other-known sources (9.4%) than for the blood or spinal fluid isolates (1.0%). (Table 3) Similarly, the Group X, Y, and Z strains and those reacting in serum A4317 were encountered more frequently in the pharynx (10.4%), sputum (35.4%), and other-known sources (12.2%) than from blood and spinal fluid (4.1%). (Table 4)

In Table 5 the total numbers of strains isolated from blood or spinal fluid are listed by year isolated and the per cent in each serological group. The total numbers of meningococcal cases (including civilian and military) reported to the Center for Disease Control<sup>14,15</sup> for each year are also shown. The military cases accounted for from 6% to 13%

of the total.<sup>15,19</sup> In our study, Group B strains were isolated most often (63.0%), next were Group C (27.9%), then Group Y (3.4%); other serogroups accounted for <0.1 to 0.7% of the isolates. Group B was the predominant serogroup from 1964 through 1968; Group C predominated over Group B in 1969 and 1970. There was no marked trend in any other serogroup; however, the percentages in Group Y remained rather constant from 1967 through 1970, varying from 4.4% to 5.5%.

Figure 1 shows the trend of Group B and C isolates from blood and spinal fluid (meningococcal cases), when the incidences are compared graphically by the per cent of the total strains studied from 1964 through 1970.

Table 6 gives the per cents of serogroup B and C strains isolated from blood or spinal fluid sources by geo-

**Table 3—Isolates, from Various Clinical Sources, Which Cross-Agglutinated or Autoagglutinated**

Clinical source	Total isolates	Cross-reacting		Autoagglutinating		Total % isolates cross-reacting or autoagglutinating
		No.	%	No.	%	
Blood or spinal fluid	2260	16	0.7	6	0.3	1.0
Pharynx	489	26	5.3	65	13.3	18.6
Sputum	113	4	3.5	11	9.7	13.2
Other—known	74	1	1.3	6	8.1	9.4
Total	2936	47	1.6	88	3.0	4.6

**Table 4—Meningococcal Isolates which Grouped as X, Y, Z or A4317 by Their Clinical Sources**

Clinical source	Total isolates	Number grouped X, Y, Z or A4317	
		Number grouped X, Y, Z or A4317	Per cent grouped X, Y, Z or A4317
Blood or spinal fluid	2260	93	4.1
Pharynx	489	51	10.4
Sputum	113	40	35.4
Other—known	74	9	12.2
Total	2936	193	6.6

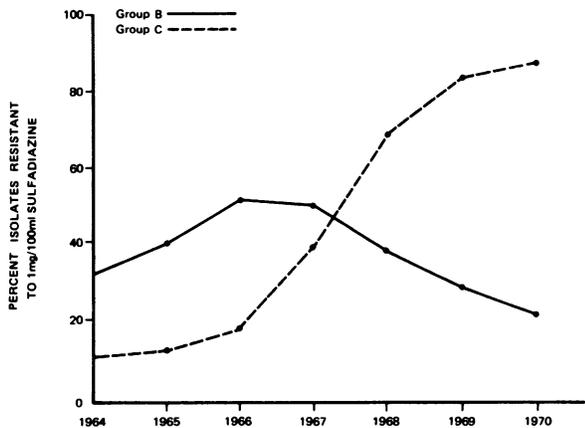
**Table 5—Per cent Meningococcal Isolates from Blood or Spinal Fluid by Year Isolated and Serogroup**

Year	Total cases reported	Serogroup											Total isolates	
		A	B	C	D	X	Y	Z	A4317	Cross	Auto-agglut. Smooth	Not tested		
1964	2826	0	78.1	17.2	0	0	0	0	0	0	0	0	4.6	151
1965	3040	1.7	75.4	11.0	0	.2	.2	0	.2	.2	.2	1.2	9.3	407
1966	3381	.4	80.3	11.0	0	0	2.5	0	.2	1.6	.4	1.2	2.0	479
1967	2161	.9	66.4	22.6	0	.6	5.5	.3	1.8	.3	0	0	1.2	323
1968	2623	0	56.6	36.2	0	0	5.1	0	.6	.3	.3	0	.6	309
1969	2951	0	42.7	50.6	0	0	4.4	.3	0	1.2	.3	0	.3	316
1970	2483	1.0	33.0	58.9	0	0	5.4	.3	.3	.3	.3	0	.3	275
Total number of isolates		15	1426	631	0	3	76	3	11	16	6	11	62	2260
Per cent		.7	63.0	27.9	0	.1	3.4	.1	.5	.7	.3	.5	2.7	99.9

