PARAMENINGOCOCCUS AND ITS ANTISERUM.*

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In the course of the treatment of epidemic meningitis with the antimeningococcic serum it became evident that a proportion of cases of the disease treated with the serum failed to react favorably. In some of these cases the meningococcus contained in the cerebrospinal exudate survived, continued to multiply, and failed to be phagocyted by the leucocytes, in contradistinction to what happened in the majority of instances. That the meningococci occurring in the resistant cases were in part resistant, or fast, to the antiserum was suspected, and two possible kinds of fast strains were recognized: first, strains originally fast at the time the serum treatment was begun; and, second, strains developing fastness in the course of the serum treatment.[1] The second group embraced those instances in which meningococci at first reacted to the serum but later failed to do so, leading to a relapse which continued to a fatal termination.

A more precise definition of the fast strains has not thus far been made. However, Dopter[2] has studied a special class of the cocci obtained from the nasal secretion and called by him parameningococci, which while resembling the common meningococcus in fermentative and cultural properties differs from it in certain immunological reactions. The parameningococcus, so called, has now been found by Dopter and other French writers to invade the meninges and the blood and is believed by them to be one of the causes of cerebrospinal meningitis.[3] According to the French ob-

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[1] Flexner, S., *Jour. Exper. Med.*, 1913, xvii, 553.

[2] Dopter, Ch., *Compt. rend. Soc. de biol.*, 1909, lxvii, 74.

[3] The literature has been reviewed by Dr. Flexner in his article on antimeningococcic serum (Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, 2d edition, Jena, 1914 (in press)).

servers, when acute meningitis arises through the presence of the parameningococcus it is not controllable by means of the ordinary antimeningococcic serum, but does respond to a special antiserum prepared with cultures of the parameningococcus.

The subject of the atypical strains of the meningococcus is an important one since it affects the question of a better control of cases of epidemic meningitis by means of a serum containing antibodies for the unusual varieties. On that account we have made a study of two cultures of the parameningococcus obtained from Dr. Dopter through the kindness of Dr. Louise Pearce, who made a hurried journey to Paris to secure them, since it was found that the cultures sent by post did not survive the journey.

IMMUNITY REACTIONS OF THE MENINGOCOCCUS AND PARAMENINGOCOCCUS.

The meningococcus is subject to the several immunity reactions of agglutination, complement deviation, and opsonization. All the reactions are specific, although certain strains react not at all or imperfectly. As regards agglutination, it may be said that considerable variations arise affecting strains of meningococci relatively or absolutely inagglutinable. And yet agglutination is perhaps the most trustworthy guide in the identification of the meningococcus through specific serum reactions.[4] While immune sera may possess high agglutinating value for meningococci, normal sera possess either none, or very little; the inagglutinable strains react to immune sera of high value no more than they do to normal sera.

On the one hand, the inagglutinable strains react differently to a given meningococcic serum, while again a given agglutinable strain may react unequally to immune sera prepared from different strains of meningococci.[5] It should further be noted that while homologous sera tend to agglutinate corresponding strains best, exceptions occur in which they agglutinate heterologous strains better.[5,6]

[4] Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, *loc. cit.* Elser, W. J., and Huntoon, F. M., *Jour. Med. Research,* 1909, xx, 371.

[5] Eberle, J., *Arch. f. Hyg.,* 1908, lxiv, 171.

[6] St. Baecher, in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, Jena, 1911, supplement 1, 80.

Notwithstanding the variations indicated, it is obviously still desirable to determine the degree in which parameningococci differ from normal meningococci in respect to immunity reactions, and to ascertain whether they form a special class or merely constitute variations from the normal type. For this purpose two cultures, L and M, of the parameningococcus secured from Dr. Dopter have been subjected to the reactions of agglutination, complement deviation, and opsonization, respecting which they have been compared with several strains of normal meningococci. The immune sera employed consisted of a polyvalent antimeningococcic serum prepared by the Department of Health of the City of New York, and several monovalent sera produced in the rabbit by immunization with single strains of the microörganisms.

