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Comments on a Newly Recognized Group of the Meningococcus.

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For practical purposes, meningococci may be considered to fall into two broad groups, I and II. Group I, which includes the Types I and III of Gordon and Murray, has apparently been responsible for all of the epidemics of which there is serological knowledge for the past 20 years,[1] whereas Group II strains have been most common in sporadic cases and in chronic carriers.

The last peak in the number of cases of meningococcus meningitis was in 1936, and 90% of the strains sent to the National Institute of Health during that year for typing were found to belong to Group I, and only 9% to Group II. In 1937 the epidemic wave began to subside, and the number of Group I cases rapidly decreased. Group II strains increased to 16% during 1937, to 46% in 1938, and to 55% in 1939. During this time conspicuous differences among so-called Group II strains were noted. Pittman noted that there were no antibodies for certain strains in many polyvalent antimeningococcus sera sent routinely to the Institute.

For several years it has been obvious that most polyvalent antimeningococcus sera have given little protection against Group II infections.[2] It seems that either members of this group were poor antigens, the wrong animals and methods were used, or else the strains comprising the group differed among themselves more than had been known.

During the 1937-40 period when most of the cases of meningococcus meningitis were sporadic, the absence of protective antibodies for Group II became even more evident. It seemed essential to find really antigenic strains.

With this end in view, monovalent sera were prepared by immunizing rabbits, in duplicate, with 12 newly isolated Group II strains. Virulence was maintained by occasional passage through mice. For immunization, carefully graduated doses of 5-hour blood agar slant cultures were given intravenously on 3 successive days of each week, with a week's rest period and a trial bleeding at the end of

[1] Branham, S. E., and Carlin, Sadie A., *J. Bact.*, 1937, **34**, 275.

[2] Branham, S. E., Proc. 29th Annual Meeting of the Drug Mfg. Assn., 1940, p. 210.

6 weeks. The first 3 injections contained approximately 10,000,000 meningococci each. Sera from the trial bleedings were studied by agglutination at 37°C, by "halo" production,[3] and by mouse protection.[4]

In rabbits immunized with 4 of the strains, protective antibodies could be demonstrated within a few weeks. In the others, practically none could be found, even for the homologous strains, after a year and a half of immunization.

The 4 strains that seemed to be good antigens were studied further. Sera prepared with them protected mice against homologous strains to a high degree—*i. e.*, 0.001 cc protected against 100,000 minimum fatal doses. Moreover, they protected equally well against each other but not against any other strains that were included in the first experiments.

It was obvious that these 4 strains were immunologically alike though they came from widely separated localities, and that they were different from the other Group II strains. From this time on all new meningococci that seemed to fall into Group II by agglutination were studied with these sera by means of the mouse protection test.

Twenty-four of 43 Group II strains (53%) received since that time have been found to be immunologically identical with these 4. Protection tests have been definite and clear-cut. No other sera have protected against them and no strains other than these 24 have been responsive to the sera from 4 original strains. A summary showing the sources is given in Table I.

Although these meningococci may be agglutinated to some extent by any broad Group II serum, it is easy to prepare specific agglutinating serum if smooth strains and brief immunization periods are used. It has been possible to classify them by specific agglutination when they are newly isolated using 37°C incubation for 2 hours, followed by ice-box storage overnight. This "typing" is usually confirmed by mouse protection. Plate precipitation or "halo" production is distinctive also when horse serum is used, but immune rabbit sera have a tendency to show halos with all Group II strains.

The heterogenicity of Group II meningococci has been noted by others. A positive "Quellung" reaction has been observed[5] by some strains with appropriate sera whereas the regular Group II strains

[3] Pittman, Margaret, Branham, Sara E., and Sockrider, Elsie M., *U. S. Public Health Rep.*, 1938, **53**, 1400.

[4] Branham, Sara E., and Pittman, Margaret, *U. S. Public Health Rep.*, 1940, **55**, 2340.

[5] Cohen, Sophia M., *J. Inf. Dis.*, 1940, **67**, 74.

TABLE I.
Source and Distribution of II Alpha Strains of Meningococci.

