HUMAN IMMUNITY TO THE MENINGOCOCCUS

II. Development of Natural Immunity

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Serum from most young adults contains antibodies to pathogenic strains of meningococci (1). The strongest evidence that these antibodies are protective derives from the fact that resistance to meningococcal disease and the presence of antimeningococcal antibodies, as determined by the serum bactericidal reaction, are closely correlated at all ages (1). Of particular importance is the observation (2, 3) that infants in the neonatal period are highly resistant to meningococcal disease, but that they are extremely susceptible by 6 months of age. The curves of increasing susceptibility to systemic meningococcal infection during the first 6 months of life and of the development of physiological hypogammaglobulinemia (4) are reciprocally related.

Results of the preceding study (1) indicate that natural immunization against meningococcal disease occurs during childhood. Thus, between ages 2 and 12 yr there is a progressive increase of approximately 5% per year in the number of children having serum bactericidal activity to strains of pathogenic meningococci. In the present study, it will be shown that immune sensitization occurs, in large part, as a result of the asymptomatic carriage of meningococci in the nasopharynx. Group specific, cross-reactive, and type-specific meningococcal antigens are seen to participate in the immunizing process. It will be further shown that immunity in the newborn infant is associated with the transplacental passage of gamma G antimeningococcal antibodies.

Methods

Bacteriological Techniques.—Bacteriological techniques, serum bactericidal reaction, and indirect immunofluorescence were performed as described previously (1).

Absorption of Serum and Gamma Globulin.—Human sera were obtained from the same sources as described previously (1).

Pooled human gamma globulin, Cohn fraction II, 10 mg/ml (lot No. 2191, E. R. Squibb, Inc., New Brunswick, N.J.), was prepared as before (1). More than 95% of the dry weight of gamma globulin was IgG as determined by quantitative radial immunodiffusion (*vide infra*). IgM and IgA antibodies were not detectable (less than 0.3% by weight).

All absorptions were carried out at 4°C, using chilled reagents and equipment. Hemolytic

complement levels of serum were found to decrease less than 10% under these conditions. Absorptions were performed within 24 hr of the time at which the sera or gamma globulin preparations were to be tested.

Absorption with meningococci: 5 hr cultures of meningococci were removed from the surface of the "chocolate" agar medium with a cotton swab moistened in Dulbecco's phosphate-buffered saline (PBS) (5). The bacteria were washed once in PBS and added to serum in a ratio of one part packed bacteria (45,900 *g* for 15 min) to nine parts serum, as recommended by Landy et al. (6). As a rule, the bacterial growth from a single Petri dish (4 inches in diameter) was used to absorb 2 ml of serum. Absorption was carried out for 2 hr with frequent mixing of the suspension. Bacteria were removed by centrifugation, and the absorbed serum was sterilized by passage through a 0.45 μ Millipore membrane. Repeated absorptions were not found to be necessary.

Absorption of gamma globulin was carried out as for serum.

Absorption with group specific meningococcal polysaccharides: 1 mg of purified, large molecular weight, group A or C meningococcal polysaccharide (7) was adsorbed to 0.9 ml of aluminum hydroxyde gel (0.05 M $AlCl_3$ solution neutralized to pH 6.6 with NaOH). Absorption was allowed to proceed for 30 min at room temperature, by which time all of the polysaccharide had been removed from solution, as confirmed by capillary precipitin tests.

1 ml of serum or pooled gamma globulin was absorbed with 100 μg of meningococcal polysaccharide (0.1 ml of polysaccharide-alum suspension) for 2 hr. The polysaccharide (and alum) was removed by centrifugation at 45,900 *g* for 20 min.

It is important to note that alum per se removed significant amounts of complement activity when added to serum at 4°C, whereas alum coated with meningococcal polysaccharide did not. Furthermore, there was no nonspecific absorption of antimeningococcal antibodies by the polysaccharide-alum complex.

Quantitative Determination of Immunoglobulins.—Levels of immunoglobulins G, M, and A in human sera were determined using the technique of quantitative radial immunodiffusion (8). Agar containing rabbit antibody against human IgG, IgM, or IgA (Partigen Plates, Hoechst Pharmaceutical Co., Kansas City, Mo., manufactured by Behringwerke, Marburg-Lahn, Germany) and pooled human serum containing known amounts of immunoglobulins (standardized and stabilized human serum, Behringwerke; distributed by Certified Blood Donors Service, Inc., Woodbury, N. Y.) were used in the assay.

Hemagglutination Tests.—Titration of antibodies to meningococcal group A and C polysaccharides was done by passive hemagglutination of polysaccharide-coated human erythrocytes as described by Gotschlich et al. (7).

RESULTS

Immunity in the Adult: The Carrier State as an Immunizing Process.—Serum from each of 85 military recruits (Fort Dix, N. J., 1966 and 1968; Fort Benning, Ga., 1968) was obtained before and after the recruit became a carrier of a strain of meningococci. 78 (92%) of the men developed increased serum bactericidal activity against their own (homologous) meningococcal isolates after onset of the carrier state.

Table I records the reciprocal bactericidal titers of paired sera from 10 of the recruits who became carriers of meningococci during the first 4 wk of basic training. The results show that carriers of *Neisseria meningitidis* of serogroups B, C, and Bo (9) develop increased titers of bactericidal activity against the homologous organism. Exogenous complement was not added in these titrations, as a common source of complement was not available for all the meningococcal

strains tested. It is our experience (unpublished observations) that, in unsupplemented serum, complement becomes the limiting factor in serum dilutions of 1:32 and above. Addition of complement to such serum frequently increases

TABLE I

Meningococcidal Activity of Sera from Carriers of Meningococci‡*

Recruit No.	Serogroup of meningococcus	Reciprocal bactericidal titer of serum§	
		Day 1	Day 24
5	C	4	16
30	C	4	8
32	Bo	4	16
45	Bo	< 4	8
48	Bo	4	16
61	B	4	16
64	B	4	32
65	C	4	8
106	Bo	4	32
123	Bo	4	32

* D-7-2 Company, Fort Benning, Ga., 1968.