It will conduce to clearness and simplicity of presentation to describe briefly the different strains of meningococci which were studied.

The parameningococci consisted of Gram-negative diplococci indistinguishable from ordinary meningococci in form, staining properties, and fermentative reactions. They were also subject to autolysis in the manner of normal meningococci.

Twenty other strains of meningococci were employed for comparison. Four, HP, MA, B, and 138, came from the Pasteur Institute, having been secured by Dr. Pearce along with the parameningococci. They were regarded as normal strains. It may be noted here that all four exhibited irregularities of agglutination, and B proved inagglutinable.

One strain, W, came from Great Britain in 1908, and was isolated from a case of posterior basic meningitis. It was a normal strain. The remaining fifteen strains were obtained in New York, partly from the stock of The Rockefeller Institute, partly from the Department of Health.[7] They have been classified as follows: Eight are normal strains agglutinating regularly. They are designated F, 20, 25, 28, 35, 45, 48, and 49. Cultures 25 and 48 were derived from rapidly fatal fulminating cases of meningitis; 28 is from a severe case terminating fatally on the fourth day; 45 is from a fatal case developing basic symptoms; 35 is from a mild case becoming chronic, in which hydrocephalus developed before death; and 49 is from a mild case which recovered.

Five are normal strains agglutinating irregularly. BH was derived from a fatal case in an infant; 9 and 18 were without history; and 37 was obtained from a case which recovered under serum treatment. Two strains, 7 and 42, gave agglutination reactions similar to the parameningococci, and had been employed in the manufacture of the antimeningococcic serum by the New York Department of Health. Table I furnishes a means of ready reference.

[7] For the Board of Health cultures I am indebted to Dr. Phoebe Du Bois and Dr. Marie Grund.

TABLE I.

Source of Cultures.

Designation.	Source.	Nature of strain.	Type of agglutination.
L	Dopter	Para	
M	Dopter	Para	
HP	Pasteur Institute	Normal	Irregular.
MA	Pasteur Institute	Normal	Irregular.
138	Pasteur Institute	Normal	Irregular.
B	Pasteur Institute	Normal	Inagglutinable.
W	Great Britain	Normal	Regular.
F	New York	Normal	Regular.
18	New York	Normal	Regular.
25	New York	Normal	Regular.
28	New York	Normal	Regular.
35	New York	Normal	Regular.
45	New York	Normal	Regular.
48	New York	Normal	Regular.
49	New York	Normal	Regular.
BH	New York	Normal	Irregular.
I	New York	Normal	Irregular.
9	New York	Normal	Irregular.
20	New York	Normal	Irregular.
37	New York	Normal	Irregular.
7	New York	Normal	Para-like.
42	New York	Normal	Para-like.

Besides the results of agglutination, other immunity reactions were studied; those, namely, of opsonization, complement deviation, and protection. These reactions are subject also to irregularities and variations, and notably that of complement deviation,[8] which has been generally given up as a method of determining the value of the antimeningococcic serum in therapeutic immunity principles.

AGGLUTINATION.

Several immune sera were employed for determining the immunity reactions. One, that of the New York Health Department, was prepared in the horse from many strains of meningococcus and preserved with 0.3 per cent. tricresol. The monovalent sera were made by immunizing rabbits with single strains. The inoculations were conducted over periods of several months. For the general

[8] Flexner, S., in Kraus, R., and Levaditi, C., Handbuch der Technik und Methodik der Immunitätsforschung, *loc. cit.*

TABLE II.

