INFECTION AND IMMUNITY, Mar. 1970, p. 226-231
Vol. 1, No. 3

Relationship of Serogroups of *Neisseria meningitidis*

I. Microagglutination, Gel Diffusion, and Slide Agglutination Studies of Meningococcal Antisera Before and After Absorption with RAS-10 Strain of Meningococci

LEONARD F. DEVINE AND CLINE R. HAGERMAN

Bacteriology Division, Naval Medical Research Unit No. 4, Great Lakes, Illinois

Received for publication 17 October 1969

Microagglutination tests were used to show the relationship of a nongroupable strain of *Neisseria meningitidis* (RAS-10) to other serological groups. RAS-10 antiserum has been prepared and studied for the first time. Antibodies to the RAS-10 strain were shown to be present in many grouping antisera obtained from different sources. These antibodies were absorbed from antisera to heterologous serogroups with the RAS-10 strain. This procedure was shown to make antisera more specific by eliminating serological cross-reactions and false grouping of RAS-10 strains. Antisera before and after absorption with RAS-10 cells were studied by using double diffusion in gels. An antigen-antibody precipitation line for the RAS-10 meningococci was shown to be removed by this procedure. Antiserum to group 29E meningococci was absorbed with group Z cells, and precipitation lines for Z cells were removed. Group 29E antiserum agglutinated group 29E and group Z cells in the slide agglutination test but was specific for group 29E cells in this test after absorption with group Z cells.

Many investigators have reported on new serological groups of meningococci since Branham (4) reviewed the serological relationships of the species in 1953. Subsequently, numerous reports have appeared in the literature in which new serological groups have been given different designations by various authors.

An extensive, continuous epidemiological survey for determining nasopharyngeal meningococcal carrier rates at Great Lakes was begun in March 1967. Hundreds of cultures that have been identified as meningococci by cultural, morphological, and biochemical characteristics were found to belong to these newly described serological groups or were agglutinated in more than one antisera to serological groups A, B, C, and Bo. Many investigators have reported these cross-agglutinating strains (1, 2, 4, 8, 9, 15, 17, 18).

The following is a résumé of recent reports on meningococcal serogroups. Jyssum described a new serological group N in 1956 (10). In 1958, Branham (5) studied approximately 2,200 strains of *Neisseria meningitidis* and selected neotype strains to serological groups A, B, C, and D. Slaterus in 1961 and 1963 (17, 18) reported on three new groups and provisionally labeled them X, Y, and Z. Bacterial cells of these three serogroups and a 4th strain (Z′) were not agglutinated by antisera to meningococci of serological groups A, B, C, and D. However, antisera to Z′ strain agglutinated group Z cells, but Z′ cells were not agglutinated by Z antisera. Hollis, Wiggins, and Schubert (9), in 1968, recommended that X, Y, and Z be designated as groups E, F, and G, respectively, and did not refer to the Z′ strain of Slaterus. Kingsbury, using deoxyribonucleic acid homology studies, suggested that group Z may not be a meningococcus (13). Evans, Artenstein, and Hunter, in 1968 (7), reported on three new groups, Bo, 29E, and 135. The Bo strain was described as being apparently identical with the Y strain of Slaterus.

In 1968, Vedros, Ng, and Culver (19) reported a new serological group of meningococci and proposed that it be designated group E. These strains are now considered to be of the same group as the Y strain of Slaterus (17, 18) and Bo strains of Evans (7).

This study was conducted to identify the serological relationships of the strains isolated in our epidemiological surveys with the new serogroups recently described in the literature. Strains of the new serological groups were obtained, and RAS-10, a representative cross-agglutinating strain, was selected for study. The latter strain was not agglutinated in 0.15 M NaCl; it was agglutinated by many other heterologous meningococcal antisera and was highly antigenic in rabbits.

Slide agglutination tests, a modification of the microagglutination test of Vedros and Hill (20) and Ouchterlony's gel diffusion technique, as described by Björklund (3), were used in this study.