**Figure 1—Per cent Group B and C Isolates from Meningococcal Cases 1964-1970**



**Figure 2—Per cent Group B and C Isolates Resistant to 1 mg/100 ml Sulfadiazine from Meningococcal Cases 1964-1970**



graphical division and year of isolation. From 1964 through 1967, Group B strains accounted for 63.4% to 90.0% of the strains from all the areas. In 1967, the per cent of Group B isolates from all the areas declined. The per cent of Group C isolates was less than 26% in all areas until 1968 when the per cent in the Eastern division markedly

increased. By 1969 over 47% of the isolates from all the areas were Group C, and in 1970 Group C strains still were predominant in all the areas except the North Central, which had 42.8% Group C and 45.7% Group B.

In Figure 2, the per cent of strains of Groups B and C from blood or spinal fluid which were resistant to 1 mg/100 ml sulfadiazine are shown. From 1966-1967,  $\geq 50\%$  of Group B strains were resistant. In 1968 this per cent began to decline, and by 1970 only 21% of the Group B strains were resistant. The resistance of Group C strains increased steadily from 1964 and 1965 with 8 and 9% to 1970 when 89% of the strains were resistant.

Figure 3 shows the per cent of Group B and C strains from blood or spinal fluid that were resistant to 1 mg/100 ml sulfadiazine from different geographical divisions by the years isolated. Group B strains were resistant in a range of 40-60% in all the divisions in 1966 and 1967, and by 1969 the per cent of resistant Group B strains from all the areas declined. The per cent of Group C strains resistant to sulfadiazine was greater than 50% in all the divisions between 1967 and 1968, and by 1969 the per cent was over 70%, and in 1970, over 85% in all the divisions.

### Discussion

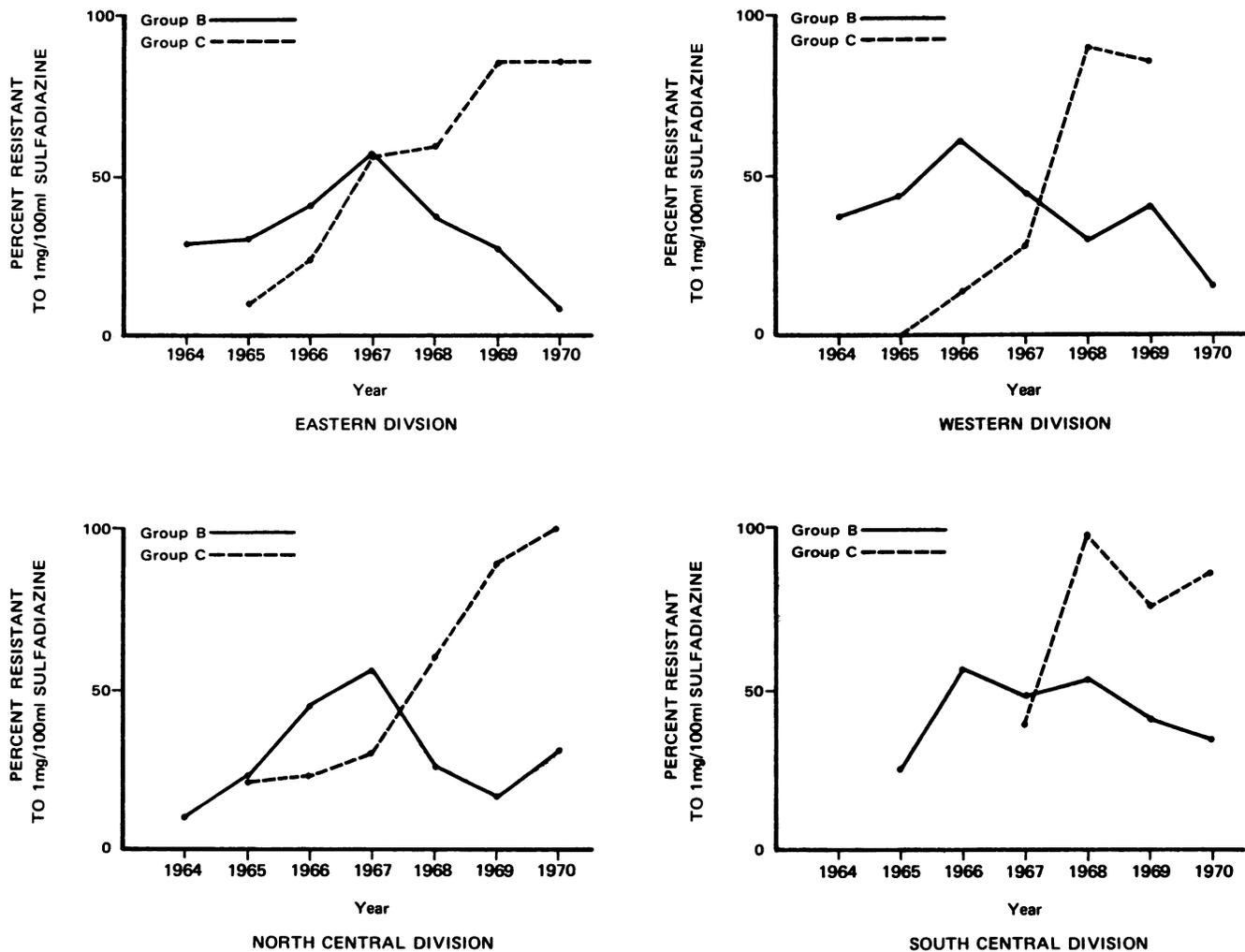
Branham reported in 1956<sup>20</sup> that during all the epidemic periods in the United States where grouping records were available, 95-96% of the strains from cases were Group A, whereas Group B isolates were responsible for most of the cases in nonepidemic years. She reported that Group A carriers were frequently found during epidemics; however, Group B strains were usually found in chronic carriers during epidemic periods, and in carriers during interepidemic periods. Groups B and C strains were responsible for sporadic cases, and Group D strains were rarely encountered. Since 1956 the outbreaks which have occurred in this country have been due to Group B and, more recently, to Group C organisms.