Monovalent Rabbit Serum Immune to Parameningococcus L. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
Para L	−	++	++	++	++	++	+
Para M	−	++	++	+	+	+	−
48	−	+	+	±	−	−	−
45	−	+	−	−	−	−	−
49	−	++	+	±	−	−	−
42	−	++	++	++	+	+	−
35	−	++	−	−	−	−	−
37	−	+	+	+	+	−	−
138	−	+	+	±	−	−	−
28	−	++	±	−	−	−	−
25	−	++	++	+	±	−	−
20	−	++	++	++	+	±	−
18	−	++	++	++	+	±	−
9	−	++	++	+	±	−	−
7	−	++	++	+	+	−	−
B	−	−	−	−	−	−	−
MA	−	+	+	+	−	−	−
HP	−	+	+	+	−	−	−
W	−	++	+	+	±	−	−
I	−	+	+	+	−	−	−
BH	−	+	+	+	+	−	−
F	−	+	+	−	−	−	−

TABLE III.

Monovalent Rabbit Serum Immune to Parameningococcus M. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
Para M	−	++	++	++	++	+	+
Para L	−	++	++	+	+	±	−
48	−	+	±	−	−	−	−
45	−	+	+	+	−	−	−
49	−	++	++	+	±	−	−
42	−	++	++	++	+	+	−
35	−	+	±	−	−	−	−
37	−	++	++	+	±	−	−
138	−	+	+	+	−	−	−
28	−	+	+	−	−	−	−
25	−	+	+	±	−	−	−
20	−	++	++	++	+	−	−
18	−	++	++	++	+	−	−
9	−	++	++	++	+	−	−
7	−	++	++	++	+	+	−
B	−	+	−	−	−	−	−
MA	−	+	+	+	+	±	−
HP	−	+	+	+	+	+	−
W	−	++	+	±	−	−	−
I	−	++	++	++	+	+	−
BH	−	+	+	+	+	+	−
F	−	+	+	±	−	−	−

work the two Dopter strains of parameningococci and normal strains 35 (mild case), 45 (basic case), and 48 (fulminating case) were used.

At the expiration of three months the titer of the rabbit sera did not exceed 1 to 500. But as normal rabbit serum is inactive in dilutions greater than 1 to 10, the specific effects could be readily followed. A few exceptions with normal strains occurred. Thus strains 45 and 138 agglutinated in 1 to 20, and strain 37 in 1 to 50. Normal horse serum is inactive above 1 to 20. Here again certain normal strains, namely 25, 45, and 138, were somewhat more sensitive and reacted in 1 to 50 to 1 to 100 dilutions.

Parameningococcus.—The polyvalent antimeningococcic horse serum was almost wholly inactive upon the two Dopter strains of parameningococci, while it agglutinated the two para-like strains 7 and 42 in dilutions 1 to 200. It should be recalled that the two latter strains were employed in the preparation of the serum.

The monovalent parameningococcus rabbit sera exhibited varying titers according as they acted upon the homologous or heterologous strains. With the former the limit was 1 to 500, with the latter 1 to 200. No difference was noted between the sera prepared from strains L or M (tables II and III).

On the other hand, the two para-like strains, 7 and 42, gave slightly different reactions according to the source of the immune parameningococcus serum. With para serum L, strain 42 agglutinated at 1 to 200, and strain 7 at 1 to 100; with para serum M, both agglutinated at 1 to 200. In other words, strains 7 and 42 behave as heterologous para strains against these two sera.

If we turn now to a monovalent serum prepared from normal strain 48 which agglutinated its own and two other normal strains in 1 to 500, the two Dopter para strains, L and M, agglutinated in 1 to 20, and the two para-like strains at 1 to 20 (strain 42) and 1 to 50 (strain 7) (table IV).

However, the Dopter para sera are not without agglutinating effects on normal strains of meningococcus, and both those that agglutinate regularly and irregularly. With para serum L, among the former, strain 18 reacts in 1 to 100, strains 25 and W in 1 to 50 dilutions; among the latter, strains 37 and BH react in 1 to 100,

strain 20 in 1 to 100 dilutions. With para serum M regular strains 18 and 49 react in dilutions of 1 to 100 and 1 to 50, respectively, irregular strains HP, I, and BH react in 1 to 200, and strains 9 and 20 in 1 to 100 dilutions.

TABLE IV.

