THE SERUM REACTIONS (COMPLEMENT FIXATION) OF THE MENINGOCOCCUS AND THE GONOCOCCUS.

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THE present communication contains a record of further attempts to establish by serological methods some criterion for the differentiation of the *Meningococcus* from the *Gonococcus.*

According to the usually accepted views of the classification of the Gram-negative cocci, an organism which occurs on what may be called neutral ground, *e.g.*, the human wrist-joint, and which resembles the Meningococcus in morphology and staining reactions and will not grow on artificial media except at a temperature above 22° C., must be regarded as a Meningococcus or a Gonococcus or may possibly belong to the group of Gram-negative cocci called Pseudo-meningococci, amongst which should perhaps be included the *Micrococcus pharyngitidis flavus* III. of v. Lingelsheim.

The Pseudo-meningococcus as described by Kutscher (1906), Lieberknecht (1908), Elsler and Huntoon (1909) and others, is an organism or group of organisms which occurs fairly frequently in the healthy human naso-pharynx and which resembles the Meningococcus culturally. According to these writers it can, however, be distinguished with varying degrees of certainty from the true Meningococcus by the agglutination test. My own observations (Arkwright, 1909) on the agglutination of different strains of Meningococcus derived from the central nervous system, lead me to place little reliance on this method as a sure test to apply to an unknown strain from a doubtful source.

Dopter (1909 II.) described strains of Meningococcus-like organisms occurring in the naso-pharynx, which were not agglutinated by a meningococcal serum, but which gave a complement-fixation reaction with the same serum. To these strains he gave the name of Parameningococcus. Recently the same writer (Dopter, 1911) has

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described seven cases of sporadic meningitis from all of which he isolated the 'Parameningococcus.' Probably Dopter's strains of 'Parameningococcus' belong to the same category as the strains of Meningococcus which have been isolated by various workers (Eberle, Trautmann, Arkwright, etc.) from the meninges in cases of meningitis, but which have not been found to react with meningococcal sera prepared with other strains of Meningococcus.

It seems, however, reasonable to expect that it would be much easier to distinguish by serological methods the Gonococcus from the Meningococcus than the latter from an organism resembling it so closely as the Pseudo-meningococcus.

In spite of the usually fairly obvious cultural differences between the Gonococcus and the Meningococcus and the sharp line that is to be drawn between the sources and pathological associations of these two organisms, experience does not bear out the anticipation of wide divergence as regards serum reactions.

Brief review of the methods advocated for the differentiation of the Meningococcus from the Gonococcus.

I. Cultural and biochemical tests.

(1) Meningococci grow on media with a much wider range of alkalinity than do Gonococci, and after the first few subcultures Meningococci will almost invariably grow well on ordinary neutral agar. This is not the case with the Gonococcus which requires either serum-agar or a specially prepared agar distinctly alkaline to litmus as recommended by Thalmann (1900, 1902) or a medium prepared with phosphate of soda as used by Blair Martin (1910). It can, however, easily be shown that different strains of Gonococcus prefer different degrees of alkalinity, and many observers have noticed that some strains of Gonococcus quickly become accustomed to ordinary neutral agar.

(2) On serum-agar, or agar of a suitable reaction, the colonies after 24-hours' growth in the case of the Gonococcus are always discrete and very small or pin point, whereas the colonies of the Meningococcus on the same medium are frequently confluent or, if few and discrete, are of much larger size.

These cultural characters on solid media appear to the writer to be the most constant features which differentiate cultures of the Gonococcus from those of the Meningococcus—an opinion also expressed by Wollstein (1907).

(3) Growth on carbohydrate media has been widely used and found of considerable value as a means of differentiation. Most observers, *e.g.* v. Lingelsheim, Elsler and Huntoon, Blair Martin and others have laid stress on the inability of the Gonococcus to ferment maltose, but I have not found this to be a constant distinguishing feature. Some strains of Gonococcus produce a distinct acidity when grown on serum-peptone-water or serum-agar to which pure maltose has been added (Arkwright, 1907, 1909; Wollstein, 1907; Gurd, 1908), and I have met with undoubted strains of Meningococcus from the cerebro-spinal fluid of cases of meningitis which did not ferment maltose. In considering these sugar reactions it must be borne in mind that the growth of Gonococcus on culture media neutral to litmus is usually very feeble and the production of acid from glucose is also less than in the case of the Meningococcus.

