THE MENINGOCOCCUS (NEISSERIA INTRACELLULARIS)

SARA E. BRANHAM

National Institute of Health, United States Public Health Service, Washington, D. C.

During the last decade there have been some interesting developments in our knowledge of the meningococcus. The purpose of this paper is to summarize these findings. It is necessary first to give a brief survey of our older knowledge of this microörganism in order to give perspective to our newer information.

This is not a monograph and is by no means comparable to the classic report of Elser and Huntoon (32) in 1909, or to the intensive review made by Murray (81) in 1929. It might in one sense be termed a "progress report" since much work is being done at present that may later clarify some of our greatest problems.

No attempt will be made to give a complete bibliography of all the work that has been done either during this decade or before, but for the sake of brevity reference will be made only to those papers that illustrate the facts under discussion.

This paper is written from a bacteriological point of view. Clinical and therapeutic discussions are not included as such, but occur only as bacteriological findings bear directly upon them.

HISTORY OF OUR EARLY KNOWLEDGE OF THE MENINGOCOCCUS

First recognition of meningococcus meningitis as a definite clinical entity goes back to the early days of the 19th century (101, 25), but it was to be many years before the causative organism became known.

In his *Handbook of Geographical and Historical Pathology*, published in 1886, Hirsch (56) devoted an interesting chapter to epidemic cerebrospinal meningitis. He discussed the epidemiology in great detail, described the occurrence of four major

epidemic cycles during the preceding fifty years, told of its seasonal preference, and mentioned the effect of fatigue and lowered resistance on susceptibility. He was one of the first to point out that the infection is especially apt to occur in children and in soldiers, and that the disease has a tendency to occur in waves, with a peak of incidence occurring about every ten years. He concluded that the disease must undoubtedly be of an infective nature, "transportable" rather than "contagious."

It was in the next year, 1887, that Weichselbaum (103) published his description of the meningococcus as recovered from six cases of acute cerebrospinal meningitis, and named it *Diplococcus intracellularis meningitidis*. Weichselbaum's description of the meningococcus is so accurate that there has been no occasion to dispute any of his findings in the years since.

During the following decade there appeared many case reports in which Weichselbaum's observations were confirmed. In many instances the meningococcus was merely seen in spinal fluid and in meningeal exudate at autopsy (70, 71, 97). In other instances it was actually cultivated (44, 55, 59, 66). In 1898, Councilman, Mallory and Wright (24) demonstrated the meningococcus in 31 out of 34 cases of meningitis. These reports securely established the organism as the cause of acute cerebrospinal meningitis.

Many fundamental facts about the meningococcus were established by these early students. They described its occurrence in pairs with flattened adjacent sides,—"two hemispheres separated by an unstained interval." The organism was apt to occur within the leucocytes in spinal fluid, but it might occur singly, in pairs, or in tetrads in cultures. It was described as being consistently gram-negative although the individual cells varied in the intensity of their staining. They found that it grew best on solid media, that it did not usually grow at room temperature, and that it was necessary to make fresh transplants at least every 2 days on the Loeffler blood-serum medium which they found best suited to it among the media then available. Weichselbaum found fresh cultures pathogenic for guinea pigs and rabbits, when given intraperitoneally, but Councilman,

Mallory and Wright were struck by the feeble pathogenicity of their strains for these animals and reported successful infection only in a goat.

The first decade of the 20th century brought further careful work on the meningococcus. A lively controversy was waged at one time by Albrecht and Ghon (1), on the one hand, and Jaeger (61, 62) on the other, concerning the gram-staining properties of this microörganism. Albrecht and Ghon felt that Jaeger's criterion of a meningococcus was too broad, and they collected evidence to show that the organism is definite in morphology and consistently gram-negative. The evidence presented by Albrecht and Ghon was further supplemented by Elser's report of 135 cases (31).

Flexner (37) studied especially the biology and pathogenicity of the meningococcus. He showed that virulence deteriorates rapidly and may disappear within a few days, and that "endotoxins" may be a greater factor in the death of animals than multiplication of the bacteria when large inocula are used. Even freshly isolated cultures were found to be of uncertain virulence. He produced a true meningitis in monkeys (38) by intraspinal inoculation, but preferred young guinea pigs, injected intraperitoneally, for experimental work. He paid considerable attention to the action of salt and believed the usual "physiological salt solution" to be very toxic for meningococci.

Wollstein (104) called attention to the close relationship of the meningococcus to the gonococcus. She stated that aside from the pathological effects in man, the chief difference between these organisms is cultural, and that demonstrable antibodies are largely common to both—a relationship which does not exist among other species in the genus *Neisseria* and which is generally overlooked. In 1909 the classic studies of Elser and Huntoon were published (32). In the same year, Arkwright (6) demonstrated that epidemic and sporadic strains were alike culturally and biochemically. He found some evidence of serological groups, but made no attempt at classification on this basis.

The period of classification really began in 1909, when Dopter

(27) found that the strains of meningococci studied by him fell into two serological groups. He called these "meningococci" and "parameningococci." Wollstein (105) made an intensive study of Dopter's strains and others, confirming his observations as to the existence of two groups indistinguishable except by serological methods. Furthermore, she found these serological differences to be supported by protection experiments with guinea pigs and monkeys, which were indicative but not absolute proof. It was about this time that Dopter and Pauron (28, 29) found that their parameningococci could be further divided into at least three subgroups, which they designated as α, β, and γ. This last very important finding seems to have been lost sight of in the years since.

THE WORLD WAR EPIDEMICS

The World War period of 1914–18 brought serious epidemics of meningococcus meningitis to the army camps of all nations involved. A tremendous amount of work was done on the meningococcus during this time and the literature of the period is voluminous. Much was done on the question of classification and on the relation of carrier strains to case strains, as ground work for control and therapy.

A confirmation of the existence of two main groups of meningococci resulted from the studies of most of the British workers. Griffith (51, 52), Scott (94), and Andrewes (5) designated these as Group I and Group II. Ellis (30) called them Type I and Type II. Arkwright (7) referred merely to "two main groups." It is interesting to note that Fildes (36), whose work was done several years later, realizing that the use of the terms "Type" and "Group" interchangeably was causing confusion, used the expressions "Super Group I" and "Super Group II" in order that his recognition of 2 groups might be perfectly clear.

A further division of these two groups into 4 types (I, II, III, IV), on the basis of agglutinin-absorption, was made by Gordon and Murray (46) in 1915, and a large number were typed on that basis in 1917–18 by Tulloch (99, 100) in Britain and in 1918–20 by Hitchens and Robinson (58), and by Butterfield and

Neill (21) in this country. During this same period the French workers, Nicolle, Debains and Jouan (83), described 4 types: 2 principal types (A and B) and 2 rare types (C and D). In the United States, Evans (33) classified meningococci according to their tropin reactions. She found 4 distinct groups, and a fifth group which was related to the others.