MATERIALS AND METHODS

Strains. Group A strains MK-01 (A_1) and MK-716 (A_2) were received from W. Sanborn, Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, United Arab Republic. Group A strain A-10 (A_3), group 29E strain 112 ($29E_1$), group Z strain 17 (Z_1), and nongroupable RAS-10 strains [RAS-10 (RAS-10_1), 331 (RAS-10_2), and 351 (RAS-10_3)] were each isolated from a carrier at Great Lakes. A group B strain ATCC 13090 (B_1) and a nongroupable strain ATCC 13804 (NG_1) were received from the American Type Culture Collection. The group C strain PTS5 (C_1) was obtained from N. Vedros, NAMRU-1, Berkeley, California. The group B strain Will-B (B_2) and group Bo strain Jor-Bo (Bo_1) were isolated from spinal fluid of a patient at Great Lakes. Strains of groups Bo, 135, 29E, X, and Z are designated as Bo-WR (Bo_2), 135-WR (135_1), 29E-WR ($29E_2$), X-WR (X_1), and Z-WR (Z_2), respectively. These strains were received from Walter Reed Army Institute of Research, Washington, D.C. Strains X_1 and Z_2 were previously obtained by M. Artenstein from K. W. Slaterus (17, 18). Gonococcal strain P-948 (GC_1) was received from Communicable Disease Center (CDC), Atlanta, Georgia.

Description of antisera. The antisera used in microagglutination tests were prepared against meningococcal strains shown in Tables 1 and 2. Antisera used in gel diffusion tests were prepared from the following strains: RAS-10_2 in rabbit 632, RAS-10_3 in rabbit 629, C_1 in rabbit 543, and $29E_2$ in rabbit 555. The last two antisera were also used in the microagglutination tests.

Preparation of antigens for microagglutination tests. Cells were prepared as described by Vedros and Hill (20), except that Mueller Hinton Broth (Difco) was used as a growth medium.

Preparation of antigen; for gel-diffusion tests and in situ absorptions. Mueller Hinton Agar plates were heavily seeded with meningococci and grown 16 to 18 hr in an 8% carbon dioxide atmosphere. The cells on each plate were harvested in 1 ml of 0.15 M NaCl in phosphate buffer (PBS; *p*H 7.2).

Preparation of antigens for absorption in test tube. Antigens were prepared for absorption in test tubes by the same preparation as for gel diffusion tests, except that the cells were centrifuged at 600 × *g* for 15 min prior to use.

Rabbit antisera preparation. Antisera was prepared by the method of Edwards and Devine (6).

Absorption in test tubes. A volume of packed cells equal to 15% of the volume of the antisera was used for absorptions. The absorptions were carried out at

TABLE 1. *Titer[a] of normal pooled rabbit serum and RAS-10_1 antiserum for bacterial cells used in microagglutination tests*

Strain	Serogroup	Normal rabbit serum	RAS-10_1 antiserum
A_1	A	<4	4
B_2	B	<4	4
C_1	C	<4	32
Bo_2	Bo	<4	4
135_1	135	<4	32
$29E_2$	29E	<4	<4
X_1	X	16	128
RAS-10_1	Nongroupable	8	2,048

[a] Reciprocal of dilution.

TABLE 2. *Titers[a] of antisera to various serogroups to homologous bacterial cells and the RAS-10 cells before and after absorption with RAS-10 cells*

Serogroup	Strains used to		Unabsorbed sera titer		Absorbed sera titer	
	Produce antisera	Titrate antisera	RAS-10	Homologous	RAS-10	Homologous
A	A_3	A_1	1,024	64	<4	64
B	B_1	B_2	512	128	4	64
C	C_1	C_1	512	2,048	4	512
Bo	Bo_1	Bo_2	1,024	1,024	<4	128
29E	$29E_1$	$29E_2$	1,024	256	<4	128
135	135_1	135_1	2,048	1,024	4	128
A[b]	M952, M1027, M1894[c]	A_1	32	1,024	ND[d]	ND
B[b]	M997, M2091, M2092[c]	B_2	512	128	ND	ND
C[b]	M1166, M1054, M1628[c]	C_1	512	256	ND	ND
29E	$29E_2$	$29E_2$	32	256	ND	ND

[a] Reciprocal of dilution.
[b] Prepared at CDC, Atlanta, Ga.: group A, CDC lot 3 (1/7/64); group B, CDC lot 6 (2/23/68); group C, CDC lot 4 (4/27/67).
[c] NIH strain numbers.
[d] Not done.