II. Serological tests.

I. *Agglutination.* Though many writers, v. Lingelsheim (1906), Kutscher (1906), Krumbein and Schatiloff (1908) and others have described very uniform results obtained with meningococcal serum and different strains of Meningococcus, this has not been by any means the universal experience of workers.

The objection to placing reliance on agglutination as a means of recognising the members of the different groups is based on two sets of facts: (1) the want of uniformity obtained with different members of the same group, *e.g.*, the Meningococcus, when a meningococcal serum is used (Trautmann and Fromme, 1908; Eberle, 1908; Ditthorn and Gildermeister, 1907; Lieberknecht, 1908; Elsler and Huntoon, 1909; Arkwright, 1909), and (2) the fact that some strains of Meningococcus will agglutinate with a gonococcal serum and *vice versâ* (Vannod, 1906; Dopter and Koch, 1908 VII.; Elsler and Huntoon, 1909; Wollstein, 1907). In the last case the experiments are further complicated by the difficulty of obtaining good uniform emulsions of the Gonococcus.

I found that, when working with a monovalent meningococcal serum, the number of strains agglutinated was very limited, and even when a polyvalent serum of a titre of 1–1000 made by injecting twelve different strains of Meningococcus was employed, strains of Meningococcus were easily found which were not agglutinated more highly by the specific serum than by normal serum (Arkwright, 1909).

Possibly the greater uniformity obtained by some observers has been due to the use of strains all of which occurred in the same epidemic

and to the employment of polyvalent sera made with many such strains.

It has been claimed by Dopter and Koch (1908 VII.) that by the use of the method of absorption of agglutinins a specific agglutination reaction can be demonstrated, even when a given serum agglutinates both the Meningococcus and the Gonococcus equally before absorption.

Since, however, a meningococcal serum may agglutinate some strains of Gonococcus but affect only a limited number of strains of Meningococcus, the method of absorption can only have a very restricted application.

Moreover absorption experiments (Arkwright, 1909) with different strains of Meningococcus and a meningococcal serum gave a differentiation between the strains of Meningococcus used similar to that obtained by Dopter and Koch between the Meningococcus and the Gonococcus. Torrey (1907) obtained results of the same kind in his studies on the agglutination of the Gonococcus.

II. By means of the Opsonic index Houston and Rankin (1907) claimed to be able to distinguish epidemic from sporadic strains of Meningococcus. This method according to their results would be quite useless for distinguishing the Gonococcus from the Meningococcus. Wollstein (1907) was unable to distinguish by opsonic experiments the Gonococcus from the Meningococcus.

III. The Precipitin reaction has been advocated by Dopter and Koch (1908 X.) and Dopter (1909 I.) as a means of differentiation in this group of organisms, but the experiments recorded by them were too few in number to justify the deduction of definite conclusions. Dopter carried the differentiation further by absorbing the precipitins.

IV. Complement fixation when applied for the same purpose has given very varying results. Vannod (1906) and Krumbein and Schatiloff (1908) consider this reaction specific for the Meningococcus and for the Gonococcus with their respective sera. Krumbein and Schatiloff used a polyvalent meningococcal serum and also gonococcal serum. Watabiki (1910), though not obtaining such distinct results with the two groups of cocci, maintained that the Gonococcus could be differentiated from other cocci by this reaction. Arkwright (1909) found this method in no way superior to agglutination as a means of distinguishing the Meningococcus from the Gonococcus. Wollstein (1907) working with monovalent sera found no distinction between the Meningococcus and the Gonococcus. Colombo (1911) has recently published a series of observations on complement fixation with these two organisms and

has found no specific difference between them. He used almost entirely polyvalent sera.