The relation of these different classifications to each other is shown in table 1. Some of the material in this table is taken from one published in 1929 by Murray in his classic monograph.

From this comparison of the different classifications that developed during this World War period, we can see that there

TABLE 1

Correlation of various classifications of meningococci

DOPTER (26)	NICOLLE, DEBAINS, AND JOUAN (83)	GRIFFITH AND SCOTT (51, 52, 94)	GORDON AND MURRAY (46)	EVANS (33)
Meningococcus	A	I	I III	R
Parameningococcus	B	II	II IV	S–U Z
	C D			T?

is a general agreement that meningococci fall into two main groups. The further division of these two groups into 4 types, as done by Gordon and Murray with the absorption of agglutinin technique, is laborious and requires sera made from very carefully chosen strains. As Fildes (36) pointed out, "Types I and III and Types II and IV of Gordon and Murray are so closely related that a change in standards could result in a change of type. The classification of meningococci is real and is not dependent upon arbitrary selection of standards. But selecting standards for determining the subgroups is arbitrary and may lead to much confusion if one and the same standard is not maintained."

In spite of its complications this 4-type classification of Gordon and Murray obtained great popularity at once and has been used

in nearly all English-speaking and South American countries from then to the present day. On the European continent, the French Groups A and B are more generally recognized.

There has apparently never been any uniformity in the terms used to designate the serological groups of the meningococcus by those accepting the Gordon and Murray classification. In their original report, Gordon and Murray (46) referred to Groups I, II, III and IV, using the term "Group" (capitalized) and with Roman numerals. In subsequent papers, Gordon (47) has referred to Types 1, 2, 3 and 4, using "Type" instead of "Group," and Arabic numerals. In one paper (48) he uses both Types 1, 2, 3 and 4 and Types I, II, III and IV. Most of those who used Gordon and Murray's classification in typing referred to Types I, II, III and IV and thus that form of designation is the one that has come into common use.

It was not only in classification that this activity in the study of the meningococcus was found. Extensive surveys of carriers were made in the effort to control the spread of the infection. These resulted in studies of the biology, physiology, and cultural features of the organism, as the nasopharyngeal and spinal fluid strains were compared.

Much attention was given, too, to the study of therapeutic serum. Jochman (64) had made the first antimeningococcus serum in 1906, and Flexner and Jobling had established its value in therapy (41, 42) in time for such serum to be used in treating patients during the severe army camp epidemics. During these epidemics it was found that all lots of serum were not equally effective. Much time was therefore given to the study of toxicity, pathogenicity, and antigenicity of the meningococcus, and to preparation of antiserum and its standardization.

Jochman (64) had standardized his first antiserum by a method involving the protection of guinea pigs and mice. Flexner (39) had used guinea pigs and monkeys for this purpose at first; but he did not consider such a method of standardization satisfactory because the meningococcus cultures were of such low virulence for these animals that the very large doses used seemed to kill from toxicity rather than by invasion. Flexner and Jobling

later used a complement-fixation test (40) and the neutralization of an autolysate in guinea pigs. Kraus and Doerr (67) and Dopter (26) had advocated protection tests against endotoxin as a basis for standardization.

Houston and Rankin (60) first studied phagocytosis with meningococci, and Neufeld in 1908 (82) was apparently the first to apply this technique to standardization of meningococcus serum. Jobling (63) considered specific phagocytosis the best method of standardizing antimeningococcus serum. He used the methods of Neufeld (82) and of Leishman (72). Kraus and Baecher (68) advocated this test also. A most interesting note in those early days was Ruppel's (92) report of a meningococcus culture of such virulence that 1 ml. of a 1:1,000,000 dilution was uniformly fatal to mice; this he used in standardizing serum. Here we seem to have the beginning of the mouse-protection test, as early as 1906.

All of these studies were made before serological differences among meningococci were recognized, and therefore before it could be known that many of these tests, such as complement fixation, were tests not only for all meningococci but for gonococci and some other members of the genus *Neisseria* as well. With the recognition of serological differences among meningococci, it was realized that not even tests that indicated the meningococcus alone would suffice for serum testing.

With the numerous epidemics of meningococcus meningitis that followed the World War came realization of the need of a uniform method of standardizing serum, one which would take the differences among meningococci into account. Amoss (2) was a leader in this field in this country. He used a number of strains of meningococcus in immunizing horses, employing cells and their autolysates, and tested the resulting sera for agglutination, phagocytosis, complement fixation, and protective value in young guinea pigs. He considered phagocytosis and complement fixation to be inadequate because they did not distinguish between types. Protection tests were found to give irregular results because of the low virulence of his cultures. He (3, 4) decided that agglutination gave the best idea of the value of the

serum and of its polyvalency. An agglutination test was finally adopted officially by the United States Public Health Service, and a standard technique for its performance was worked out by McCoy and Leake (75) in 1918.

In 1916 Hitchens and Robinson (57) described a mouse protection method of standardizing serum which they felt was a truer measure of therapeutic effectiveness of serum than agglutination. In 1920 (33) Evans described a technique based on the specific tropin groups of her classification, and their specific antibodies.

Meanwhile, in England, Gordon had become convinced that the "endotoxins" of the meningococcus were very important in the pathogenesis of this microörganism and that serum, in order to be useful, should contain "antiendotoxin." He described a method (49) in 1918 for determining the antiendotoxic content of the horse sera, using mice, and studied means of producing sera with higher antiendotoxin content. Gordon found considerable clinical evidence to support his views, but his test was not simple to perform and results were apt to be irregular. The success of the mouse protective test of Hitchens and Robinson depended upon high virulence of the meningococci used and cultures with this quality were not generally available. The technique of Evans' bacteriotropin test was very exacting. Thus the agglutination test was soon generally used. Those advocating it did not think it to be an ideal method but considered that it at least indicated the presence of antibodies and the polyvalency of the serum.

It will not be profitable to refer in further detail to the vast volume of literature of this World War period, as it would only leave the reader in a state of confusion. A tremendous amount of work was done, and the resulting literature was both voluminous and confusing. Then, with the coming of peace, meningococcus meningitis suddenly ceased to be so common. Cases of meningitis occurred only sporadically; meningococci isolated from them were more difficult to type. Both experimental work and preparation of antiserum were done with old strains carried over from the former period of intensive study.

INTEREPIDEMIC YEARS

From this World War period we emerged with a belief that our skirmishes with the meningococcus had led to advances along at least three lines: Meningococci were classified into 4 serological divisions, antiserum was polyvalent, and it was conveniently standardized by an agglutination test. During 1923 the first edition of Bergey's Manual (8) was published and the meningococcus appeared as *Neisseria intracellularis*, by which name it has since been known.