The isolates from blood or spinal fluid which are included in this report represent 10.7% to 14.9% of the number of meningococcal cases reported to the Center for Disease Control<sup>14,15</sup> for the years 1965 through 1970. The per cent for 1964 was 5.3%. Of the total reported cases in 1965 through 1970, approximately 87-94% were civilian.<sup>15-19</sup> Serological grouping data on these strains showed

**Table 6—Per cent Group B and C Isolates from Blood or Spinal Fluid by Geographical Division 1964-1970**

Year	Serogroup B Geographical division				Serogroup C Geographical division			
	Eastern	Western	North Central	South Central	Eastern	Western	North Central	South Central
1964	88.8	82.4	83.3	-	11.1	17.6	16.6	-
1965	74.5	90.0	69.2	80.0	22.3	6.8	15.4	20.0
1966	79.7	82.8	81.8	86.5	9.8	12.9	13.6	2.7
1967	63.4	68.1	72.7	68.9	25.8	22.9	22.7	17.8
1968	49.1	58.9	58.0	67.0	45.9	25.6	36.0	28.7
1969	43.6	37.7	34.6	48.7	49.3	51.1	59.1	47.5
1970	25.7	41.8	45.7	34.8	68.9	44.1	42.8	56.0

**Figure 3—Per cent Group B and C Isolates Resistant to 1 mg/100 ml Sulfadiazine from Meningococcal Cases by Geographical Divisions**



that Group A strains represented less than 1% of the total, only 15 strains being received during the test period. Group B strains were the predominant strains from 1964 through 1968 (56.6% to 80.3%), and for the years 1969 and 1970, this group waned to 42.7% and 33.0%. Group C strains accounted for less than 25% of the strains received from 1964 through 1967; in 1968, this per cent increased to 36.2% and in 1969 and 1970, Group C strains were the predominant serogroup from meningococcal cases.