56 C for 30 min and then at 37 C for 2 hr. Antisera of low specificity often required two or three absorptions.

Antisera absorptions were done in situ in some gel diffusion experiments by a modification of the technique of Björklund (3). The routine test was preceded by adding meningococcal cells to the central well and replacing them with more cells after 8 hr. These were removed by suction 16 hr later. Antiserum was added in the central well, and bacterial cells in the peripheral wells.

Microtiter agglutination procedure. A modified microagglutination technique of Vedros and Hill (20) was used. After 30 min of treatment with 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), Formalin was added to make a final concentration of 1.9% formaldehyde. The cells were stored for 24 hr at 4 C, separated by centrifugation, washed once in sterile distilled water, resuspended in 0.19% formaldehyde in water, and stored at 4 C. Prior to use, the formaldehyde was removed from the bacteria by centrifugation, and the cells were resuspended to an optical density of 0.7 (520 nm) in PBS (*p*H 7.2).

The antisera dilutions were made in PBS containing 0.25% pooled normal rabbit sera in test tubes (13 by 100 mm) and added to the microtiter plates before addition of the bacteria.

Considerable difficulty was encountered in the microagglutination test. These tests were successful only when meningococcal cells were morphologically intact, i.e., free of autolysis and free from clumping when observed microscopically. No group Z cells that met these qualifications could be grown. When meningococcal cells satisfied these criteria, occasional spontaneous agglutination in the microagglutination test was prevented by using 0.25% pooled normal rabbit sera in the test. All tests had two controls; a series of doubling dilutions of pooled normal rabbit serum plus meningococcal cells, and doubling dilutions of antiserum to the RAS-10 strain plus heterologous meningococcal cells. The test was considered specific when both controls were free of agglutination above a final dilution of 1 to 4.

Gel-diffusion tests. "Ionager" no. 2 was obtained from Oxoid Division of Oxo, Ltd., London, England. A 1% agar gel with a final concentration of 0.01% Merthiolate and 0.014% sodium borate as preservative was used for double-diffusion tests. A standard microscopic slide (1 by 3 inches) was overlaid with a thin layer of agar and dried, and then overlaid with 2.5 ml of agar. A circular pattern around a central well was used in all immunodiffusion tests (L.K.B. Instruments, Inc., Washington, D.C.). Thick suspensions of meningococcal cells in 0.15 M NaCl in PBS (*p*H 7.2) containing 0.01% Merthiolate were placed in the wells with Pasteur pipettes. Precipitation lines were observed at 24 and 48 hr.

Slide agglutination tests. A drop of 0.15 M NaCl in PBS (*p*H 7.2) containing 0.5% phenol was placed on a microscope slide. Cells were added and mixed thoroughly with a bacteriological loop to make a homogeneous, turbid suspension. A loopful of antisera was added and mixed, and agglutination was observed within 30 sec at 10× magnification with a dissecting microscope. It is important that the dissecting microscope be used to assure that the original suspension is homogeneous and also to distinguish between autoagglutination and the fine agglutination characteristic of RAS-10. True autoagglutination is irreversible. However, an apparent autoagglutination may occur either before or after addition of the antisera, and can be distinguished from RAS-10 agglutination by its reversibility upon stirring. Generally, recognized groups of meningococci agglutinate in larger clumps.

RESULTS

Table 1 shows the titers of the pooled normal rabbit serum and the antiserum to the RAS-10 strain for all the meningococcal cells used in the experiments shown in Table 2.