Monovalent Rabbit Serum Immune to Meningococcus 48. Agglutination Reactions Made at 55° C.

Strains.	C	10	20	50	100	200	500
48	−	++	++	++	++	+	+
Para M	−	++	+	±	−	−	−
Para L	−	++	+	−	−	−	−
45	−	+	+	+	+	−	−
42	−	+	+	−	−	−	−
49	−	++	++	++	+	−	−
35	−	++	++	++	++	+	+
37	±	++	++	++	++	+	−
138	−	++	++	++	++	+	−
28	−	++	++	+	−	−	−
25	−	++	++	++	++	++	+
20	−	++	+	+	−	−	−
18	−	++	++	++	++	+	±
9	−	++	++	+	±	−	−
7	−	++	++	±	−	−	−
B	−	+	+	±	−	−	−
MA	−	+	+	+	+	−	−
HP	−	+	+	+	+	+	−
W	−	++	++	++	+	±	−
I	−	++	+	+	±	−	−
BH	−	++	+	+	+	+	−
F	−	++	++	++	+	−	−

The conclusion to be drawn from this series of tests is not to the effect that parameningococcus strains are strictly different as regards agglutination from normal strains of meningococcus, but that they nevertheless display a certain relative specificity.

Normal Meningococcus.—Two classes of normal meningococci have been recognized. They have been denominated "regular" and "irregular" according as they agglutinate in all or only in part of the normal immune sera. The variations in regard to agglutinability among normal strains are wide, as is exhibited in table V. It is this great variability that makes it impracticable on the basis of agglutination alone to separate certain strains as being a distinct group or species. It remains true, however, that the group distinguished by the name of "para" departs even more widely from the normal

standard than do the several irregular strains studied. And this difference reappears in respect to other immune reactions to be described.

TABLE V.

Agglutination Reaction at 55° C. Limit Dilutions for Complete Agglutination.

Strain.	Sera.				
	Board of Health.	Para M.	Para L.	Normal 48.	Normal 35.
Para L	1 : 10	1 : 100	1 : 500	1 : 20	1 : 10
Para M	1 : 10	1 : 500	1 : 200	1 : 20	1 : 20
Para-like 7	1 : 200	1 : 200	1 : 100	1 : 20	1 : 50
Para-like 42	1 : 100	1 : 200	1 : 200	1 : 20	1 : 50
Normal regular W	1 : 500	1 : 20	1 : 50	1 : 100	1 : 100
Normal regular F	1 : 50	1 : 20	1 : 20	1 : 100	1 : 50
Normal regular 18	1 : 50	1 : 100	1 : 100	1 : 200	1 : 100
Normal regular 25	1 : 50	1 : 20	1 : 50	1 : 500	1 : 200
Normal regular 28	1 : 100	1 : 20	1 : 10	1 : 50	1 : 50
Normal regular 35	1 : 10	1 : 10	1 : 10	1 : 500	1 : 500
Normal regular 45	1 : 50	1 : 50	1 : 10	1 : 100	1 : 200
Normal regular 48	1 : 50	1 : 10	1 : 20	1 : 500	1 : 50
Normal regular 49	1 : 100	1 : 50	1 : 20	1 : 100	1 : 100
Normal irregular BH	1 : 500	1 : 200	1 : 100	1 : 200	1 : 200
Normal irregular I	1 : 100	1 : 200	1 : 50	1 : 50	1 : 100
Normal irregular 9	1 : 200	1 : 100	1 : 50	1 : 50	1 : 50
Normal irregular 20	1 : 50	1 : 100	1 : 100	1 : 50	1 : 100
Normal irregular 37	1 : 100	1 : 50	1 : 100	1 : 200	1 : 100
Normal irregular 138	1 : 200	1 : 50	1 : 20	1 : 200	1 : 50
Normal irregular HP	1 : 20	1 : 200	1 : 50	1 : 200	1 : 200
Normal irregular MA	1 : 200	1 : 100	1 : 50	1 : 100	1 : 100
Inagglutinable B	1 : 20	1 : 10	0	1 : 20	0

Hence it is apparent that a clean cut classification into parameningococcus and meningococcus strains has been possible with thirteen only of the twenty-two strains whose agglutination reactions have been studied. The remaining nine act either irregularly or so nearly alike in all the sera tested that definite discrimination is not possible.