‡ Meningococcus acquired between the 1st and 24th day of basic training.

§ Sera from each recruit tested against his own meningococcal isolate.

TABLE II

Antimeningococcal Antibodies of Immunoglobulin Classes G, M, and A in Sera from Carriers of Meningococci‡*

Recruit No.	Serogroup of meningococcus	Reciprocal immunofluorescence titer§					
		IgG		IgM		IgA	
		Day 1	Day 24	Day 1	Day 24	Day 1	Day 24
5	C	4	32	<4	4	<4	8
30	C	4	16	<4	8	<4	8
61	B	4	16	4	16	<4	16
64	B	4	16	4	16	4	32
106	Bo	4	16	<4	8	4	8
123	Bo	16	64	<4	8	4	32

* D-7-2 Company, Fort Benning, Ga., 1968.

‡ Meningococcus acquired between the 1st and the 24th day of basic training.

§ Sera from each recruit tested against his own meningococcal isolate.

the bactericidal titer 2 to 8-fold. Thus, the bactericidal titers of some of the 24th day sera in Table I are minimal values.

Results in Table II show that the increased bactericidal activity which accompanies the carrier state is associated with the production of antimeningococ-

cal antibodies. Sera from six of the meningococcal carriers in Table I were tested for the presence of antibodies to the homologous organisms. In every instance significantly increased titers of specific IgG, IgM, and IgA antibodies, as determined by indirect immunofluorescence, were found in the 24th day serum.

In addition to forming bactericidal antibodies to their own strains of *N. meningitidis*, carriers of meningococci also produce *cross-reacting* antibodies to heterologous strains of pathogenic meningococci.

Table III shows the change in bactericidal activity of sera from representative military recruits who became carriers of meningococci (serogroups C and

TABLE III

Bactericidal Activity of Sera from Carriers of Meningococci against Pathogenic Meningococci*

Recruit No.	Meningococci isolated from nasopharynx		Serum bactericidal titer 1:4 or greater against strain (serogroup)					
			A1 (A)		B11 (B)		C11 (C)	
	Serogroup	Day acquired	Day 1	Day 35	Day 1	Day 35	Day 1	Day 35
130	Bo	3	−‡	+§	+	+	+	+
96	C	10–14	−	+	−	+	+	+
90	C	14–20	+	+	−	+	+	+
219	C	21–27	−	+	−	+	−	+
155	Bo	28–35	−	+	+	+	−	+
2	Noncarrier		−	−	−	−	+	+
199	"		−	−	−	−	−	−

* I-2 Company, Fort Dix, N. J., 1967.

‡ Minus sign (−); bactericidal titer less than 1:4.

§ Plus sign (+); bactericidal titer greater than 1:4.

Bo) during a 35 day study period. Weekly nasopharyngeal cultures were taken, so that the approximate date of acquisition of the meningococcal strain is known. It is clear from the data that, in each instance, there was increased bactericidal activity to one or more of the three test strains of meningococci (groups A, B, and C) originally isolated from cases of meningitis. The immune response occurred within 14 days of acquisition of the carrier strain, and in recruit 155 within 7 days of acquisition. Of 123 carriers of serogroups B, C, or Bo in four training companies (Fort Dix, 1966–1968) whose serum originally lacked bactericidal activity to at least one of the three pathogenic test strains, 107 (87%) developed such activity after onset of the meningococcal carrier state. 27 recruits whose sera were not bactericidal to one or more of the test strains and who did not become carriers of meningococci during the study failed to show any change in spectrum of bactericidal activity.

As in studies with homologous strains of meningococci, the development of

cross-reactive bactericidal activity to heterologous meningococci is accompanied by the appearance of specific IgG, IgM, and IgA antibodies, as determined by indirect immunofluorescence. Thus, within 2 wk of conversion from carrier-negative to carrier-positive status (serogroups B, C, or Bo), 15 recruits who were tested developed increased titers of antibodies of the three major immunoglobulin classes to all the pathogenic test strains (groups A, B, and C). Eight of these recruits had neither detectable bactericidal activity nor antibodies to one or more of the meningococcal test strains prior to onset of the carrier state but developed both shortly after becoming carriers. 10 recruits who did not become carriers of meningococci showed no change in pattern or titer of specific antibodies to meningococci.

The dynamics of the cross-reactive immune response to the meningococcal carrier state was studied more closely in recruits who were cultured twice each week and bled weekly for 7 wk. Results in Table IV show that the peak bactericidal titer to the heterologous test strain A1 was reached 10 to 14 days after conversion from a carrier-negative to a carrier-positive state (recruits 1, 3, 9, 21, 25). This titer was maintained for the duration of the study. Recruit 18 was a meningococcal carrier throughout the study and maintained a constant, elevated bactericidal titer. In contrast, recruit 41, also a carrier for the 7 wk of study, showed an initial increase in titer, suggesting that he had acquired the meningococcal strain shortly before the study began. Recruit 48 converted from a carrier-positive to a carrier-negative state at the end of the 1st wk of study. His elevated cross-reactive bactericidal titer remained constant over the ensuing 6 wk. Recruit 14, who was carrier-negative during the study, showed neither increase nor decrease in bactericidal titer during the 7 wk of observation. This was true for sera from four other noncarriers who were studied.

The cross-reactive immune response induced by the meningococcal carrier state appears to be of long duration. Two members of our laboratory (E. W. and J. W.), after carrying meningococci of serogroups B and C respectively, became carrier-negative. Elevated cross-reactive bactericidal titers against meningococcal strains A1 and B11 remained constant for a minimum of 4 and 6 months after loss of the carrier strain. Significantly, antimeningococcal IgG titers remained constant during this period, while IgM and IgA levels decreased progressively after 4 wk.

Immunity in the Newborn.—More than 50% of sera from newborn infants have bactericidal activity against pathogenic strains of meningococci of serogroups A, B, and C (1). The present experiments show that this bactericidal activity is due to transplacental passage of IgG antibodies from mother to fetus.