Table 2 shows that all nonabsorbed antisera, except group C antiserum to strain C_1, group A (CDC lot 3), and group $29E_2$, had titers to RAS-10_1 equal to or greater than the cells of their homologous groups. All antisera absorbed with cells of the RAS-10_1 strain reduced the titers of the antisera to 4 or less for RAS-10_1 cells. Homologous titers were reduced by eightfold or less for the homologous meningococci. All antisera shown in Table 2 agglutinated RAS-10_1 and their homologous cells in slide agglutination tests before absorption except group A (CDC lot 3) and group $29E_2$. These two antisera had microagglutination titers of only 1:32 for RAS-10_1 cells. All antisera absorbed with the RAS-10_1 cells strongly agglutinated the homologous strains in slide agglutination tests and failed to agglutinate cells of the RAS-10_1 strain.

Except for agglutination of RAS-10_1 cells, antisera with rare exceptions were group-specific when tested by the slide agglutination method. Antisera were also group-specific by the microagglutination method when the pooled normal rabbit serum and the RAS-10 antiserum failed to agglutinate the cells in dilutions above 1:4. Any increase above this level (in nonspecific agglutination) usually was accompanied by an apparent cross-agglutination between serological groups in microagglutination tests.

Normal rabbit sera neither agglutinated smooth suspensions of groupable meningococci in the microagglutination test nor formed precipitation lines in gel diffusion tests with either meningococci or gonococci. Specific precipitation lines were not found with group B antisera and group B cells. Antisera to the RAS-10 strains consistently produced a characteristic precipitation line to the homologous cells near the antigen well (Fig. 1). However, heterologous antisera that strongly agglutinated RAS-10 cells in the slide agglutination test usually formed a similar or identical line

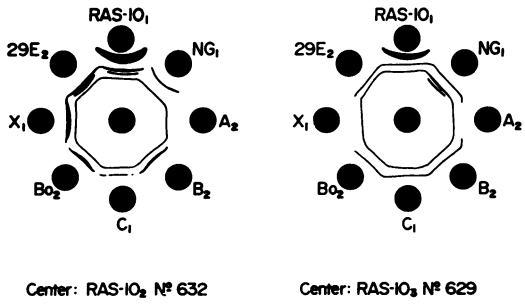

FIG. 1. *Double-immunodiffusion plates showing reactions between eight strains of N. meningitidis and antisera to RAS-10₂ and RAS-10₃. Characteristic precipitin lines for RAS-10 occur near the RAS-10₁ antigen well. Precipitation lines common to other N. meningitidis strains are also present.*

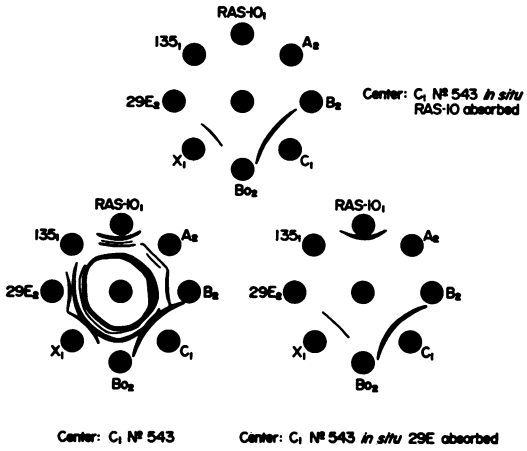

FIG. 2. *Double-immunodiffusion showing reactions between eight strains of N. meningitidis and antiserum to group C₁. Left, unabsorbed group C₁ antiserum showing a group-specific line, RAS-10 line, and common precipitation lines. Middle, RAS-10₁ cells added to the center well prior to performing the test prevented nearly all the nonspecific and the RAS-10 lines from forming. The group-specific line remains. Right, 29E₁ cells added to the center well prior to performing the test prevented nearly all the nonspecific lines from forming. The RAS-10 and C precipitation lines remain.*

in the same relative position next to the antigen well (Fig. 2, left).