Attention is directed also to the fact that lack of agglutination by polyvalent antimeningococcic horse serum is insufficient evidence for the classification of meningococci into para and normal strains, since even normal meningococci do not invariably agglutinate in such a serum in high dilution, and some strains fail to agglutinate in dilutions greater than 1 to 20.

OPSONINS AND COMPLEMENT DEVIATION.

Opsonins.—The opsonin content is employed extensively for determining the therapeutic value of antimeningococcic serum. It was desirable therefore to test the specificity of this reaction upon normal and para strains of meningococci. For this purpose the Neufeld technique was employed. The result is shown in table VI, which tends again to isolate the two parameningococcus strains of Dopter from the strains of normal meningococcus employed.

TABLE VI.

Opsonization.

Serum.	Strain.	Control.	1:10	1:20	1:50	1:100	1:200	1:500	1:1,000	1:2,000	1:5,000
Board of Health	Para L	−	+	−	−	−	−	−	−	−	−
	Para M	−	+	−	−	−	−	−	−	−	−
	48	−	+	++	+	+	+	±	−	−	−
	35	±	++	+	+	±	−	−	−	−	−
	I	−	++	++	++	++	+	+	+	+	±
Fulminating case	48	−	+	+	+	+	+	±	−	−	−
	35	−	++	++	++	+	+	±	−	−	−
	Para L	−	+	+	+	−	−	−	−	−	−
	Para M	−	+	+	−	−	−	−	−	−	−
	I	−	+	+	+	±	−	−	−	−	−
Para M	Para M	−	+	+	+	+	+	+	−	−	−
	Para L	−	+	+	+	+	−	−	−	−	−
	48	−	+	+	±	−	−	−	−	−	−
	I	−	+	+	±	−	−	−	−	−	−
Para L	Para L	−	+	+	+	+	+	±	−	−	−
	Para M	−	+	+	+	+	−	−	−	−	−
	48	−	+	+	+	−	−	−	−	−	−
	I	−	+	±	−	−	−	−	−	−	−
Mild case	35	−	++	++	++	++	+	±	−	−	−
	48	−	++	++	+	+	±	−	−	−	−
	Para L	−	+	+	−	−	−	−	−	−	−
	Para M	−	+	+	−	−	−	−	−	−	−

Complement Deviation.—The degree of deviation of complement exerted by an antimeningococcic serum was recommended by Kolle and Wassermann to estimate its therapeutic value. Since, however, it appears that the reaction is subject to considerable and unexplained fluctuations it has not been generally adopted. None the less, it was desirable to determine the degree of specificity of the reaction as applied to para and normal strains.

Antigens were prepared by the method of Schwartz and McNeil.[9] When the antigens made with the parameningococci of Dopter were tested against the polyvalent antimeningococcic horse serum, complement was bound. When, however, the monovalent parameningococcic sera were titrated against normal meningococci, complement deviation occurred only in low dilutions. When the parameningococci antigens were titrated against monovalent sera prepared with normal meningococci strains 48 and 35, no binding was obtained. On the other hand, antigens of strains 48 and 35 deviated complement of the homologous sera in low dilutions (table VII). The action of the polyvalent antimeningococci serum corresponds with the results obtained by Dopter,[10] who, however, also noted that the sera of patients suffering from parameningococcal infection deviated complement in the presence of parameningococci but not of normal meningococci. If this is strictly true, then it must be held that the monovalent rabbit sera are less specific than the serum of patients.

The conclusion to be drawn from the tests of complement deviation is again to the effect that the para strains deviate from the normal strains, although the distinction cannot be said to be absolute.