In this country antiserum was made in a number of localities by immunizing horses with the available cultures representing the 4 types of Gordon and Murray, and these antisera were standardized against a "control" serum by an agglutination test, using standard type strains as antigens. Some laboratories produced antiserum by immunizing horses with many strains, on the basis of Amoss' theory (2) that a great variation occurred among strains, and to be truly polyvalent many should be represented. Other laboratories used no more than 4 to 6 strains, on the basis of Wadsworth's theory (102) that a few well-chosen strains of good antigenicity were adequate. Apparently satisfactory antiserum was prepared by both methods.

Most of the actual and experimental work of this period was done, necessarily, with old strains of meningococci. They were, for the most part, both avirulent and non-specific. There was much "crossing" between types in agglutination, and good typing sera were difficult to prepare. Protection tests, using small animals, were thought to be out of the question. Bacteriologists who had worked much with the meningococcus recognized that the cultures had changed culturally, serologically, and antigenically. Many present workers can remember the ringing tones with which the late Doctor Krumwiede denounced these "degenerated meningococci," from the floor of bacteriological meetings. Occasionally promising new strains from some of the few cases of meningococcus infection would be obtained, but by the time studies with them were well under way, virulence was gone. There was, on the whole, not very much lively

interest in the meningococcus during this time. There were no extensive or severe epidemics. It was generally felt that serum therapy in meningococcus meningitis was well established and on a satisfactory basis. Plenty of antiserum was being made and standardized. There seemed no special cause for concern.

Suddenly, reported cases of meningococcus meningitis leaped in number from 1700 in 1926 to 3000 in 1927, and to 10,551 by 1929. Mortality was very high—as much as 70 to 80 per cent in some localities—and serum therapy seemed entirely inadequate. Something was evidently the matter. Why should serum therapy, which had been so successful during the years immediately after its introduction, be so useless now? Were the invading strains of the meningococcus different? Was serum being made by the wrong methods? Was the method of standardization at fault?

EPIDEMIC PERIODS OF 1928–30 AND 1935–36

Again the meningococcus became the subject of intensive interest. For a time there was abundant material for study. Literally, hundreds of fresh new cultures of meningococci were available.

Typing sera were prepared in rabbits, using the old standard type cultures representative of the Gordon classification. These cultures had "spread" antigenically, and typing was not always easy. By far the greatest number of the new strains fell into Types I and III, though a few were of Type II, and in the Chicago epidemic Type IV was abundant. It was realized very early that typing was easier if done soon after isolation of the strain, and that antigenicity soon began to "spread" and cross-agglutination became marked.

The Type IV cultures were easily separated from the others by simple agglutination. Everyone was surprised to find these Type IV strains a clear-cut, serologically, homogeneous group, with practically no antigenic affinity for Type II. Gordon's original Type IV strains did, indeed, cross-agglutinate with Type II, and antisera prepared from them agglutinated the new American Type IV strains, but the new Type IV strains were not agglutinated by any Type II antiserum.

All Type IV strains recovered during these epidemic years could be traced to a single outbreak in Chicago. None was found elsewhere, either here or abroad, and not more than two or three Type IV strains have been isolated since that time anywhere. So a comparative study of these Chicago Type IV strains with others has not been possible, and we can not yet know whether we have an "American Type IV" which is a "subtype" of the original Type IV, or whether these Chicago Type IV cultures represent merely one variety or strain that was responsible for that one epidemic. That the latter is a possibility is suggested by our knowledge that meningococcus strains of any type vary a great deal in serological pattern, and that those recovered from a given epidemic may be exactly alike in this respect, especially if the epidemic is an explosive one and the microörganism is passed from person to person rapidly. There is much that is mysterious about the Type IV meningococcus that can not be cleared up until new strains are found. The Chicago Type IV strains comprised approximately 8 per cent of all meningococci recovered and typed by us during the epidemic years of 1928–1930 (11). It was apparently the predominant type of the Chicago outbreak. In Chicago, too, a number of spinal fluids yielded a pigmented member of the genus *Neisseria* which was not a meningococcus. This was named *Neisseria flavescens* by the author (9) who studied 26 strains of it. Epidemiologically this was very interesting. Although other members of the genus *Neisseria* have been found in occasional sporadic cases of meningitis, no such organism had, so far as known, been involved in an epidemic before or since.

Type II strains were also easily separated from other types by simple direct agglutination. There was occasionally some cross-agglutination with Types I and III, but not enough to obscure the true identity, and not more, certainly, than would be expected with antiserum made with old strains. Less than 6 per cent of the spinal fluid strains studied during the 1928–30 epidemics were of Type II. They showed no cross-agglutination with any of the Type IV strains described above. These Type II strains came from widely separated localities and were not identical in sero-

logical pattern. They did not form as homogeneous a serological group as did the Type IV strains.

More than 80 per cent of the strains recovered during these days fell into Types I and III. About 64 per cent of both the Type I and the Type III strains could be identified as such by simple agglutination, whereas for 36 per cent of each type the laborious absorption of agglutinins was necessary for identification. Every degree of interrelationship seemed to occur. Some

TABLE 2

Apparent change in type of some strains of meningococci when different typing sera are used

NUMBER	STRAIN	SERUM Ia (178)						SERUM IIIa (146)						INDICATED GROUP	SERUM Ib (270)						SERUM IIIb (153)						INDICATED GROUP
		100	200	400	800	1600	3200	100	200	400	800	1600	3200		100	200	400	800	1600	3200	100	200	400	800	1600	3200	
1	304	4	4	4	3	1	0	4	4	1	0	0	0	I	0	0	0	0	0	0	4	4	3	2	0	0	III
2	321	4	4	4	3	3	1	4	4	4	2	1	0	I	3	3	2	1	0	0	4	4	4	3	2	0	III
3	328	4	4	4	4	3	2	4	4	4	2	0	0	I	3	3	2	0	0	0	4	4	4	3	2	0	III
4	335	4	4	4	4	3	0	4	4	3	1	0	0	I	1	1	1	0	0	0	4	4	4	4	2	0	III
5	337	2	2	1	1	0	0	4	4	4	4	3	0	III	3	3	3	3	3	0	3	3	3	2	0	0	I
6	348	4	4	4	4	2	0	4	4	4	4	4	3	III	4	4	4	4	4	3	4	4	4	2	2	0	I
7	350	4	4	4	3	1	0	4	4	4	1	0	0	I	0	0	0	0	0	0	4	4	4	3	1	0	III
8	357	4	4	4	3	2	1	3	3	2	1	1	0	I	2	2	2	1	0	0	4	4	4	3	2	1	III
9	366	4	4	4	4	3	1	4	4	3	1	0	0	I	4	4	4	0	0	0	4	4	4	4	3	0	III
10	178*	4	4	3	3	3	2	2	2	1	0	0	0	I	4	4	3	3	2	1	3	1	0	0	0	0	I
11	146*	3	3	2	1	0	0	4	4	4	4	3	2	III	3	3	3	2	2	0	4	4	4	4	3	2	III
12	270*	4	4	3	2	1	0	2	2	1	0	0	0	I	3	3	3	2	1	0	1	1	1	0	0	0	I
13	153*	2	2	0	0	0	0	3	3	3	3	0	0	III	2	2	2	1	0	0	3	3	3	2	2	0	III

* Control.

strains were antigenically "narrow," showing a relation to relatively few other strains; others were very "broad," crossing with nearly all strains tested. Many had to be done over and over; and with a few the interrelationship was so close that whether they were to be called Type I or Type III could not be determined. With such a situation it can be seen that a change of the strains used as standards for making the typing sera could result in an apparent change of type. Table 2 shows actual examples of such occurrence (12). Similar results were obtained by all who attempted typing of meningococci on a large scale at this time.