Bactericidal activity of eight matched pairs of maternal and fetal (cord) sera was determined against eight pathogenic strains of meningococci (serogroups A, B, C, and 135 [9]). Results in Table V show that, with only two exceptions

(both paired sera No. 3) in 64 comparisons, the spectrum of bactericidal activity of maternal and fetal serum is identical. Also, titers of bactericidal activity to each organism are generally the same for maternal and cord sera. In five in-

TABLE IV

Bactericidal Activity of Sera from Carriers of Meningococci against a Strain of Pathogenic Meningococci (A1)*

Recruit No.	Parameters measured	Day of study														
		1	3	7	10	14	17	21	24	28	31	35	38	42	45	49
1	Carrier status‡	—	—	—	—	—	—	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo
	Bactericidal titer§	4		4		4		4		16		16		16		16
3	Carrier status	—	—	—	—	—	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo
	Bactericidal titer	16		16		16		16		64		64		64		64
9	Carrier status	—	—	—	—	—	—	—	—	—	—	Bo	Bo	Bo	Bo	Bo
	Bactericidal titer	4		4		4		4		4		4		32		32
18	Carrier status	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Bactericidal titer	64		64		64		64		64		64		64		64
21	Carrier status	—	—	—	—	—	—	—	—	—	C	C	C	C	C	C
	Bactericidal titer	4		4		4		4		4		4		32		32
25	Carrier status	—	—	—	—	B	B	B	B	B	B	B	B	B	B	B
	Bactericidal titer	4		4		4		16		16		16		16		16
41	Carrier status	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo	Bo
	Bactericidal titer	16		64		64		64		64		64		64		64
48	Carrier status	B	B	B	—	—	—	—	—	—	—	—	—	—	—	—
	Bactericidal titer	64		64		64		64		64		64		64		64
14	Carrier status	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Bactericidal titer	4		4		4		4		4		4		4		4

* I-3 Company, Fort Dix, N. J., 1966.

‡ Minus sign (—) indicates negative culture; letter indicates serogroup of meningococcal strain isolated from nasopharynx.

§ Reciprocal bactericidal titer against meningococcal strain A1 (serogroup A).

stances where bactericidal titers differed, that of fetal serum was higher than maternal serum.

The relation of bactericidal activity in fetal serum to the presence of IgG antibodies was determined by indirect immunofluorescence. In all instances in Table V in which bactericidal activity was present against a particular strain

of meningococcus, IgG antibodies to that organism were detected. No IgG antibodies were found when bactericidal activity was absent (i.e., bactericidal titer less than 1:4). Neither IgM nor IgA antibodies were detected in fetal serum by immunofluorescence, even when the serum was bactericidal.

Quantitative immunoprecipitation showed that the fetal sera had 6–73% more immunoglobulin G than did maternal sera, a finding consistent with published reports (10). This presumably accounts for the tendency of the fetal sera to have higher bactericidal activity than the maternal sera, and for the apparent discrepancy in the spectra of bactericidal activity of the paired sera No. 3 in

TABLE V

Bactericidal Activity of Maternal and Fetal Sera against Pathogenic Strains of Meningococci

Meningococcal strains (serogroup)	Reciprocal bactericidal titers of paired sera*															
	1		2		3		4		5		6		7		8	
	M‡	F§	M	F	M	F	M	F	M	F	M	F	M	F	M	F
A1 (A)	8	8	4	8	<4	4	8	8	8	16	4	4	4	8	8	8
129-EUR (A)	4	4	<4	<4	<4	<4	4	4	4	4	<4	<4	<4	<4	4	8
130-EUR (B)	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4	<4
153-I (B)	8	8	<4	<4	<4	4	8	8	>16	>16	<4	<4	4	4	16	16
166-IV (B)	16	16	4	4	<4	<4	4	4	<4	<4	<4	<4	<4	<4	8	16
107-VI (C)	8	8	>16	>16	<4	<4	4	4	4	4	<4	<4	4	4	8	8
C11 (C)	4	8	>16	>16	<4	<4	8	8	4	4	<4	<4	<4	<4	<4	<4
122-Misc. (135)	16	16	8	8	<4	<4	8	8	4	4	<4	<4	<4	<4	16	16

* Exogenous complement added.

‡ Maternal serum.

§ Fetal (cord) serum.

Table V. IgM was detected in low concentration in two of eight fetal sera (9 and 11 mg/100 ml); IgA was not found in fetal sera.

Immunity in the Child.—After the occurrence of physiological hypogammaglobulinemia, the increasing prevalence among children of bactericidal antibodies to pathogenic strains of meningococci (1) suggests that a process of active immunization is operative. As shown above, immune sensitization in the adult occurs in response to carriage of meningococci in the nasopharynx. It would seem reasonable that this is a basic mode of immunization which also occurs throughout childhood. The chief objection to such a hypothesis is the expectation that children who lack antibodies to pathogenic meningococci would develop systemic disease upon exposure to these organisms. Such a prediction is based on our study of meningococcal meningitis among military recruits (1). The following studies provide a possible explanation of this apparent paradox.

Two surveys were conducted to determine the prevalence and kinds of men-

ingococci carried by the children in the mid-Atlantic region of the United States. The results were compared with those obtained from young adult males arriving for basic training at Fort Dix, N. J. All surveys were conducted during spring and summer, 1968. Table VI shows the distribution of meningococcal strains within the various age groups. 55 children, ages 3 months to 6 yr, were cultured upon their arrival at Junior Village, Washington, D. C., a municipal home for orphaned and abandoned children (11). 13 of the children (23.6%) carried organisms compatible with meningococci in the nasopharynx. Of the isolates, four were classical meningococci and 13 were lactose-fermenting strains (12). The

TABLE VI

Strains of Meningococci Isolated from the Nasopharynx of Carriers in Various Age Groups

Ages*	No. cultures	No. of meningococcal strains isolated						
		Total	Classical‡	Lactose§	Typable	Serogroups identified		
						B	C	Bo
					%			
3 months–6 yr	55	13	4	9	30.8	4	0	0
6 months–12 yr	137	23	13	10	4.4	1	0	0
Total children.....	192	36	17	19	13.9	5‖	0	0
19–26 yr	150	36	36	0	66.7	8	10	6

* Populations studied: 3 months–6 yr, Junior Village, Washington, D. C., 1968; 6 months–12 yr, Walter Reed Army Medical Center, 1967; 19–26 yr, obtained from three separate groups of military inductees immediately upon their arrival at Fort Dix, N. J., 1966–1968.