PROTECTION EXPERIMENTS.

In the end the existence of profound differences between the para and normal strains of meningococci will be determined not so much by the immunity reactions already described, as by the results of protection tests, since in practice it is this test that determines whether special account should be taken of the para strains in the preparation of the antimeningococcic serum. For the purpose of the protection tests guinea pigs and monkeys were employed.

That guinea pigs weighing about 125 grams are especially susceptible to inoculation with cultures of the meningococcus was noted by Flexner.[11] I am able to confirm his observation. The tests were conducted with lethal doses by intraperitoneal injection of the

[9] Schwartz, H. J., and McNeil, A., *Am. Jour. Med. Sc.*, 1912, cxliv, 815.
[10] Dopter, *Bull. et mém. Soc. méd. d. hôp. de Paris*, 1911, xxxi, series 3, 590.
[11] Flexner, S., *Jour. Exper. Med.*, 1907, ix, 105.

TABLE VII.

Complement Deviation.

Complement, guinea pig serum, 1:40 dilution, in c.c.	Immune rabbit serum Para M, in c.c.	Para M antigen, in c.c.	Sheep corpuscles, 1:20 dilution, in c.c.	Anti-sheep rabbit serum, 1:100 dilution, in c.c.	Result.
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.2	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
		Culture 48 antigen.			
0.1	0.1	0.25	0.1	0.1	Incomplete hemolysis.
0.1	0.2	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
	Immune serum, fulminating case.				
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	No hemolysis.
		Culture 35 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	No hemolysis.
		Para L antigen.			
0.1	0.1	0.25	0.1	0.1	Complete hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Para M antigen.			
0.1	0.1	0.25	0.1	0.1	Complete hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
	Board of Health serum.				
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Complete hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Para L antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.
		Culture 48 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	Incomplete hemolysis.
0.1	0.01	0.25	0.1	0.1	Incomplete hemolysis.
		Culture 35 antigen.			
0.1	0.1	0.25	0.1	0.1	No hemolysis.
0.1	0.1	0.15	0.1	0.1	No hemolysis.
0.1	0.01	0.25	0.1	0.1	Complete hemolysis.

several cultures alone and combined with homologous and heterologous sera. Preliminary experiments were made to determine the effects of normal rabbit and horse serum. They were found not to protect in corresponding doses against the cultures used. The cultures alone in the doses given invariably caused death. The following is a detailed example of a protective experiment in young guinea pigs, of which table VIII presents the results in brief.

Experiment 1.—A. May 27, 1914. Two guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M.

May 28. Both guinea pigs were dead.

B. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Para M immune rabbit serum.

May 28. Four guinea pigs were living but one was ill.

May 29. One guinea pig was dead.

C. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Para L immune rabbit serum.

May 28. All four guinea pigs were dead.

D. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of immune rabbit serum 48.

May 29. Three guinea pigs were dead.

E. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of immune rabbit serum 35.

May 28. All four guinea pigs were dead.

F. May 27. Four guinea pigs weighing 120 gm. each were injected intraperitoneally with 0.2 c.c. of a 24-hour old sheep serum agar culture of Para M, followed at once by 0.5 c.c. of Board of Health antimeningitis serum.

May 28. One guinea pig was dead.

What is noticeable is the general fluctuation of protection in that each monovalent serum, while being most perfectly protective for its homologous organism, exerts, also, some, if variable, amounts of protection against other or heterologous organisms. In this regard it cannot be said that the para strains of Dopter acted more regularly and specifically than the normal strains. On the whole, and as was probably to be expected, the Board of Health polyvalent serum showed the greatest regularity of action. In other words, this polyvalent serum carried protective immune bodies in about equal amount for the normal and para strains.

It was deemed desirable to ascertain the protective value of mono-

TABLE VIII.

Serum Protection Experiments.