Workers do not use the same designations for the new serological groups. Slaterus<sup>3</sup> described three groups which he called types X, Y, and Z. Bories et al.<sup>5</sup> confirmed that strains of X and Y were *N. meningitidis* and suggested that, after confirmation by other workers, these provisional groups be called E and F. In 1968, Hollis et al.<sup>6</sup> found that a significant number of the ungroupable smooth strains in this country were agglutinated by antisera prepared from Slaterus' X, Y and Z strains and proposed that these strains should more appropriately be called Groups E, F, and G. During the same year, Evans et al.<sup>7</sup> reported three new serogroups called "Boshard," 29E, and 135 and stated that the "Boshard" group was the same as Slaterus' Group Y. Also in 1968, Vedros et al.<sup>8</sup> described a new serological group which included a "Bo" strain and called this Group

E. Slaterus,<sup>4</sup> after his original publication, described a strain which did not group as X, Y, or Z; however, when antiserum was produced from this strain, the Z strain would react in this antiserum. He called this strain Z'. Devine and Hagerman<sup>9</sup> reported that Evans' 29E strain was related or identical to the Z' strain. Because of the confusion caused by this different nomenclature, we have continued to use the designation Y for strains of the Y or Boshard group, and for strains that react with either, or both Z and Z' antisera, we have used the designation Z. Evans' strain 135 appears to be related to the strains in this report which are designated as A4317 (unpublished data), and both of these strains are agglutinated by some high-titered Y antisera. Since our studies of these relationships are not complete, we have continued to use the designation A4317.

Artenstein et al. (Bull. Wld. Hlth. Org., *in press*) recently reported on the Army's experience with meningococcal isolates from 1964 through May 15, 1970. Their results with Group B and C isolates from case sources are compared with those from this study in Table 7. The same trend in prevalence occurred in the military group as in the civilian group; however, the change from Group B to C strains was more marked and occurred sooner in the military group. Reports from other countries indicate that the

serological trends seen in this country are not the same as those encountered elsewhere. Group A strains have been reported to be the cause of epidemics in Africa.<sup>21,22</sup> Severin et al.<sup>23</sup> studied strains from the Netherlands isolated between 1958 and 1968 and reported that Group B strains by far prevailed (67.7% to 85.5%). They found 6.5% of the strains from patients were Group A. Abbott et al.<sup>24</sup> reported on strains isolated from patients in the United Kingdom. Although 50% of the strains were ungroupable, 11.4% were Group A and 36.4% were Group B.

None of the isolates from cases were grouped as D in this study. Four per cent of the strains were grouped as Slaterus X, Y, Z or as A4317. Only three blood or spinal fluid strains were grouped as X and three, as Z, whereas 76 and 11 were grouped as Y and A4317, respectively. One per cent of the strains were ungroupable because of autoagglutination or cross-reactions. Artenstein et al. (Bull. Wld. Hlth. Org., *in press*) reported that 3% of the strains from Army cases were due to Groups Y, 29E, or 135, and only 0.2% of the strains were nongroupable.

Since the strains studied by different workers may be serotypes within the same broad groups, it is timely that a committee, such as has been formed,<sup>25</sup> carry out comparative studies on these strains to establish a uniform grouping system.

In this study the strains from the pharynx probably are not as representative of the population as those from meningococcal cases; however, several observations about these strains seem worthwhile. Two Group D strains, which are apparently very rarely isolated, were found from pharynx sources. The per cent of strains grouped within the new serological groups was greater than the per cent of strains from case sources. Others,<sup>26,27</sup> Artenstein et al. (Bull. Wld. Hlth. Org., *in press*) have observed that the new serogroups are encountered in carrier surveys in this country and that Y is frequently isolated from this source and in some surveys has been the predominant strain isolated.

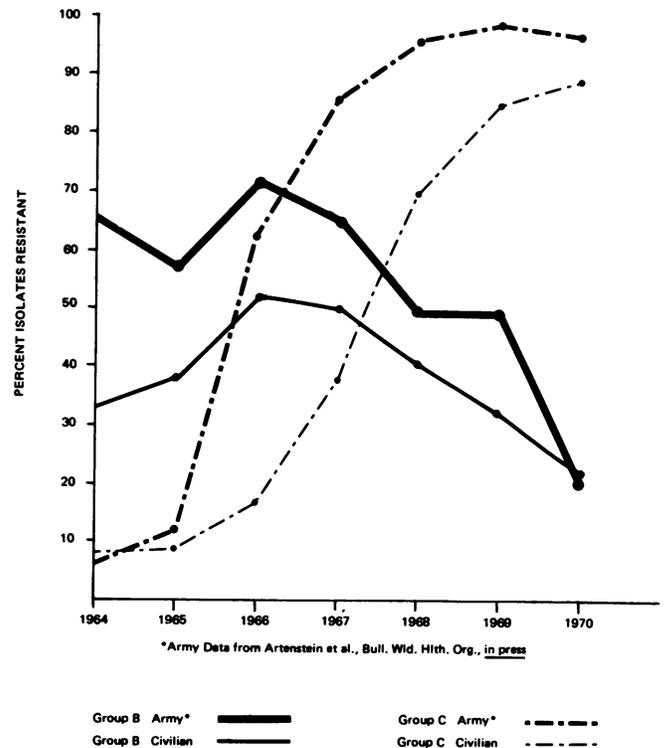
Although strains of Group A which are sulfadiazine resistant have been reported in other countries,<sup>21,22</sup> no Group A strains were found in this study which were resistant to 1 mg/100 ml. The per cent of Group B strains resistant peaked in 1966 with greater than 50% resistant, and the per cent resistant declined to 21% in 1970. Group C strains increased in the per cent resistant to sulfadiazine as the prevalence of these strains increased. In 1970, 89% of the