‡ Ferment dextrose and maltose.

§ Ferment dextrose, maltose, and lactose.

‖ Two classical and three lactose-fermenting strains.

former had typical colonial morphology, were oxidase-positive, did not grow at 22°C, and fermented dextrose and maltose; the latter were similar, but fermented lactose in addition to dextrose and maltose. For convenience, the lactose-fermenting organisms will be referred to as meningococci, although their relation to true meningococci has not been definitively established. Only one of the classical meningococci and three of the lactose-fermenting organisms were typable (all serogroup B).

A survey of healthy children seen at the outpatient clinic, Walter Reed General Hospital, Washington, D. C., produced somewhat different results, although the trend was similar. Here the children ranged in age from 6 months to 12 yr, with the great majority being over 2 yr of age. As seen in Table VI, 23 (16.8%) of 137 children studied harbored meningococci. The ratio of classical to lactose-fermenting strains of meningococci was 13:10. Only one typable or-

ganism was isolated (serogroup B), this being a strain with a classical fermentation pattern.

A summation of the meningococcal carrier experiences of the two pediatric populations shows that of 192 children, ages 3 months to 12 yr, 36 (18.8%) carried meningococci; 17 classical and 19 lactose-fermenting strains. Only five (14%) of the 36 isolates were typable (all group B). A similar carrier rate (24%) was found among 150 military inductees (Table VI). However, in contrast to results of the pediatric study, there were no lactose-fermenting organisms among the 36 meningococcal isolates, and 67% of the strains were typable (serogroups B, C, and Bo).

Results in Table VI indicate that the population of meningococci which infants and young children acquire is different from that carried by young adults. It was of interest, therefore, to determine if the carriage of nontypable and lactose-fermenting organisms produced cross-reacting antibodies to known pathogenic strains of meningococci. To this end 109 children at Junior Village, Washington, D. C. were cultured every 2 wk for periods up to 4 months.[1] The population under study at any point numbered approximately 60, with an average of five children admitted to the group and five discharged from the group every 2 wk. According to the regulations of the institution, all children were bled on admission, but only sporadically thereafter. Sera were stored at −20°C.

During the study, nine children converted from carrier-negative to carrier-positive status. By special permission, serum was obtained from six of these children within 1 to 3 wk after acquisition of the meningococcal strain. Table VII shows the cultural data and reciprocal bactericidal titers of the six children from whom paired sera were available. Two of the six carriers developed increased bactericidal activity against one or more of meningococcal strains A1, B11, and C11. Neither of the carrier strains was typable with available antisera.

Because of the wide disparity in time between the first bleedings and acquisition of the meningococcal strains (5 and 7 months respectively), it was impossible to be certain that the carrier strains were indeed the cause of the increased bactericidal activity in sera from the second bleedings. Therefore, the second sera were absorbed with the homologous meningococcal isolates. In both instances the cross-reactive bactericidal activity was removed. Hemolytic complement levels were reduced less than 10% by these absorptions.

Other investigators (13) have noted that, during interepidemic periods, meningococci isolated from asymptomatic carriers among the civilian population are more susceptible to the bactericidal action of blood from normal adults than are meningococci from cases of meningitis. In the present experiments, a similar

[1] Material for this study was gathered by personnel from the Rhinovirus Laboratory (Dr. Albert Z. Kapikian, Director), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md., as part of a continuing survey of infectious diseases at Junior Village.

relationship was found using sera from children. Two meningococcal strains (G-2-81 and C-52) isolated from healthy carriers were exposed to sera from the panel of 282 children previously tested against three case strains of meninococci (1). Like the case strains, the carrier strains were from adults. However, unlike the situation with the known pathogenic strains, where only sera from newborn infants and children over the age of 4 yr were bactericidal in significant numbers, more than 85% of sera from children of all ages were able to kill the two carrier strains in titers of 1:4 or greater.

TABLE VII

Meningococcocidal Activity of Sera from Children before and after Carriage of Meningococci*

Subject	Age	Meningococcal isolate			Reciprocal bactericidal titer‖						
					Serum No. 1				Serum No. 2¶		
		Serogroup‡	Lactose§	Date isolated	Date obtained	A1	B11	C11	A1	B11	C11
	yr										
L. K.	2½	NT	+	7/22/68	6/11/68	<4	16	8	<4	16	8
C. W.	5	B	−	7/22/68	1/10/67	<4	<4	<4	<4	<4	<4
N. W.	2½	NT	−	7/22/68	2/13/68	<4	<4	<4	16	32	16
M. H.	1½	NT	−	8/5/68	1/16/68	<4	<4	<4	<4	32	16
L. S.	2	NT	+	9/3/68	4/23/68	<4	<4	<4	<4	<4	<4
J. A.	2½	NT	−	9/3/68	2/27/68	<4	<4	<4	<4	<4	<4

* Junior Village, Washington, D. C., 1968.

‡ NT, nontypable.

§ Plus sign (+), ferments lactose in addition to dextrose and maltose.

‖ Tested against three pathogenic strains of meningococci: A1 (group A), B11 (group B), and C11 (group C).

¶ Obtained 7 to 21 days after isolation of meningococcal strain.

Antigenic Determinants of Immunity to N. meningitidis.—

Group-specific antigens: The group-specific meningococcal polysaccharides were first isolated by Rake and Sherp (14). These polysaccharides have recently been isolated in large molecular weight form and chemically defined by Gotschlich et al. (7) and Liu et al.[2]

In order to determine whether group specific polysaccharides contribute to the immune response which accompanies the meningococcal carrier state, sera from 45 recruits at Fort Dix, N. J. 1967 (I-2 Company) were tested for the presence of antibodies to meningococcal A and C substances. Sera were obtained during the 3rd and 8th wk of basic training. Nasopharyngeal cultures were taken twice each week. Results in Fig. 1 show that 31 of 38 recruits who acquired a group C meningococcus between the first and second bleedings de-

[2] Liu, T. Y., J. K. Jönsen, and E. C. Gotschlich. Manuscript in preparation.

veloped increased hemagglutination titers to erythrocytes coated with C polysaccharide. There was a mean increase of 16-fold in hemagglutination titer to the group C–specific antigen. Base line titers were 0–4 (median 0); compared with 4–256 (median 16) in the second serum. The immune response was detectable within 2 wk of acquisition of the meningococcus. Six recruits who did not become carriers, and eight who carried a meningococcal strain other than serogroup C had no change in hemagglutination titer to C substance. There were no increases in hemagglutination titer of sera from carriers of group C meningococci to red blood cells coated with group A polysaccharide.