Serum.	Cultures employed.				
	Para L.	Para M.	Culture 48.	Culture 35.	Culture 45.
Para L	Protected all	Protected none	Protected 8 of 10	Protected all	Protected none.
Para M	Protected 4 of 10	Protected 7 of 10	Protected 5 of 10	Protected none	Protected none.
Culture 48	Protected 6 of 10	Protected 4 of 10	Protected all	Protected 9 of 10	Protected none.
Culture 35	Protected 4 of 8	Protected none	Protected 5 of 6	Protected all	Protected none.
Board of Health	Protected 5 of 6	Protected 5 of 6	Protected 5 of 6	Protected 3 of 6	
Normal horse	Protected none	Protected none	Protected none	Protected none	
Normal rabbit	Protected none	Protected none	Protected none	Protected none	

valent sera upon monkeys infected by intraspinal inoculation and treated in the same manner. For this purpose parameningococcus L (Dopter) was employed for infection. The culture proved to be of low virulence, necessitating large doses in order to set up fatal infection. The method was to inject the culture and then immediately afterwards the immune sera. The following small series of experiments was made.

Experiment 1.—Control. A *Macacus rhesus* received intraspinally the surface growths of two sheep serum water agar slant cultures suspended in normal saline. Three hours after the injection the animal became ill; death occurred in twenty hours. At autopsy the meninges were congested and edematous; cultures of parameningococcus L were recovered.

Experiment 2.—A second *Macacus rhesus,* having received a similar dose of the suspended culture, was given five minutes later 1.5 c.c. of parameningococcus rabbit serum L. Slight symptoms of illness only developed. Twenty-four hours later lumbar puncture yielded turbid fluid containing polynuclear leucocytes enclosing diplococci; no diplococci were found outside of cells. A second dose of 1.5 c.c. of the immune serum was administered. At the expiration of another twenty-four hours the animal appeared well and the cerebrospinal fluid was clear.

Experiment 3.—A third *Macacus rhesus* was inoculated with the established dose of parameningococcus L and five minutes later was given 1.5 c.c. of immune rabbit serum prepared from normal meningococcus 48. No protection was afforded, and death occurred within twenty hours.

Experiment 4.—The fourth and last test was made with parameningococcus L and immune rabbit serum prepared with parameningococcus M. It was a repetition of experiment 2. The animal recovered completely.

The series of tests with monkeys is of value in supporting the group distinction between the normal and para meningococci. It is highly improbable that in a larger series of experiments some degree of cross-protection should not have been found between the normal and para organisms. On the other hand, the experiments indicate that para sera L and M are equally effective for protection against a given parameningococcus as in the case of para organism L.

DISCUSSION.

The study of *Diplococcus intracellularis* or meningococcus and allied organisms has led to the setting up of four classes as follows: (1) pseudomeningococci found by von Lingelsheim;[12] (2) diplococci derived from cases of posterior basilar meningitis described by Houston[13] and other English workers; (3) S strains isolated by Friese and Müller[14] from the nasopharynx of patients not having meningitis, and classified by Sachs-Müke[15] as pseudomeningococci; and finally (4) the diplococci described by Dopter as parameningococci. Von Lingelsheim's cocci are so readily differentiated from true meningococci by their morphological and cultural characteristics that they require no further mention. The other three classes, however, are described as being morphologically and culturally indistinguishable from true meningococcus, differing only in serum reactions, especial stress being laid upon differences in agglutinating power.

The diplococci from cases of basilar meningitis have been shown to be true meningococci,[16] a fact further substantiated by the tests

[12] von Lingelsheim, W., *Klin. Jahrb.*, 1906, xv, 373.
[13] Houston, T., and Rankin, J. C., *Brit. Med. Jour.*, 1907, ii, 1414.
[14] Friese, H., and Müller, H., *Klin. Jahrb.*, 1909, xx, 321.
[15] Sachs-Müke, *Klin. Jahrb.*, 1911, xxiv, 425.
[16] Wollstein, M., *Jour. Exper. Med.*, 1909, xi, 579.

made in the present study with diplococci from two personal cases of chronic basilar meningitis, and by one strain sent by Dr. Houston in 1908.