Gradually the conviction grew that although the information obtained about the intricate serological patterns of the meningococci was extremely interesting and might have some important applications, the effort spent in trying to separate Types I and III from each other was not actually profitable from a practical and routine standpoint. It seemed as though a much more satisfactory procedure would be to consider Types I and III together as a broad group, and to designate them as Group I, as had been done previously by Griffith (51, 52), Scott (94), and others (5, 30, 7, 36).

EPIDEMIC AND SPORADIC GROUPS OF MENINGOCOCCI

There are kinds of evidence other than serological for considering Types I and III to belong together in Group I. These are epidemiological, clinical, chemical, and immunological.

Records of the typing of meningococci go back to about 1915 in England and to about 1918 in this country. Since then, three great epidemic waves have occurred. The majority of strains isolated and typed during this period fell into the Types I and III, the percentages in this country during the 1928–31 period being 81 and 96, respectively. During interepidemic years the percentage of these fell as low as 6. The percentage of Type II strains, on the other hand, was as low as 4 and 5 during the 1928–31 epidemic period, and as high as 40 to 43 in interepidemic times. Thus we see that Group I (composed of Types I and III) has been responsible for all of the large epidemics of which we have serological information. This conclusion is illustrated in table 3. From the number of reported cases it can be seen that the peak epidemic years have been 1918, 1929, and 1936, with low incidence of meningococcus meningitis in the intervening endemic years. Hedrich (53) has discussed most interestingly this rhythmical recurrence of waves of meningococcus meningitis.

Thus epidemiological evidence indicates that we have an epidemic meningococcus, Group I, and another, Group II, responsible for sporadic cases. Group II meningococci are more common also in non-epidemic carriers; in fact, the percentage of

carriers of this microörganism may sometimes be quite high, and such persons may harbor them for many months or even years.

TABLE 3

Incidence of meningococcus infection and type distribution of meningococci in the United States since 1915

YEAR	NUMBER OF STATES REPORTING	NUMBER OF CASES	STRAINS STUDIED	TYPE DISTRIBUTION (IN PER CENT OF THE STRAINS EXAMINED)			
				I and III	II	IV	Other
1915	?	1,403					
1916	?	1,748					
1917	30(?)	4,705					
1918	30(?)	5,749	63	58.6	25.8	2.3	13.3
1919	30(?)	2,417					
1920	30	2,258					
1921	30	2,002	27	31.3	18.7	6.3	43.7
1922	30	1,527	15	6.7	0	13.3	80.0
1923	35	1,506					
1924	35	1,223					
1925	35	1,253					
1926	35	1,700					
1927	38	3,001	4				
1928	40	4,996	57	86.1	5.5	7.6	5.9
1929	46	10,551	89				
1930	44	8,384	82				
1931	41	5,518	26	96.0	4.0		
1932	41	3,102	19	68.0	32.0		
1933	44	2,913	7	57	43	0	0
1934	45	2,500	10	60	40	0	0
1935	43	5,736	91	84.4	13.2	0	2.2
1936	45	6,528	299	89.1	8.7	0	2.7
1937	44	5,484	132	84.0	16.0	0	0
1938	44	2,788	28	53.6	46.4	0	0
1939	49	2,051	33	45.4	54.6	0	0

Nasopharyngeal strains are often so non-specific that it is difficult to group them by agglutination, although they are agglutinated by polyvalent antiserum and have typical cultural and fermentation reactions. During epidemics Group I carriers are more apt to be found than in non-epidemic times, and there is reason to believe that it is usually these Group I carriers that transmit the infection. It is not so much the number of carriers

in a population that is important, as the kind of meningococcus that is being carried. Unless studies on this aspect can be carried through, as by the methods of Rake (91), carrier surveys are worse then useless.

Laybourn (69) has stated his belief that the chronic carriers of Group II strains are responsible for the sporadic cases, intermittently transmitting heavy infections to contacts who might be able to resist small numbers of these strains of lower virulence, but who succumb to the massive doses.

From a clinical standpoint, Group I strains of meningococci act alike. There has been no hint of any difference in clinical manifestation that would indicate the division of this Group into Type I and Type III.

The Group II strains, on the other hand, sometimes tend to produce a clinical picture that is different from the typical epidemic case. Such strains often do not localize in the meninges, but cause a generalized blood-stream infection. These cases often show a rash, which may be more or less purpuric, and they are sometimes mistaken for other infectious diseases, e.g., typhus or Rocky Mountain spotted fever. Although the microörganisms often remain for some time in the blood stream, there are few cases of fatal endocarditis due to Group II meningococcus; fatal meningococcus endocarditis is usually found to be caused by Group I strains.

In many cases in which Group II strains cause a typical meningitis, the illness tends to be prolonged, and sometimes runs a chronic course. Of course, Group I infections may also occur in a septicemic form without meningeal symptoms and with a purpuric rash. But such cases are more apt to be fulminating, and run a quick course.

Chemical studies of the meningococcus have indicated that there is no reason to divide Group I into Types I and III. Rake and Scherp (89) have demonstrated a specific carbohydrate which is common to the two types. This carbohydrate is a sodium salt of a polysaccharide acid (93). They have been unable to demonstrate a comparable carbohydrate in the Group II strains which they have studied.

The close relationship between Types I and III can be demonstrated also by the plate-precipitin, or "halo" method. This was first described by Petrie in 1932 (84), studied further by Kirkbride and Cohen (65), and later developed quantitatively by Pittman, Branham, and Sockrider (85) into a method for making a preliminary evaluation of immune serum. The relationship is also shown by the "quellung" reaction obtained with the Group I strains. This was first demonstrated by Clapp (22). We shall see later that the relationship between Types I and III is also shown by protection experiments.