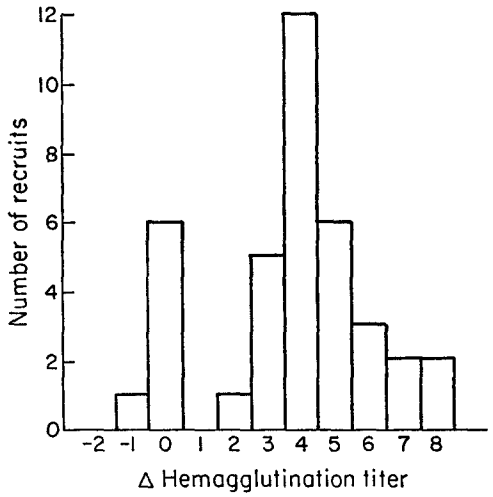

FIG. 1. Distribution of change in hemagglutination titer against group C meningococcal polysaccharide of sera from carriers of group C meningococci, I-2 Company, Fort Dix, N. J. 1967. Meningococcal strains acquired between first and second bleedings (5 wk apart). "Δ Hemagglutination titer" is the number of serial 2-fold dilutions (2^n) increase or decrease in reciprocal hemagglutination titer between first and second sera.

The relation of the rise in group-specific hemagglutination titer to the increase in bactericidal activity which accompanies the carrier state is shown in Table VIII. Sera obtained from recruits in I-2 Company after they acquired a group C meningococcus were absorbed with meningococcal C polysaccharide. It is clear from the results that when anti-polysaccharide antibodies were present in high titer, as detected by hemagglutination, these antibodies comprised the major part of the bactericidal response against group C meningococci. When there was little antibody response to C polysaccharide the increase in bactericidal titer was considerably less and presumably due to antibodies to other antigens. Bactericidal titers to serogroup A (strain A1) and B (strain B11) meningococci were unaltered by absorption with C polysaccharide.

Some idea of the prevalence of antibodies to group-specific meningococcal polysaccharides in the general population of the United States was gained by measuring the bactericidal activity of pooled gamma globulin (approximately 2000 donors) before and after absorption with A and C polysaccharides. Results in Table IX show that the bulk of the bactericidal activity to the prototype C meningococcus, (C11), was directed against the C polysaccharide. Absorption of concentrated gamma globulin (10 mg/ml) with 100 μg/ml meningococcal C substance reduced the bactericidal titer to strain C11 8-fold, but did not change the titer against the group A organism (A1). In contrast, absorption of gamma

TABLE VIII

Absorption of Sera from Carriers of Group C Meningococci‡ with Meningococcal Group C Polysaccharide*

Recruit No.	Reciprocal bactericidal titer§			Reciprocal hemagglutination titer¶		
	15 February	22 March		15 February	22 March	
		Unabsorbed	Absorbed‖		Unabsorbed	Absorbed‖
60	4	256	16	0	128	4
96	4	256	16	0	128	16
184	8	256	32	0	256	2
197	8	1024	128	2	512	64
75	<4	16	16	0	0	0
191	<4	8	4	0	0	0

* I-2 Company, Fort Dix, N. J., 1967.

‡ Meningococcal strain acquired between 15 February and 22 March.

§ Tested against meningococcal strain C11 (group C).

‖ 100 μg group C meningococcal polysaccharide/ml serum.

¶ With cells sensitized with group C polysaccharide.

globulin with group A polysaccharide produced only a 2-fold decrease in bactericidal activity against strain A1. This is within the range of error of the titration, and its significance cannot be assessed. Reabsorption with group A polysaccharide was ineffective in further lowering the bactericidal titer.

Cross-reactive antigens: The development of bactericidal antibodies to serogroups of meningococci other than that of the homologous carrier strain indicates that antigens in addition to group-specific polysaccharides are involved in immune sensitization to meningococci during the carrier state. The existence of such antigens was further demonstrated by cross-absorption studies.

Table X shows the bactericidal activities of normal human serum (J. W.) before and after absorption with eight meningococcal strains of various serogroups. It is clear from the results that in many instances antigens are shared by heterologous strains of meningococci. Thus, all organisms listed in Table X

had the ability to remove bactericidal activity to one or more meningococcal strains. Further, most strains seemed to share at least two common antigens. This was shown by the ability of all strains to remove bactericidal antibodies to strain A1, and of all strains except A1 to absorb bactericidal activity to strain 130-EUR. However, none of the eight strains tested appeared to have identical antigenic patterns. For example, strain 70-II absorbed bactericidal antibodies to strains B11 and 130-EUR, but neither of the latter two strains completely removed bactericidal activity to strain 70-II. Similarly, strain B11 removed bactericidal activity to strain 130-EUR, but the reciprocal absorption was ineffective.

TABLE IX

Absorption of Pooled Human Gamma Globulin with Groups A and C Meningococcal Polysaccharides*

Treatment of gamma globulin	Reciprocal bactericidal titer‡ against meningococcal strains	
	A1 (group A)	C11 (group C)
Untreated	32	64
Absorbed with C (100 μg/ml)	32	8
Absorbed with A (100 μg/ml)	16	64

* Cohn fraction II, 10 mg/ml. Prepared from blood collected in the spring of 1968.

‡ Exogenous complement added.