Antiserum to C_1 with a homologous titer of 1:2048 and a RAS-10_1 titer of 1:512 (Table 2) showed a homologous and a RAS-10_1 precipitation line in the gel diffusion test (Fig. 3, left). The same antiserum absorbed with intact RAS-10_1 cells had titers reduced to 1:512 and 1:4, respectively, for homologous and RAS-10 cells (Table 2). This antiserum, absorbed with intact RAS-10_1 cells, was tested again in gels against the same antigens (Fig. 3, right). The antiserum still formed a homologous precipitation line and two nonspecific precipitation lines but failed to form the RAS-10 line.

When C_1 antiserum containing RAS-10 antibody was absorbed with RAS-10 cells in situ (Fig. 2, center), the RAS-10 cells had an opportunity to autolyze, and the RAS-10 antigen-antibody precipitation line, as well as most other precipitation lines, failed to develop. Nevertheless, the homologous precipitation line developed just as with the unabsorbed serum.

Generally, when groupable strains of meningococci are used to absorb antisera to heterologous groups in situ, only the homologous and RAS-10 precipitation lines develop (illustrated in Fig. 2, right, for absorption of C_1 antisera in situ with $29E_2$).

Group $29E_2$ antiserum with a homologous microagglutination titer of 1:256 (Table 2) formed nonidentical lines of precipitation with a gonococcal strain and with meningococcal cells of groups 29E and Z in gels (Fig. 4, left). The same antiserum absorbed with whole cells of group Z in a test tube neither agglutinated Z cells in the slide agglutination test nor formed lines of precipitation with Z cells (Fig. 4, right). Antisera to group Z did not agglutinate 29E cells in slide agglutination tests.

Meningococcal group-specific antigen-antibody precipitation lines were also found in gel diffusion tests for serogroups A, Bo, X, Z, and 135 (*not shown*).

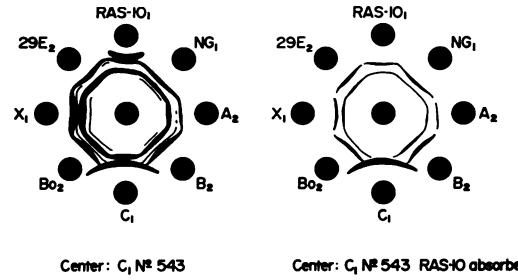

FIG. 3. *Double-immunodiffusion showing the reaction between eight strains of N. meningitidis and antiserum to group C₁. Left, unabsorbed group C₁ antiserum showing a group-specific line to C₁, RAS-10 lines, and common precipitation lines. Right, group C₁ antiserum absorbed in a test tube with intact cells of RAS-10₁. The group C specific line and two common precipitation lines remain. The absence of the RAS-10 precipitation line coincides with the failure of these antisera to agglutinate the RAS-10 cells in slide and microagglutination tests.*

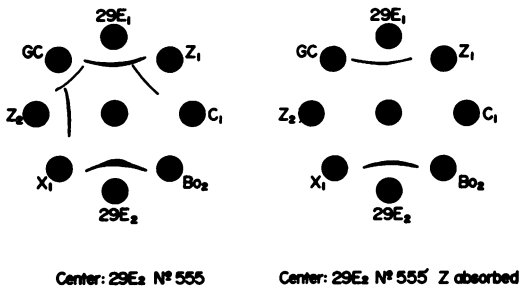

FIG. 4. *Double-immunodiffusion showing the reaction between seven strains of N. meningitidis, one strain of N. gonorrhoeae, and antiserum to group 29E$_2$. Left, unabsorbed 29E$_2$ antiserum showing 29E group-specific precipitation lines and precipitation lines to Z and the gonococcal cells. Right, 29E$_2$ antiserum absorbed in a test tube with intact cells of Z$_2$. Precipitation lines to Z and gonococcal cells are absent while the 29E group-specific line remains. The absence of the precipitation line to Z coincides with the failure of this antisera to agglutinate Z cells by means of slide agglutination.*