Group II is much less homogeneous than Group I. The complexity of the group was first noted by Dopter (28, 29), who reported finding α, β and γ types of his "Parameningococcus." Many workers since that day have commented upon this heterogeneity. Group II strains seem closely related in agglutination at 56°C., but when other tests are employed differences among strains are very striking. These differences among Group II strains, as well as the contributions made by the "halo" technique and the "quellung" reaction to our knowledge of the meningococcus will be discussed more fully later.

Henceforth in this paper the terms "Group I" and "Group II" will be used to designate the two principal broad groups into which meningococci fall. The term "Type" will be used only where it is intended to designate some subdivision of a group in a special sense.

NEW KNOWLEDGE OF THE BIOLOGY OF THE MENINGOCOCCUS

Rake (87) has made a study of the biological properties of freshly isolated meningococci as compared with old stock strains. The older ones showed rough colonies, small and rather dry, whereas those newly isolated gave smooth, moist, glistening colonies. As these smooth colonies were subjected to usual laboratory maintenance, small rough variants gradually appeared and the two forms of colonies occurred side by side on plates. In time, the rough colonies became more and more numerous. The rough forms also became antigenically nonspecific and were agglutinated nearly equally by all monovalent

sera. Apparently Group II strains assumed the rough form more readily than those of Group I. Rake found that nasopharyngeal strains were often of the rough colony variety. Smooth colonies are usually 2 to 4 mm. in diameter, but the author has seen them a centimeter or even more.

Two other types of colonies must be mentioned. One is a minute rough colony which appears only occasionally side by side with other kinds, often in fresh cultures. Fishing and replating these colonies has always resulted in the appearance of both rough and smooth colonies, in the hands of the author, and repeated efforts to isolate a minute colony strain proved unsuccessful.

The fourth type of colony noted by Rake (87) and by the author also, is the mucoid type which occurs in a few strains. These colonies may be relatively large, much raised and cushiony. They are very mucoid and stringy and do not emulsify well.

Although he was unable to demonstrate a true capsule by the staining methods which he used, Rake (87) showed that the clear zone so often seen in stained preparations was wider in newly isolated strains than in stock strains, and that the rough variants showed no such zone at all. Since then, Clapp (22) has demonstrated a definite capsule on smooth Group I meningococci by means of the Neufeld quellung-reaction.

Gibbard (45) has shown in his electrophoresis studies that fresh strains have a higher P.D. than stock strains, and this finding has been confirmed by Branham (10).

Rake (88) also investigated the agglutinogenic properties of fresh as compared with stock strains of meningococci, preparing monovalent antisera in rabbits and studying the agglutination obtained with homologous and heterologous antigens. Differences of great importance were found. Sera prepared with fresh, smooth strains agglutinated the homologous antigens specifically and without crossing if the test was incubated for 2 hours at 37°C. and then placed in the icebox over night. When the test was performed with the usual 56°C. incubation over night, crossing was quite pronounced, and results only in the highest dilutions seemed significant. Sera prepared from rough stock

strains did not agglutinate well at 37°C., but gave good agglutination at 56°C. over night. This agglutination was not clear-cut, however, and much crossing occurred.

Apparently specific agglutination occurs first (88), and if smooth specific strains are used for immunization, clear-cut results can be obtained at the lower temperature. If the strains used for immunization are not smooth and specific, specific agglutinins will be lacking in the antisera and little agglutination occurs at 37°C. It is easy to understand how agglutination at 56°C. came to be the usually accepted technique during the years when fresh strains of meningococcus were rarely available. And it is also easy to see that the agglutination titers obtained at this higher temperature and with the overnight incubation time led to a false sense of security about the value of serum to be used therapeutically.

Chemical studies done during this time were enlightening. Rake and Scherp (89, 90), in their studies on the antigenic complex of the meningococcus, separated three fractions. One was a carbohydrate common to all meningococci, and present in some other microörganisms also, which they termed the "C" substance. This is, no doubt, the same carbohydrate which Zozaya (107) had recovered, in less purified form, from other members of the genus *Neisseria* and from certain other microörganisms as well; and it is also related to the alcohol-precipitable carbohydrate found by Miller and Boor (79) in the gonococcus and the meningococcus. It is probably the same as the "C" substance obtained by Tillett and Francis (98) from some of the pneumococci.

Another fraction obtained by Rake and Scherp (90) was protein in nature, and was likewise common to gonococci and to Type III pneumococci. This fraction was very toxic for animals, especially rabbits. Rake and Scherp termed this the "P" fraction.

The third fraction was found to be "Type"-specific and not to be common to all meningococci (89). In Types I and III, this fraction was carbohydrate in nature and identical for the Type I and the Type III strains studied, thus giving chemical evidence for the identity of these two types. Scherp (93) purified the Type I fraction and found it to be a sodium salt of a polysaccharide acid. The Type II specific substance was different in

nature since it gave only a weak Molisch reaction. This substance has been studied recently by Menzel and Rake (76), and they have found it to be protein in nature and antigenic. A "Type"-specific carbohydrate seems to be absent from this group according to these studies.

Recently isolated strains of Type IV have been unavailable for these chemical studies.

Evidence indicates that the specific carbohydrate is responsible for the highly specific precipitation reaction obtained with Group I strains of meningococcus on agar plates containing immune serum. Petrie (84) described the "halos" of specific precipitate which are formed by the interaction of the antibody in the serum with the specific polysacchride of the meningococcus when colonies of the latter are allowed to grow on agar plates containing the serum. This reaction is not merely species-specific but distinguishes clearly the Group I strains (Type I and Type III) from the Group II strains which do not produce the specific carbohydrate. Petrie found that he could distinguish between rough and smooth colonies in this way. Newly isolated strains of Group I meningococci gave the specific reaction whereas rough, older stock cultures did not. Kirkbride and Cohen (65) confirmed Petrie's observations in a study of many strains. They showed that the length of time during which a culture is maintained in a laboratory is not, in itself, responsible for its loss of specific substance, but rather the conditions under which the culture is kept. Some strains which they have cultured for many years are still smooth. The individual strains vary greatly, too, in their tendency to become rough. The degree of halo production seemed to correlate with the degree of smoothness or roughness, and with the degree of "type-specificity" of the strain as determined by this and other methods.

Maegraith (74) plated out very old Group I strains and found that individual colonies varied greatly in halo production, according to their smoothness or roughness, some giving good halo and others none at all. Pittman, Branham, and Sockrider (85) have utilized this specific halo reaction for preliminary evaluation of therapeutic sera.

Most Group II meningococci have not been found to produce

halos with the majority of polyvalent sera. The significance of this occurrence is not yet clear. It has just been pointed out that no specific carbohydrate has been found in Group II meningococci; nevertheless, some Group II strains do produce halos with sera containing a high content of specific Group II antibodies. It must also be remembered that most polyvalent sera contain very few antibodies that are specific for Group II. If better Group II antisera are produced, it is possible that it will be easier to demonstrate specific halos for Group II strains.