The S cocci described by Friese and Müller were not obtained from meningitis patients nor from persons who had been in contact with cases of meningitis, and all the cultures differed markedly in agglutination reactions from strains of true meningococcus. In the absence of other serum tests it is not possible to bring these cocci into relation with parameningococci.

Dopter's parameningococci remain, then, in a class by themselves, differing serologically more or less from other diplococci. Although Dopter first found them in the nasal mucus of persons who had been in contact with meningitis patients, other observers soon demonstrated their presence in the blood and cerebrospinal fluid. Thus in 1910 a case of purpura fulminans with septicemia was observed by Carnot and Marie,[17] from which the organism was isolated from the blood. No meningitis was present in this case. Menetrier[18] was the first to report a case of meningitis due to parameningococcus. The patient was an infant and Menetrier noted a marked difference between the apparent mildness of the symptoms and the intensity of the infection as evidenced by the character of the cerebrospinal fluid, in which the majority of the cocci were extracellular. Injections of the usual antimeningococcic serum instead of causing amelioration of symptoms and fall in temperature were followed by intensification of symptoms. Seven cases of meningitis due to the parameningococcus having come under Dopter's observation, he recommended the therapeutic use of ordinary antimeningococcic serum to be followed later by an antiparameningococcic serum if laboratory examination showed the presence of parameningococcus infection. Dopter[19] prepared such an antiparameningococcus serum in 1912, and its use was followed by the recovery of cases of meningitis caused by the parameningococcus

[17] Carnot and Marie, P.-L., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1911, xxxi, series 3, 74.

[18] Menetrier, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1911, xxxi, series 3, 82.

[19] Dopter, *Semaine méd.,* 1912, xxxii, 298.

(Salin and Reilly,[20] Mery, Salin, and Wilborts,[21] Menetrier and Avezou,[22] and Hallé[23]). In these cases two or three injections of ordinary antimeningococci serum were said to have been without effect, while the injection of the antiparameningococcus serum was followed by prompt improvement. Hallé, noting that only extracellular cocci were present in the cerebrospinal fluid after the ordinary serum had been given, did not wait for cultures of the diplococcus before resorting to the antiparameningococcus serum. He believes that a mixture of para and true meningococcus serum will give good results in the treatment of meningitis, though he agrees with Netter[24] that polyvalent serum is best. Netter uses a mixture of the two sera, but believes that a polyvalent serum, like that made in America, and which has given him excellent results, fulfills all requirements.

SUMMARY AND CONCLUSIONS.

The parameningococci of Dopter are culturally indistinguishable from true or normal meningococci, but serologically they exhibit differences as regards agglutination, opsonization, and complement deviation.

Because of the variations and irregularities of serum reactions existing among otherwise normal strains of meningococci it does not seem either possible or desirable to separate the parameningococci into a strictly definite class. It appears desirable to consider them as constituting a special strain among meningococci not, however, wholly consistent in itself.

The distinctions in serum reactions between normal and para meningococci are supported by the differences in protective effects of the monovalent immune sera upon infection in guinea pigs and monkeys.

[20] Salin, H., and Reilly, J., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1913, xxxv, series 3, 423.

[21] Mery, H., Salin, H., and Wilborts, A., *Bull. et mém. Soc. méd. d hôp. de Paris,* 1913, xxxv, series 3, 411.

[22] Menetrier, P., and Avezou, J., *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 45.

[23] Hallé, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 149.

[24] Netter, *Bull. et mém. Soc. méd. d. hôp. de Paris,* 1914, xxxvii, series 3, 53.

It is therefore concluded that it is highly desirable to employ strains of parameningococcus in the preparation of the usual polyvalent antimeningococcic serum. It remains to be determined whether it is better to employ the parameningococci along with normal meningococci in immunizing horses, or to employ normal and para strains separately in the immunization process and to combine afterwards, in certain proportions, the sera from the two kinds of immunized horses.