DISCUSSION

A method was sought for preparing cell suspensions that would retain group-specificity in microagglutination tests for several days. No consistently reliable method was found; however, on several occasions, representative strains of all serogroups, except Z and D, were grown and treated with formaldehyde, from which smooth nonaggregated cell suspensions were obtained. The test results were considered to be specific when cell suspensions were not agglutinated in titers above 1:4 by anti-RAS-10 serum or normal rabbit serum. However, these cell suspensions deteriorated in a few days, lost their specificity, agglutinated in anti-RAS-10 serum in high titer, and eventually autoagglutinated in normal rabbit serum (illustrated in Table 1 with group X cells).

In other experiments not reported, group X cells, which did not agglutinate with normal rabbit serum and anti-RAS-10 antiserum, have been prepared from time to time. Cells of serogroups C and 135 were agglutinated by RAS-10 antiserum in dilutions of 1:32 in the experiments shown in Table 1 and indicate a moderate degree of nonspecificity of these cells in these particular tests.

The unabsorbed antisera shown in Table 2 which agglutinated RAS-10 cells in high titer also agglutinated RAS-10 cells by slide agglutination and agglutinated deteriorated cells nonspecifically in microagglutination tests. In contrast, specific antisera of groups A and 29E with low anti-RAS-10 titers (1:32) did not agglutinate RAS-10 cells in slide agglutination tests (Table 2).

The cell suspensions of C$_1$ and 135$_1$ which agglutinated in a 1:32 dilution in RAS-10 antisera (Table 1) were agglutinated in their homologous antisera prior to absorption in dilutions of 1:2048 and 1:1024, respectively. After absorption, the homologous titers were reduced by four- and eightfold, respectively (Table 2). The high homologous titer prior to absorption may be more apparent than real, because of a combination of group-specific and non-group-specific antibodies to cells of moderate integrity. There may have been, in fact, little reduction in homologous titer. The inexplicable reduction in the homologous group Bo titer, after absorption, is puzzling. This apparent antigenic relationship is currently under investigation.

Individual lots of grouping antisera prepared at NAMRU-4, at CDC, or procured commercially vary widely in the ability to agglutinate the RAS-10 strain in slide agglutination tests. We have consistently noted that, when pairs of rabbits are immunized with the same antigen preparation at the same time in the same schedule, nonspecific RAS-10 titers of the sera vary widely between rabbits and appear to be independent of the homologous titers.

The extent to which false typing and serological cross-reactions occur depends on the occurrence of RAS-10 antibody in the series of grouping antisera and the number of RAS-10 strains among the strains being typed. The occurrence of RAS-10 strains among carriers is variable and generally unrecognized without the use of RAS-10 antisera. However, little difficulty arises in grouping meningococcal strains isolated from clinical cases, since the RAS-10 strains apparently rarely, if ever, cause clinical meningococcal disease. The nature of the RAS-10 strain is not clear at this time. It may have the internal antigens of heterologous groups as the surface agglutinating antigen and may be a mutant lacking goup-specific terminal polysaccharide units.

The RAS-10 strains may be related to the other serological groups of meningococci as the Ra or

TABLE 3. *Nomenclature used or proposed by various investigators for closely related or serologically identical strains*

Slaterus (17, 18), 1961	Evans, Artenstien, and Hunter (7), 1968	Vedros, Ng, and Culver (19), 1968	Hollis, Wiggins, and Schubert (9), 1968
X			E
Y	Bo	E	F
Z			G
Z′	29E		
	135		

chemotype I strains of *Salmonella* are related to the more complex *Salmonella* chemotypes (12). They may also be the analogous meningococcal counterpart of the transient (T) forms of *Salmonella* described by Kauffmann (11, 12). There is evidence to suggest that meningococcal strains exist which are the counterpart of the two classes of semirough (SR) *Salmonella* strains reported by Naide et al. (16).