There are some indications that the specific carbohydrate fraction of the meningococcus is associated with the capsular layer of the cell. For one thing, cell washings are peculiarly rich in the specific carbohydrate. Then, too, we know that capsules can be demonstrated by the "quellung reaction" only in smooth strains that produce halos. As a rule, these are Group I strains, although Cohen (23) has reported capsule-swelling in certain Group II strains which we, also, have found to produce halos. In general, Group II strains do not show capsular swelling, nor do they produce halos, although the failure to produce halos may be due to the condition of the experiment.

Little (73) has shown that meningococcus cultures showing capsules behave differently in agglutination from those with no capsules. He referred to capsular agglutinins in the antiserum, and to somatic agglutinins. The capsulated meningococci are agglutinated by the capsular agglutinins and the non-capsulated meningococci by the somatic agglutinins. According to this thesis an antiserum made by immunizing horses with rough, non-capsulated meningococci would contain little or no capsular agglutinin, and the capsulated organisms would not be agglutinated easily by such serum. The non-capsulated antigens were agglutinated better by such sera. Conversely, serum made by immunization with smooth capsulated strains would agglutinate capsulated strains easily and quickly. It is no doubt the presence of the specific or capsular factors that cause specific agglutination; and that is why typing freshly isolated strains is easier than typing the same strains after a period of laboratory maintenance. "Crossing" is due to somatic or non-specific factors, and becomes

more and more pronounced as the strains become less specific. The specific carbohydrate is the same for all Group I meningococci, but the somatic pattern may vary among them greatly. Thus, when rough strains are used, results like those illustrated in table 2 may be found. In typing by agglutination, changing from one strain to another as antigen, or from one serum to another, may result in an apparent change of Type if overnight incubation at 56°C. is used.

Whether we refer to them as smooth, capsulated or type-specific it is plain that meningococci possessing these qualities are very different from the rough, non-capsulated, non-specific forms that all meningococcus cultures tend to become when maintained in the usual routine way. They are different culturally, serologically, and chemically, and we shall see that they also differ markedly in virulence.

VIRULENCE OF MENINGOCOCCI

In meningococci, virulence is a transient and unstable quality. So quickly do they lose it after isolation that until very recently the statement was usually made that the "meningococcus is not pathogenic for lower animals with the exception of the monkey." Such large doses of the microörganisms were necessary to kill small laboratory animals that they were considered to have died, in all probability, from the "endotoxins" of the bacterial cells and not from actual infection. Occasionally workers were able to find virulent cultures and to maintain them in this state long enough to do some valuable animal work. Such was the mouse-protection test for antimeningococcus sera that was worked out by Hitchens and Robinson (57). Other workers, lacking virulent cultures, were unable to obtain constant results, since the "endotoxins" in the large doses of their avirulent cultures interfered with their findings. Branham, Lillie and Pabst (13, 16) found many new strains to be virulent enough to produce fatal infection in experimental animals, although relatively large doses of the bacteria were necessary. Rabbits and guinea pigs developed a typical meningitis after intracisternal injection, and in the guinea pig the meningococci were often recovered from the blood.

Mice developed a generalized infection after intraperitoneal inoculation and the meningococcus could be recovered from the heart blood. Within a few days these new cultures of meningococci lost their virulence for these animals and such large inocula were required for killing that death could be attributed to toxicity instead of true infection by the microörganisms. Zdrodowski and Voronine (106), in Russia, also reported successful experimental infection of rabbits with meningococci.

During the winter of 1934–35 there was a sharp increase in the number of meningococcus infections, and many of the strains obtained were of sufficient virulence for a time to infect mice in doses of approximately 100,000 bacteria. Branham (14) used these strains to evaluate antimeningococcus serum by a mouse-protection method. She succeeded in keeping the strains virulent for several months but finally found it necessary to lay them aside for fresher strains. No standardization of method was possible under these conditions.

Two important events occurred about this time which opened the way for rapid progress in knowledge of the meningococcus. One of these was the demonstration by Miller (78) that gastric mucin, as a medium for suspension, brought out, or protected, the virulence of meningococci so that it was possible for as few as 2 to 10 highly virulent meningococci to infect a mouse. This meant that even moderately virulent strains of meningococci could be used for study in animals, and that many multiples of a minimum fatal dose could be used in protection tests without introducing the "endotoxin" factor. Moreover, the meningococci could be kept at maximum virulence for mice by frequent mouse passage, suspended in mucin. By "maximum virulence" is meant that a dose of from 2 to 10 meningococci, in mucin, is sufficient to kill a mouse.

The other important event was the development of the lyophile method of preservation of cultures. Although preservation of cultures by freezing and drying was by no means a new idea, the perfection of the Flosdorf-Mudd (43) apparatus did much to bring this method into an easy laboratory routine. Meningococci live for several years when preserved thus. When culti-

vated again, especially after several rapid transfers, they show the same characteristics that they possessed when they were frozen and dried in vacuum. Thus freshly isolated virulent strains may be kept virulent for a long time.

Newly isolated strains of meningococci vary considerably in their initial degree and duration of virulence for mice. It is much easier to keep some strains virulent than others. Virulent cultures are always smooth, and the Group I strains always produce specific halos and have capsules. The virulence of Group II strains is especially apt to be transient. Fewer Group II strains seem to possess capsules or produce halos, though this may be actually due to the low content of Group II antibodies in serum. Virulent Group II strains are, however, always smooth.

Freshly isolated strains of meningococci differ from each other in many ways besides the duration of their virulence. They vary in the persistence of capsules, halo production, and other evidences of smoothness and specificity. Strains maintained at maximum virulence for mice vary in their susceptibility to phagocytosis. Heist and the Solis-Cohens (54), and Silverthorne (95, 96) have shown that freshly isolated virulent cultures of the meningococcus withstand the lytic action of fresh human blood better than older, avirulent strains. Branham (20) has found this to be generally true, although individual strains, kept at maximum virulence for mice, vary greatly in this respect. She found, too, that they vary in susceptibility to serum- and to drug-therapy (18) when used experimentally in mice. Either these variations are inherent in the individual strains and independent of virulence, or else there is a virulence of a kind not measurable by any of the tests yet applied.

Variation in sensitivity to serum treatment is especially important, since serum-therapy in meningococcus meningitis is so widely employed. Table 4 shows this difference in sensitivity to serum treatment. Six strains of meningococci, each of maximum virulence for mice, were included in a study with one "polyvalent" antimeningococcus serum. It can be seen that the amount of serum necessary to protect 50 per cent of the mice varied from as little as 0.0013 ml. with Strain 1041 (Group I)

to a point where 0.1 ml. gave practically no protection against Strain 1054 (Group II).