It could be argued that the patterns of antigenic determinants presented in Table X result from incomplete or nonspecific absorption of antibodies. That this is not the case is shown by the following experiments. First, the pattern was reproduced when the experiment was repeated. Second, reabsorption of some of the aliquots of serum did not alter the pattern of bactericidal activity. Third, removal of bactericidal activity to the homologous strain was consistently observed. Fourth, absorption of another human serum (I. G.) with the same eight meningococcal strains produced a similar (although not identical) pattern, which also was reproducible. Finally, removal of bactericidal activity by absorption with heterologous meningococci correlated with the removal of antimeningococcal antibodies as determined by indirect immunofluorescence. For example, absorption of the serum (J. W.) with meningococcal strain G-2-81 removed specific IgG antibodies to strains G-2-81, A1, 70-II, B11, and 130-EUR but not to strains 129-EUR, C11, or C52.

Antigenic determinants within a serogroup: Results in Table X indicate that not only are there cross-reactive antigens between the various meningococcal serogroups, but that antigenic variation exists within serogroups as well. An

attempt was made, therefore, to analyze the antigenic diversity of meningococcal strains belonging to a single serogroup.

Table XI summarizes an experiment in which cross-absorption of pooled human gamma globulin (Cohn fraction II) was carried out with 23 strains of serogroup C meningococci. The use of gamma globulin from a pool of approximately 2000 adults maximized the probability of obtaining antibodies to most meningococcal antigens. Group C organisms were chosen for study because they were most readily available. It was not desirable to rely solely on the ab-

TABLE X

Absorption of Normal Human Serum with Meningococci from Diverse Serogroups*

Serum absorbed with meningococcal strain (serogroup)	Bactericidal titer 1:4 or greater against meningococcal strains‡							
	A1	129-EUR	70-II	B11	130-EUR	C11	G-2-81	C-52
A1 (A)	−§	+	+	+	+	+	+	+
129-EUR (A)	−	−§	+	+	−	+	+	+
70-II (B)	−	+	−§	−	−	+	+	+
B11 (B)	−	+	+	−§	−	+	+	+
130-EUR (B)	−	+	+	+	−§	+	+	+
C11 (C)	−	+	+	+	−	−§	+	+
G-2-81 (Y)	−	+	−	−	−	+	−§	+
C-52 (NT)‖	−	+	+	−	−	+	+	−§
Unabsorbed¶	+	+	+	+	+	+	+	+

* Laboratory volunteer (J. W.), male, age 23 yr, recent carrier of group C meningococci.

‡ Minus sign (−) indicates strains to which bactericidal activity was removed (i.e., titer less than 1:4). Plus sign (+) indicates strains to which bactericidal activity was *not* removed (i.e., titer 1:4 or greater).

§ Homologous absorption.

‖ NT, nontypable.

¶ Original titers were 1:8 to 1:16.

sorption with bacteria to remove all antibodies to the group C polysaccharide since the relative amounts of this antigen on the surface of each of the 23 meningococcal strains used for absorption was not known. To ensure complete removal of antibodies to the group C polysaccharide, the pooled gamma globulin was first absorbed with purified C substance adsorbed to alum.

The meningococci were selected to represent as diverse a collection of strains as possible; group C organisms having been obtained from cases and carriers in civilian and military populations from Europe and widely separated areas of the United States during the period 1964 through 1968. Absorptions were done on 2 ml aliquots of gamma globulin (10 mg/ml) for 2 hr at 4°C. Bactericidal tests were performed in the presence of exogenous complement. Bacteri-

TABLE XI

Absorption of Pooled Human Gamma Globulin with group C Meningococci*

Gamma globulin absorbed with meningococcal strain‡		Bactericidal titer 1:4 or greater against meningococcal strain§																						
WRAIR Code No.	Strain No.	17	18	14	15	22	19	1	16	9	5	6	13	21	7	20	2	10	3	8	4	23	11	12
79-II	1	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
142-EUR	2	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
158-III	3	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
171-IV	4	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
101-II	5	—	18	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
115-I	6	—	18	—	—	—	—	—	—	—	—	—	13	21	—	—	—	—	—	—	—	—	—	—
138-I	7	ND‖	18	—	—	—	—	—	—	—	—	—	13	—	—	—	—	—	—	ND	—	—	—	—
166-I	8	17	18	—	15	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—
98-I	9	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	2	—	—	—	—	—	—	—
153-III	10	—	—	—	—	—	—	—	—	—	—	—	13	21	—	—	2	—	3	—	—	—	—	—
91-III	11	17	18	—	—	—	—	—	—	—	5	6	—	—	—	—	2	—	3	—	—	—	—	—
107-VI	12	17	18	—	—	—	—	—	—	—	5	6	—	—	—	—	2	—	3	—	—	—	—	—
121-EUR	13	—	—	—	—	—	—	1	—	—	5	6	—	21	7	—	2	—	—	—	—	—	—	—
47-VI	14	ND	—	—	—	—	—	1	—	—	5	6	13	21	7	—	2	—	—	ND	—	—	—	—
51-III	15	—	—	—	—	—	—	1	—	—	5	6	—	21	7	20	2	—	—	8	—	—	—	—
82-IV	16	—	—	—	—	—	—	1	—	—	5	6	—	21	7	20	2	—	—	8	—	—	—	—
32-I	17	—	—	—	—	—	—	1	—	—	5	6	13	21	7	20	2	—	—	8	—	—	—	—
38-IV	18	—	—	—	—	—	—	1	—	—	5	6	13	21	7	20	2	—	—	8	—	—	—	—
74-III	19	ND	—	—	—	—	—	1	—	—	5	6	13	21	7	20	2	—	—	ND	—	—	—	—
140_a-I	20	17	18	—	—	—	—	1	—	—	5	6	13	21	—	—	2	—	3	—	—	—	—	—
126-EUR	21	17	—	—	15	—	—	1	—	—	5	6	—	—	7	—	2	—	3	8	—	—	—	—
59-EUR	22	17	18	—	15	—	—	1	—	—	5	6	13	21	7	20	2	10	3	8	—	—	—	—
C11(60-EUR)	23	17	18	—	15	—	—	1	—	—	5	6	13	21	7	20	2	10	3	8	—	—	—	—
Unabsorbed¶....	.....	8	8	4	8	16	8	16	8	4	16	16	8	8	8	8	16	8	8	4	8	4	4	8

* Cohn fraction II, 10 mg/ml. Same pool as used in Table IX.