Slide agglutination tests, microagglutination tests, and gel diffusion tests indicate that serogroups A, B, and C are legitimate classifications. Nearly all antisera tested in our laboratory by slide agglutination were group-specific when tested against the recognized serological groups, except 29E antisera, which always agglutinated group Z strains. However, group Z antisera did not agglutinate 29E strains. This relationship of 29E and Z is identical with the description of Slaterus (18) of the serological relationship of Z′ to Z. Other proposed serological groups are probably related to each other (Table 3).

LITERATURE CITED

1. American Public Health Association. 1950. Diagnostic procedures and reagents, 3rd ed. American Public Health Association, Inc., New York.
2. Beno, D. W., L. F. Devine, and G. L. Larson. 1968. Identification of *Neisseria meningitidis* carbohydrate fermentation patterns in Mueller-Hinton broth. J. Bacteriol. 96:563.
3. Björklund, B. 1962. Specific inhibition of precipitation as an aid in antigen analysis with gel-diffusion method. Proc. Soc. Exp. Biol. Med. 79:319–324.
4. Branham, S. E. 1953. Serological relationships among meningococci. Bacteriol. Rev. 17:175–188.
5. Branham, S. E. 1958. Reference strains for the serologic groups of meningococcus. Int. Bull. Bacteriol. Nomen. Taxon. 8:1–15.
6. Edwards, E. A., and L. F. Devine. 1968. A genus-specific complement fixation antigen from *Neisseria meningitidis*. Proc. Soc. Exp. Biol. Med. 128:1168–1173.
7. Evans, J. R., M. S. Artenstein, and D. H. Hunter. 1968. Prevalence of meningococcal serogroups and description of three new serogroups. Amer. J. Epidemiol. 87:643–646.
8. Goldschneider, I., E. C. Gotschlich, and M. S. Artenstein. 1969. Human immunity to the meningococcus. II. Development of natural immunity. J. Exp. Med. 129:1327–1348.
9. Hollis, D. G., G. L. Wiggins, and J. H. Schubert. 1968. Serological studies of ungroupable *Neisseria meningitidis*. J. Bacteriol 95:1–4.
10. Jyssum, K. 1956. Serological relationship among meningococcal strains in Norway. J. Immunol. 76:433–440.
11. Kauffmann, F. 1956. A new antigen of *Salmonella paratyphi* B and *Salmonella typhimurium*. Acta Pathol. Microbiol. Scand. 39:299–304.
12. Kauffmann, E. 1961. Die Bakteriologie der *Salmonella* Species. Munksgaard, Copenhagen.
13. Kingsbury, D. T. 1967. Deoxyribonucleic acid homologies among species of genus *Neisseria*. J. Bacteriol. 94:870–874.
14. Lüderitz, O., A. M. Staub, and O. Westphal. 1966. Immunochemistry of O and R antigens of *Salmonella* and related *Enterobacteriaceae*. Bacteriol. Rev. 30:192–256.
15. Miller, C. P., W. G. Beadenkopf, D. Peck, and M. W. Robbins. 1944. A survey of chronic meningococcus carriers in semi-permanent population. J. Infec. Dis. 74:212–224.
16. Naide, T., H. Nikaido, P. H. Mäkelä, R. G. Wilkinson, and B. A. D. Stocker. 1965. Semirough stains of Salmonella. Proc. Nat. Acad. Sci. U.S.A. 53:147–153.
17. Slaterus, K. W. 1961. Serological typing of meningococci by means of micro-precipitation. Antonie van Leeuwenhoek J. Microbiol. Serol. 27:304–315.
18. Slaterus, K. W. 1963. Types of meningococci isolated from carriers and patients in a non-epidemic period in the Netherlands. Antonie van Leeuwenhoek J. Microbiol. Serol. 29:265–271.
19. Vedros, N. A., J. Ng, and G. Culver. 1968. A new serological group (E) of *Neisseria meningitidis*. J. Bacteriol. 95:1300–1304.
20. Vedros, N. A., and P. R. Hill. 1966. Microagglutination technique for *Neisseria meningitidis*. J. Bacteriol. 91:900–901.