Table 5 shows the differences in response of these same 6 strains of meningococci to sulfanilamide (18). All mice were protected from Strains 1041 (I), 1037 (I), and 1054 (II) by the

TABLE 4

Difference in sensitivity to one antiserum of 6 strains of meningococci, all of maximum virulence for mice

(100,000 M.F.D.)

STRAIN	GROUP	AMOUNT SERUM B PROTECTING 50 PER CENT MICE
1027	I	0.0056 ml.
1037	I	0.0015 ml.
1041	I	0.0013 ml.
963	II	0.0065 ml.
1054	II	No protection
1108	II	0.020 ml.

TABLE 5

Variation in sensitivity of 6 strains of meningococci to sulfanilamide

STRAIN	GROUP	PERCENTAGE OF DEATHS ACCORDING TO AMOUNT OF SULFANILAMIDE GIVEN				
		1 mgm.	2 mgm.	4 mgm.	8 mgm.	No drug
1027	I	80	10	10	0	100
1041	I	0	0	0	0	100
1037	I	40	0	0	0	90
963	II	60	60	0	0	100
1054	II	60	0	0	0	100
1108	II	80	60	60	60	90

doses used, whereas a small percentage was protected against 1108 (II). Similar results were obtained with sulfapyridine.

It may be mentioned here that the combination of antiserum and drug was far more effective in protecting mice than either agent alone, more even than would be accounted for by the sum of the two effects (15, 18). Apparently a synergistic action is present; and it is not virulence alone that seems responsible for this difference.

When meningococci are established at maximum virulence for mice it is relatively easy to keep them so for indefinite periods by following a routine for their maintenance. Of course, it is simple to dry them in vacuum by the lyophile method and to store them until needed. In this case several rapid transfers (e.g., twice a day) on blood-agar slants should be made before titration of the virulence of the strain (86). But often it is necessary to keep cultures on culture media at maximum virulence over long periods of time. This is especially desirable in the case of those strains used in evaluating sera by a standardized technique. The media that prolong viability for the greatest time are not those that are best for maintaining virulence; indeed, the very opposite seems to be the case as a general rule. Semi-solid agar is excellent for maintaining stock strains for at least a month, but to maintain virulence it is necessary to resort to some other method, such as transfer every two days on blood-agar slants or three times a week on serum-glucose-agar slants. It is likely that the excellent experimental results which Kirkbride and Cohen (65) have reported with old laboratory cultures are due to their having maintained their cultures in a similar fashion. Occasionally mouse passages are necessary to supplement these frequent transfers, these to be done bi-weekly or weekly according to the strain. Cultures grown for 5 or 6 hours on blood-agar slants are best for use when virulence and freedom from autolytic products are desired.

MENINGOCOCCI AS ANTIGENS

It is important that something should be said about the behavior of meningococci as antigens in the immunization of animals.

Group I strains are very much better antigens than Group II strains, and smooth strains are much better in this rôle than rough strains. In fact, if the smooth capsular antigens are not present, specific antibodies for these antigens can not be produced and only the rough, or somatic antibodies will be found.

In rabbits, the earliest agglutinins for Group I are the most specific. As immunization continues, the range of the serum-

agglutinins spreads, so that much cross-agglutination between types and groups occurs. Precipitins are much slower to appear than agglutinins.

Rabbits require much longer immunization with Group II strains to yield a serum containing measurable antibodies, whether agglutinins, precipitins, or protective antibodies. In fact, a good agglutinating serum for Group II is difficult to obtain. Precipitins and protective antibodies for Group II are even more unattainable. Individual strains of Group II vary in antigenicity even more than those of Group I. Serum from rabbits injected with one of our standard Group II strains (No. 963) over a period of 9 months showed practically no protection for mice, although the strain was of maximum virulence for mice. Experience with horses has been similar to that with rabbits.

Since Group II strains are generally such poor antigens, it is not surprising that polyvalent therapeutic antimeningococcic serum is usually very low in specific antibodies for Group II strains, and that such sera are often ineffective in Group II infections.

As long as agglutination was the only method of evaluating antisera, these shortcomings of Group II strains as antigens were not obvious. Incubation at 56°C. for 18 hours allows agglutination to occur with rough, somatic, or nonspecific agglutinins and agglutinogens as well as with the specific, smooth capsular ones. A good agglutinin titer at this temperature may thus be sometimes misleading.

Miller's (78) introduction of mucin to the field of meningococcus study has allowed the development of a mouse-protection technique which has been very revealing. This mouse-protection test has not as yet been adopted as the official method of testing antisera for obvious reasons. Every factor involved is variable-mouse, culture, mucin, medium, serum, and individual worker. Pittman has discussed these factors in detail (86). Each must be standardized as accurately as possible so that comparable results can be obtained on various testings and in different hands. After that is done, a trial period will be necessary in order that the results of testing with mice can be correlated or compared with

clinical results. The mouse-protection test has shown that most polyvalent sera have very few specific antibodies for most Group II strains.

The poor antigenicity of Group II strains, in general, and the resulting low content of specific antibodies for Group II in sera may account for the relatively rare demonstration of halos with these strains on serum agar plates. That such halos do occur with some of these strains and good sera has been demonstrated. Since no specific carbohydrate has been demonstrated for Group II, the halo reaction may be dependent on some less active fraction. When better Group II sera are prepared, more light may be shed on this phase of the problem.

It is interesting to note the close correlation that exists between mouse protection and halo production (i.e. the plate precipitin test) in the sera studied. This is especially evident with the polyvalent sera, both whole and concentrated, which are made in various localities. More than a hundred such antisera have been studied (85, 86), using mouse protection, halo production, and agglutination at both 37° and 56°C. The agglutination at 37° is more closely correlated with protection and halo production than is agglutination at 56°. This suggests that it is the specific capsular antibodies that are important in protection. Hence, on an immunological basis there seems to be no reason for separating Group I meningococci into Types. If animals (i.e., rabbits or horses) are immunized with either Type I or Type III strains, the serum from them protects mice against infection with either type. But these sera do not protect against Group II strain infections, nor does a Group II antiserum protect against the Group I (Types I and III) infections.

TOXINS OF THE MENINGOCOCCUS

Toxin-production by the meningococcus has been the subject of much discussion. That products of the meningococcus are extremely toxic for animals was recognized in some of the earliest studies on this microörganism. When cultures of low virulence were used in animal studies, the amount of "endotoxins" was often great enough to kill the animals, aside from the infection

induced. Recognizing that these "endotoxins" were the chief lethal factor of such cultures, Flexner (40) used autolysates of the meningococcus instead of living cultures in testing his sera in 1908. This procedure was also recommended by Kraus and Doerr (67). Gordon (49, 50) believed antiendotoxins necessary for good therapeutic sera and described a technique for determining their antiendotoxic potency. He discarded all sera that did not have an appreciable antiendotoxic content as measured by his tests in mice. Results obtained with "endotoxins" were found to be irregular, and this technique of Gordon did not come into general use. For a time the toxic products of the meningococcus were ignored.