**Table 7—Prevalence of Group B and C Isolates from Army\* and Civilian Cases, 1964-1970**

Year Isolated	% Serogroup B		% Serogroup C	
	Army	Civilian	Army	Civilian
1964	86.4	78.1	9.7	17.2
1965	84.5	75.4	12.3	11.0
1966	80.4	80.3	14.2	11.0
1967	35.8	66.4	54.7	22.6
1968	11.4	56.6	84.6	36.2
1969	10.4	42.7	87.3	50.6
1970	3.2	33.0	96.2	58.9

\* Army data from Artenstein et al. (Bull. Wld. Hlth. Org., *in press*) 1964 through May 15, 1970.

**Figure 4—Per cent Group B and C Isolates from Army Meningococcal Cases Resistant to 0.1 mg/100 ml Sulfadiazine Compared to Per cent Group B and C Isolates from Civilian Meningococcal Cases Resistant to 1.0 mg/100 ml Sulfadiazine**



Group C strains were resistant. Although the results of sulfadiazine resistance in the study by Artenstein et al. of Group B and C strains from Army cases are based on minimal inhibitory concentrations (MIC) of 0.1 mg/100 ml and those in this study are based on MIC values of 1.0 mg/100 ml, there is a parallel between the results of the two groups.

In the other serogroups, the per cent of strains resistant to 1 mg/100 ml sulfadiazine ranged from 2.4 to 25%.

When the geographical sources of the strains were examined, the strains received from all the areas showed the same trend toward predominance of Group C meningococci. This trend was preceded by an increase in the sulfadiazine resistance of Group C strains and a decline in the per cent of resistant Group B strains.

### Summary

From 1964 through 1970, serogrouping and sulfadiazine susceptibility tests were performed on over 3,000 meningococcal isolates from civilians residing in all geographical areas of the United States. Group A strains accounted for less than 1% of the isolates from blood or spinal fluid (meningococcal cases), and all were found to be sensitive to sulfadiazine (minimal inhibitory concentration < 1.0 mg/100 ml). Group B strains were the most frequent isolates from cases in 1964 through 1968, ranging from 56.6 to 80.3% of the strains received. During this period, 33 to 52% of the Group B strains were sulfadiazine resist-

ant. In 1969 and 1970, the per cent of Group B strains declined to 42.7 and 33.0, respectively. This decline was accompanied by a decline in sulfadiazine resistance (32% in 1969, 21% in 1970). Group C isolates in 1964 through 1967 ranged from 11% to 22.6% of the total isolates from cases, but by 1969 they were the predominant serogroup submitted to this laboratory. This increase in number was accompanied by a corresponding increase in sulfadiazine resistance. In 1966, only 17% of the Group C isolates from cases were resistant, but by 1970, 89% were resistant. When case isolates were separated by the year isolated and geographical origin, the same trends in Group B and C prevalence and sulfadiazine susceptibility were seen in all the divisions. No Group D strains were found from cases; however, two were isolated from pharyngeal sources. Strains of serogroups X, Y, and Z were isolated from 3.6% of the cases and were found frequently from other sources. The per cent of sulfadiazine resistant isolates of these groups from cases ranged from 4.2% to 25%. Only 1% of isolates from cases were not groupable because of either cross-reactions or autoagglutination, whereas 9.4% to 18.6% of isolates from other sources were ungroupable because of these reactions.

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