‡ Preabsorbed with group C meningococcal polysaccharide, 100 μg/ml.

§ Strains to which bactericidal activity is *not* removed (i.e., titers of 1:4 or greater) are indicated by appropriate number in table.

‖ ND, not determined.

¶ Reciprocal bactericidal titer.

cidal titers of 1:4 to 1:16 were obtained with unabsorbed gamma globulin against all of the meningococcal strains. After absorption, only titers of less than 1:4 were considered indicative of removal of bactericidal antibody.

It is clear from results in Table XI that there is a spectrum of antigenic patterns among the 23 strains of group C meningococci. At one extreme, strains 1, 2, 3, and 4 removed bactericidal antibodies to all organisms except strains 13 and 21; at the other extreme, strains 22 and 23 removed bactericidal activity to only 9 of 23 test strains.

In addition to the *number* of meningococcal strains with which an organism shares common antigens, the *pattern* of absorption of bactericidal activity is important. Thus, strains 22 and 23 appeared to be similar antigenically as judged by the fact that in addition to removing bactericidal activity to each other, their absorption patterns against the remaining meningococcal strains were identical (read horizontally in Table XI). Further, the panel of organisms which absorbed bactericidal activity to strains 22 and 23 was identical (read vertically in Table XI). Other organisms which seemed to have similar patterns of surface antigens are 5 and 6, and 11 and 12.

The remainder of the meningococcal strains in Table XI, while obviously having antigens in common, differed with regard to at least one antigen. For example, most organisms failed to remove bactericidal activity to strains 13 and 21. However, strains 13 and 21 are not identical, as shown by differing absorption patterns against organisms 3, 8, 15, and 17; and, most significantly, by failure of strain 13 to remove bactericidal activity to strain 21.

The completeness and specificity of the absorptions were attested by the facts that homologous absorptions removed bactericidal activity to each of the 23 strains, and that the pattern of bactericidal activity could be reproduced using the same lot of gamma globulin (half the experiment was repeated). It is of interest in this regard that strains 22 and 23 which appeared identical antigenically in Table XI were obtained within one month of each other from cases of meningitis that occurred in Frankfurt, Germany.

Summarizing results in Table XI, among the 23 group C meningococcal strains tested, there appeared to be 3 pairs of antigenically identical (or closely related) strains and 17 nonidentical strains, judged by susceptibility to bactericidal antibodies. Using the formula $C = 2^n - 1$ (15), where C equals the number of antigenic patterns and n equals the number of antigenic determinants, it is apparent that there are at least five antigens, in addition to the group-specific polysaccharide, present on the surface of group C meningococci.

DISCUSSION

Previous experiments have shown that susceptibility to systemic meningococcal disease, both in children and adults, is related to a deficiency of humoral

antibodies to meningococci (1). The present study describes two mechanisms by which such antibodies are acquired; passive immunization by transplacental passage of immunoglobulins, and active sensitization as a result of the meningococcal carrier state. The effect of passively acquired immunity is obviously restricted to the first few months of life, but is of great theoretical importance for several reasons. First, it strongly indicates that humoral antibodies are protective against systemic meningococcal disease. Second, since these maternally derived antibodies are of the immunoglobulin G class, it would appear that IgG antibodies are the ones mainly responsible for protection against meningococcal disease in the adult. This is supported by the finding that bactericidal activity could be recovered quantitatively from the IgG pool of sera from two adult males (one a carrier of group C meningococci, the other normal) when fractionated by gel filtration on a Sephadex G-200 column (unpublished observations). Such immunity would be expected to be of long duration (16). Third, the protective effect of passively transferred IgG antibodies validates the use, in the present experiments, of pooled human gamma globulin (Cohn fraction II) to discern the kinds of meningococcal antigens responsible for elicitation of protective antibodies during the carrier state.

The clearest demonstration that the meningococcal carrier state is an immunizing process is found among military recruits in basic training. Here, acquisition of a strain of meningococci results in the production, within 2 wk or less, of antibodies to meningococci. Such antibodies are of the three major immunoglobulin classes and combine with group-specific and cross-reactive antigens. In military recruits and among laboratory personnel, carrier-induced antimeningococcal antibodies have been shown to persist at high titer for a minimum of 4 to 6 months after exposure. This provides a reasonable explanation for the universal observation (17–19) that seasoned military personnel are much less susceptible to meningococcal disease than are basic recruits. Such seasoned troops have apparently been immunized during basic training by means of the meningococcal carrier state. This is corroborated by a study (1) in which 65% of a susceptible population of military recruits became carriers of meningococci other than the prevalent disease-producing strains. Such individuals subsequently developed bactericidal antibodies to the pathogenic organisms (unpublished observations).

It would appear at first that immunization by the meningococcal carrier state, so prominent among military recruits, cannot be invoked as a mechanism of naturally acquired immunity in young children. The main theoretical objection is that children, who lack antibodies to pathogenic strains of meningococci (1), would develop systemic disease rather than become carriers of meningococci. However, the fact is that most children become meningococcal carriers and not cases. As seen in the present experiments, the resolution of this paradox appears to be in the observation that children carry mostly atypical, nontypable

strains of meningococci. Such strains have been found experimentally (20, 21) and epidemiologically (22, 23) to be of low virulence.

An interesting observation was made by Fothergill and Wright (24) which bears on the present findings. In a study of susceptibility of children to *Haemophilus influenzae* meningitis, they found that sera from most children 6 months to 3 yr of age were unable to kill virulent strains of *H. influenzae*, whereas the same sera routinely killed avirulent strains. We have obtained similar results using three meningococcal strains of proved pathogenicity and two strains from asymptomatic carriers. In addition, one of the carrier strains (G-2-81) was previously shown (1) to be killed by sera from prospective cases of meningococcal meningitis among adults, while such sera has no effect on the pathogenic organisms. It is possible, therefore, although by no means proved, that meningococci isolated from asymptomatic carriers tend to be nonpathogenic because they are exquisitely sensitive to the bactericidal action (or other protective activities) of antibodies in serum. Such antibodies presumably could arise in the infant as a result of contact with cross-reactive antigens from nonmeningococcal sources.