In 1931, Ferry, Norton, and Steele (34) described a toxin which they obtained from young broth cultures of certain strains of meningococci. Only those strains which grew as a pellicle at the surface of the medium produced this toxin. After the pellicle had sunk to the bottom of the flask the culture gradually became non-toxic. They considered this to be a true soluble toxin, and produced antitoxin by immunizing horses with filtrates of these cultures. Ferry has (35) reported some neutralization of the toxin with antitoxin in monkeys.

There has been much discussion about this toxin. Some workers have disagreed with Ferry and others as to the nature of this toxin and have insisted that it is endotoxic and the product of autolysis. None of these workers has, however, followed the technique described by Ferry and his co-workers; instead, they have incubated their cultures for a number of days, long enough for abundant autolytic products to accumulate and to mask the action of any weak soluble toxin which might be present.

Laboratory proof of the true toxin nature of this poison is difficult to obtain. The usual laboratory animals are extremely resistant when it is given intravenously, intraperitoneally, or subcutaneously, and so protection with antitoxin according to the law of multiple proportions has not been demonstrable. When it is given intracisternally to rabbits, guinea pigs, or monkeys, very small amounts produce an acute purulent leptomeningitis, often fatal, which is clinically and histologically indistinguishable from

that caused by the meningococcus itself (13, 16). Since horse-serum is itself very toxic for the meninges of rabbits and guinea pigs, neutralization with antitoxin injected by the intracisternal route has not been demonstrated (17).

At present toxin is demonstrated by intradermal injection into human subjects (34). Approximately 50 per cent of individuals give a positive skin test with Ferry's toxin in high dilutions. The antitoxin is standardized by the partial neutralization of a positive skin test made with the toxin (34). This is admittedly unsatisfactory, but it is the only method of toxin-antitoxin neutralization that is now available.

It is evident that filtrates of certain strains of meningococci, grown in a special broth medium for 24 to 48 hours, contain a substance or substances highly toxic for some laboratory animals when given intracisternally, producing positive skin reactions in a high proportion of human beings. Some strains are apparently much better than others for this purpose. This toxin is produced only by cultures growing at the surface and forming a pellicle, and it seems to disappear on prolonged incubation. Later, much "endotoxin" accumulates. Although it is possible that the toxin is very dilute "endotoxin," as is claimed by some, it is also quite likely that these two toxic products are distinct from each other. Whatever the nature of this product may be, those who have followed Ferry's technique have found this toxin, although they may not have found it for all of the serological types.

Antitoxin produced by the immunization of horses with Ferry's toxin is very low in content of agglutinins, precipitins, and antibodies that protect mice against infection, but many clinicians feel much enthusiasm for it as a therapeutic agent. The discovery of some satisfactory way of demonstrating toxin-antitoxin neutralization for the meningococcus will be a welcome event.

PRESENT CLASSIFICATION OF MENINGOCOCCI

By way of a summary, and at the risk of perhaps needless repetition, we shall conclude the discussion with the following remarks.

In some ways the classification of meningococci has become

simplified. On epidemiological, clinical, chemical, and immunological grounds there seems to be no real reason for dividing Group I meningococci into Types I and III. This division can be done serologically with the majority of the strains of this group by absorption of agglutinins if enough time and effort are spent, and if the strains and sera used as a basis of the study are sufficiently specific. But the interrelationship of these two types is so close that a change of standard strains can result in a change of the resultant typing.

Epidemiologically, there are found only two main groups of meningococci, Group I and Group II—an epidemic group and an endemic group. These differ from each other in clinical manifestation, chemical composition, and immunological behavior. The endemic Group II includes most carrier strains, and a high proportion of those from sporadic cases. Group I has been responsible for most of the epidemics during the past 25 years, i.e. the period over which we have serological information.

Group II is more heterologous than Group I. This was recognized as early as 1914 by Dopter and Pauron (28, 29), who described α, β, and γ types of their "Parameningococcus." Representative cultures of these types are no longer available, so we have no way of knowing whether or not they are related to some of the peculiar Group II strains that are encountered. All of the latter strains which are agglutinated by Group II antiserum are considered to fall into Group II, though variations among them in serological pattern may, at times, be very striking. Other differences may be found among these Group II strains. Only a few of them have been found to have capsules as demonstrated by the quellung-reaction (23). These have invariably been found to produce conspicuous halos with polyvalent sera that yield no halos with other virulent, smooth Group II strains (19). Immunologically, these strains seem quite different from the other Group II strains. They are good antigens, and serum from rabbits immunized with them protect mice against the homologous and related strains, but not against the other Group II strains (19). Conversely, sera which protect against the other Group II meningococci do not protect against these capsulated

strains. There has been no chemical work reported upon this subgroup, so that at present we have no knowledge as to whether or not there may be a capsular carbohydrate specific for it. Possibly these strains may not belong to Group II at all, in spite of their agglutinogenic affinities.

Menzel (77) has worked with certain Group II strains which are serologically comparable to those used as "standard" Group II strains for typing and serum-testing. He has obtained no carbohydrate there, but has found the specific fraction to be protein in nature. Since the strains with which he has worked are typical of the usual Group II strains, he and Rake are referring to these strains as "Type" II and are considering them as a type within Group II. The capsulated halo-producing Group II strains referred to above show important differences from these.

Except for those recovered during an epidemic, nasopharyngeal strains are usually of Group II. These are frequently rough and non-specific, whereas those from spinal fluid and blood are usually smooth and specific. Some nasopharyngeal strains are so completely rough that they can not be typed. These are sometimes agglutinated equally well by both Group I and Group II antisera. Since they are probably devoid of the specific fractions, even absorption of agglutinins would not give satisfactory results.

The "Type IV" strains that have been found in this country have not had the close relation to the "Type II" strains of Gordon and Murray that their original strains had. Type IV strains are very rare in the United States now and those which are maintained here are too old for their relationships to be accurately determined. Possibly they, too, represent a subgroup or type within Group II; or they may be an independent group.

We may summarize the subject of classification of meningococci by saying that they fall into two broad serological Groups, I and II. These may be divided into subgroups or Types. The Types I and III of which Group I is composed, are alike epidemiologically, clinically, chemically and immunologically, so that there seems to be no reason for separating them as a routine procedure. Group II is apparently also composed of subgroups or types.

Immunological and probably chemical differences within this group are becoming more apparent since the development of the mouse-protection and other techniques. There seems to be at least one subgroup that is immunologically distinct, and it is possible that the separation of this subgroup may prove to be of practical clinical importance.

The relation of the "Type IV" of Gordon and Murray to the present Groups I and II is not understood.

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