The experience of various observers as regards agglutination suggested that the use of polyvalent sera for other serum reactions in this group of organisms might be delusory. Meningococci and Gonococci appear to fall, as regards agglutination, into subgroups the members of which react among themselves, but not with the members of other subgroups of the same organism (Torrey, 1907; Arkwright, 1909). Unless, therefore, the serum used were obtained by injecting all the subgroups, it might fail to produce the reaction with the cocci which were members of the remaining subgroups and which had not been injected. A polyvalent serum might give very uniform reactions with six strains but not with a seventh or eighth. On the other hand, although a polyvalent meningococcal serum gives a reaction with some strains of Gonococcus as well as with some strains of Meningococcus, it is possible that a monovalent meningococcal serum might be obtained which had no affinities with any strain of Gonococcus. If such a monovalent meningococcal serum gave uniformly positive results with all strains of Meningococcus and negative results with all the strains of Gonococcus which were available, the result would be significant and the use of such a serum for the classification of new strains might be of value. Unless, however, a serum with such strictly specific activity can be obtained, serological tests are not of much value for making a final and conclusive diagnosis of a given strain of a meningococcus-like organism.

A polyvalent serum is very unlikely to fulfil these conditions and consequently a series of monovalent meningococcal and gonococcal sera were prepared with the object of testing the specificity of the complement-fixation reaction with monovalent sera.

Preparation of immune sera.

For my experiments monovalent sera were prepared by injecting rabbits intravenously with emulsions of cocci in increasing doses. Heated cocci were used first, but for the later injections living cultures were employed. Considerable difficulty was experienced in obtaining sera of sufficient strength, as the rabbits frequently died after the later doses, especially when the Gonococci were being used.

The strains of Meningococcus and Gonococcus used for injection were grown on horse serum agar.

Eventually three monovalent meningococcal and five monovalent gonococcal sera were obtained which gave fairly well marked complement fixation with the homologous cocci or extracts.

Experiments were also made with one polyvalent serum and one monovalent serum obtained from the horse.

Strains used.

The strains of Meningococcus and Gonococcus used in these experiments were the following:

Meningococci:

M. XII	Isolated from the meninges of a sporadic case of meningitis.
M. 119	Isolated post-mortem from the spinal cord of a case of acute epidemic meningitis.
M. 135	Isolated from the cerebro-spinal fluid of a sporadic case of meningitis.
M. 141	From the cerebro-spinal fluid of a sporadic case of meningitis.
M. 162	From the cerebro-spinal fluid of a sporadic case of meningitis.
M. 164	From the cerebro-spinal fluid of a very acute sporadic case of meningitis.
M. 165	From the cerebro-spinal fluid of an adult case of meningitis occurring in an epidemic area.

Gonococci:

G. 1	Isolated from the vaginal discharge of a child suffering from vulvo-vaginitis.
G. 2	From a case of acute gonorrhoea in an adult male.
G. 3	From another case of acute gonorrhoea in an adult male.
G. 4	From a case of acute gonorrhoea in an adult male.
G. 6	From a case of vulvo-vaginitis in a child.

Preparation of the antigen extract.

Extracts of the cocci were used as "antigen" for the complement-fixation reaction and various methods of extraction were tried. The following method was found to be the best of those experimented with:

An emulsion of the growth on ascitic agar in a Roux bottle was made with 10 c.c. of salt solution. This was centrifuged and the deposit made up to its original volume with salt solution. After adding a few drops of chloroform and shaking, the emulsion was left at room temperature for three or four days. The extract was then centrifuged before use. It was found that if the deposit from the last centrifuging was again made up to the original volume with salt solution and left for a further period of two to three days, a second extract as good as the first could be obtained, and by again repeating the same process, a third

and even a fourth extract could sometimes be obtained of almost undiminished value for complement-fixation experiments.

The extract was diluted eight or in some cases sixteen times before use and the specific meningococcal or gonococcal serum was diluted eight times. The largest dose of serum or extract diluted as above was 0·5 c.c. and a tube containing a double dose of serum and another with a double dose of extract were always put up as controls. Some experiments were made in which falling doses of extract were used with a constant dose of specific serum, and other experiments in which the dose of extract was constant but the doses of serum decreased in the successive tubes. The results yielded by the two methods were on the whole alike.

Haemolytic system.