Despite the fact that carrier strains of meningococci are generally of low virulence, they contain antigens which cross-react with those of pathogenic meningococci. Thus, carrier strains G-2-81 and C-52 were able to remove bactericidal antibodies to disease-producing organisms of various serogroups. Furthermore, in a limited study of children, ages 2–5 yr, who converted from carrier-negative to carrier-positive status, two of six children developed bactericidal antibodies which cross-reacted with one or more of the pathogenic meningococcal test strains. Absorption with the homologous carrier strain removed these cross-reacting antibodies. The relatively low percentage of children who reacted immunologically to the carrier state as compared to adults may be due to a peculiarity of the immune response in children, unusual antigenicity of the infesting meningococci, the nature of the carrier state in children, or sampling artifact due to the small size of the study group. It is significant in this regard that one of the children who failed to respond immunologically had acquired a classical group B meningococcus, which in the adult would be expected to elicit prompt production of cross-reacting antibodies.

50% of "meningococci" isolated from the nasopharynx of children fermented lactose in addition to glucose and maltose. Such organisms have been described previously (12) and were found to be indistinguishable culturally from true meningococci. All had been isolated from asymptomatic carriers and were presumed to be avirulent. In our experience with several hundred strains of meningococci from cases of meningitis, none have been found to ferment lactose (unpublished observations). Obviously antigenic analyses and DNA homologies (25) are necessary to establish the identity of these lactose-fermenting *Neisseria* as meningococci. However, it was found in the present study that 4 of 19 lactose-

fermenting strains typed as group B meningococci. Furthermore, many of the nontypable lactose-fermenting organisms were agglutinated by antisera to several meningococcal serogroups.

Two major groups of antigenic determinants are responsible for eliciting the production of antibodies to meningococci: the group-specific polysaccharides and cross-reactive antigens. The presence of antibodies to group-specific meningococcal antigens has been described in human sera from cases (26, 27), carriers (28), and normal individuals (29). Recent studies have shown that group-specific hemagglutinating (30) and complement-fixing (31) antibodies can arise during the carrier state. The present experiments show that, in military recruits, hemagglutinating and bactericidal antibodies to purified meningococcal C polysaccharide appear within 2 wk of acquisition of group C organisms in the nasopharynx. Sera from carriers of group A meningococci were not available for testing against the A polysaccharide.

In order to gain some insight into the role that group-specific meningococcal polysaccharides play as natural immunogens in the general population, hemagglutinating and bactericidal antibody titers were determined on pooled human gamma globulin before and after absorption with the polysaccharides. The bulk of bactericidal antibodies to group C meningococci in gamma globulin collected in 1968 were directed against the group-specific C polysaccharide; whereas little, if any, of the activity to group A organisms were directed against the A polysaccharide. This is not unexpected, as C organisms are prevalent in the United States at the present time, while group A meningococci have been rare for 10 to 15 yr (32, 33). An attempt was made to demonstrate the role of meningococcal A polysaccharide in natural immunity to group A organisms by using a gamma globulin pool prepared in 1944, the year in which the peak of the last great A epidemic in the United States occurred. Unfortunately, this preparation of pooled gamma globulin from 1944 was totally inactive against meningococci from any of the major serogroups. It is likely that at present, antibodies to the meningococcal C polysaccharide constitute a defense against group C organisms, and that cross-reactive antibodies help to protect against group A infection.

The nature of cross-reactive meningococcal antigens is not known. Such antigens have been recognized since the beginning of meningococcal research, being the bane of attempts to classify meningococci serologically (34–36). Cross-reactive antigens in other Gram-negative bacterial species such as *Salmonella typhosa* and *Escherichia coli* have been identified with endotoxin (somatic or "O" antigens) (37, 38). It is reasonable to expect that this will prove to be the case with meningococci. Indeed, preliminary reports (39)[3] on purified meningococcal endotoxin suggest that common antigenic determinants may be shared

[3] Ivler, D., and F. Wyle. Personal communication.

across serogroup lines. We have recently found (unpublished observations) complement-fixing antibodies in normal human serum to meningococcal endotoxin isolated by the method of Westphal et al. (40). Also, in two of four instances, bactericidal antibody to meningococcal strain A1 was removed from human serum by absorption with endotoxin from strain A1.

The relationship between the multiple antigenic determinants within a single serogroup and cross-reactive antigens is not clear. Cross-absorptive studies have identified a minimum of five antigens in the former group. It is possible that the antigenic determinants within a serogroup are true type-specific antigens. However, they may be identical with the cross-reactive antigens.

Rake and Scherp (41) described a group antigen ("C" antigen) which was common to the various species of the genus *Neisseria*. The importance of this antigen has not been clearly assessed. However, the apparent specificity and selectivity of bactericidal activity in human sera to individual strains of meningococci suggests that antibodies to this antigen play a minor role in natural immunity to meningococcal disease.

SUMMARY

Results of the present study suggest that natural immunity to meningococcal disease is initiated, reinforced, and broadened by intermittent carriage of different strains of meningococci throughout life.

In young adults, carriage of meningococci in the nasopharynx is an efficient process of immune sensitization. 92% of carriers of serogroup B, C, or Bo meningococci were found to develop increased titers of serum bactericidal activity to their own meningococcal isolate, and 87% developed bactericidal activity to heterologous strains of pathogenic meningococci. The rise in bactericidal titer occurred within 2 wk of onset of the carrier state, and was accompanied by an increase in titer of specific IgG, IgM, and IgA antibodies to meningococci.

In early childhood, when few children have antibodies to pathogenic meningococci, active immunization seems to occur as a result of carriage of atypical, nonpathogenic strains.

Immunity to systemic meningococcal infection among infants in the neonatal period is associated with the passive transfer of IgG antibodies from mother to fetus.

The antigenic determinants which initiate the immune response to meningococci include the group-specific C polysaccharide, cross-reactive antigens, and type-specific antigens.

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