No.	Laboratory designation	Date received		
1	1054	6/3/37	Spinal fluid	Baltimore, Md.
2	1166	3/4/40	"	N. Y. State Lab.
3	1170	3/21	"	Syracuse, N. Y.
4	1171	3/28	"	Washington, D. C.
5	1184	12/27	"	Baltimore, Md.
6	1189	2/1/41	"	Nashville, Tenn.
7	1191	2/3	"	Seattle, Wash.
8	1194	2/14	"	Syracuse, N. Y.
10	1195	2/15	"	Galveston, Texas
11	1207	2/27	"	Nashville, Tenn.
12	1218	3/7	Nasopharynx	Camp Devens, Mass.
13	1219	3/7	"	"
14	1220	3/7	"	"
15	1223	3/7	"	Halifax, N. S.
16	1225	3/7	"	"
17	1252	5/6	Spinal fluid	Minneapolis, Minn.
18	1259	6/5	"	Washington, D. C.
19	1271	6/12	Nasopharynx	Ft. Geo. Meade, Md.
20	1279	6/30	Spinal fluid	Minneapolis, Minn.
21	1284	7/15	"	Cheyenne, Wyo.
22	1286	7/14	Blood	Syracuse, N. Y.
23	1294	10/8	Nasopharynx	Boston, Mass.
24	1296	10/27	Sputum	Baltimore, Md.

have not been found to show this phenomenon. Cohen[5] placed the capsulated strains tentatively in her "Group X" of atypical meningococci. In the 1940 report of the New York State Board of Health, Cohen[6] reported that about one-fourth (25%) of the Group II meningococci from New York State have fallen into the encapsulated group.

Undoubtedly, a distinct immunological entity is involved. It is related to Group II by agglutination but is quite distinct on a basis of protection. There is sometimes a very slight agglutinin relation to Group I.

It has been impossible to demonstrate a relationship between these organisms and the Type IV of Gordon and Murray[7] though their original type strain and other more recent strains have been studied. Rake's[8] Type VII, reported as being related to Group II, has been unavailable for comparison.

Since the meningococci just described have comprised one-fourth (25%) of the II's found in New York State, and one-half (53%) of the II's received by the National Institute of Health from the country at large, they apparently form a group of epidemiological and clinical

[6] Cohen, Sophia M., *Ann. Rep. N. Y. State Div. Lab. and Res.*, 1940, p. 27.
[7] Gordon, M. H., and Murray, E. G. D., *J. Roy. Army Med. Corps*, 1915, **25**, 411.
[8] Rake, Geoffrey, *J. Exp. Med.*, 1934, **59**, 553.

importance. Since they are a homogeneous group immunologically, and are excellent antigens, the most obvious explanation for the failure of polyvalent therapeutic sera to protect against such infections is that these strains have not been recognized as being different and have not been included in immunization of the animals which provided the serum.

In order that such strains and their significance may be recognized, it would be helpful to employ a convenient designation. The term "encapsulated II's" is awkward and also misleading, since there are often strains of this group in which capsules are not demonstrable. The term "Group X" is not sufficiently definite and does not indicate its relationship.

The first person to report immunological differences among the Group II meningococci was Dopter[9] when he described *alpha, beta* and *gamma* types of his "parameningococcus", naming them in the order of their frequency. Unfortunately, Dopter's original strains are no longer in existence but his idea offers a good precedent to follow. Since the strains just described comprise what is apparently the largest homogeneous unit among those related to Group II, they have been designated in this laboratory as Group II *alpha,* considering them tentatively as a sub-group of II. This apparent serological relation to Group II may be very superficial. Crossing in agglutination and in halo production with some rabbit sera are the only evidence for kinship.

Whether these are really a sub-group of II or something quite independent, only time and further study can tell. It will be easy to make a final classification when enough is known. Since Dopter's designation was the earliest and is applicable, it can well be brought into use for the present time.

Summary. About 50% of the strains of meningococci which have been superficially "typed" as belonging to Group II have been found to form an independent and homogeneous lot. Those who have worked with individual strains agree that many of them give a definite Quellung reaction, they are good antigens, and produce sera that protect mice well against infections by meningococci of the same group. But there is no cross protection with sera representing other groups.

Because of the close agglutinogenic relation to other Group II meningococci, it is suggested that, for the present, they be designated as Group II *alpha,* following the precedent set by Dopter in his studies of his parameningococcus since designated as Group II.

[9] Dopter, Ch., and Pauron, *C. R. Soc. de Biol.,* 1914, 77, 231.