The haemolytic system used consisted of sheep's corpuscles, rabbit-v.-sheep serum, and guinea-pig complement. The haemolytic serum was titrated each day with the complement and a double haemolytic dose of serum was used with 0·5 c.c. of a 1–10 dilution of fresh guinea-pig's serum.

No results are recorded unless the control tubes gave complete haemolysis.

The controls have been omitted from the tables for the sake of brevity.

The signs used in the tables indicate the amount of haemolysis which took place. Thus + + + = complete haemolysis; + + = partial haemolysis; + = slight haemolysis; and − = no haemolysis.

Experiment I.

In Experiment I the following sera and extracts of cocci were employed:

M.M.H.S. (*XII*)	=	Monovalent meningococcal horse serum obtained by immunisation with *Meningococcus XII.*
P.M.H.S.	=	Polyvalent meningococcal horse serum obtained by immunisation with 20 strains of Meningococcus.
M.E. 141	=	Meningococcal extract prepared from *Meningococcus 141.*
M.E. 162	=	Meningococcal extract prepared from *Meningococcus 162.*
G.E. 1	=	Gonococcal extract prepared from *Gonococcus 1.*

The meningococcal extracts are heterologous to both sera.

The dose of extract was kept constant and used with falling doses of serum.

The results of Experiment I are shown in Table I:

TABLE I.

Sera *M.M.H.S. (XII)*	Dose	Extracts *M. 141*	*M. 162*	*G. 1*
0·5	0·5	–	+	–
0·25	0·5	–	+ +	–
0·125	0·5	+	+ + +	+
0·06	0·5	+ +	+ + +	+
0·03	0·5	+ + +	+ + +	...
P.M.H.S.				
0·5	0·5	–	–	–
0·25	0·5	–	–	–
0·125	0·5	–	–	–
0·06	0·5	+	+	+
0·03	0·5	+ +	+ +	+

It will be seen that the monovalent meningococcal serum produced fixation of complement to an equal degree in the presence of the Gonococcus extract and one of the heterologous Meningococcus extracts, but in the presence of the other Meningococcus extract complement was bound only to a very slight extent. The polyvalent meningococcal serum on the other hand produces fixation of complement equally in the presence of all three extracts.

Experiment II.

In Exp. II the same two horse sera and meningococcal extracts were used, but a third meningococcal extract was also tested with these sera.

In this experiment the dose of serum was kept constant and falling doses of extract were employed.

The results obtained in Exp. II are shown in Table II. Tested in this way the difference between the reactions with *M.E. 141* and *162* were still well marked. The monovalent serum caused marked complement-fixation in the presence of only one of the extracts (*M.E. 141*) but in the presence of *M.E. 119* or *M.E. 162* it produced very slight fixation.

When the polyvalent meningococcal serum was employed, rather different results were obtained. *M.E. 119* still gave a completely negative reaction, whereas a difference was shown between *M. E. 141* and *M. E. 162*, which did not appear in Exp. I when falling doses of serum were used.

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TABLE II.

Sera	Extracts			
M.M.H.S. (XII)	Dose	*M. 119*	*M. 141*	*M. 162*
0·5	0·5	+ + +	–	+ +
0·5	0·25	+ + +	–	+ + +
0·5	0·125	+ + +	–	+ + +
0·5	0·06	+ + +	+	+ + +
0·5	0·03	+ + +	+ +	+ + +
P.M.H.S.				
0·5	0·5	+ + +	–	–
0·5	0·25	+ + +	–	+
0·5	0·125	+ + +	+	+ + +
0·5	0·06	+ + +	+	+ + +
0·5	0·03	+ + +	+ +	+ + +

Experiment III.

In this experiment two monovalent rabbit sera were used and extracts of six strains of Meningococcus and of one strain of Gonococcus.

M.M.R.S. 141 = Monovalent meningococcal rabbit serum prepared with *M. 141.*
M.G.R.S. 1 = Monovalent gonococcal rabbit serum prepared with *G. 1.*
M.E. 119 = Extract of *Meningococcus 119.*
G.E. 1 = Extract of *Gonococcus 1.*
Etc.

The doses of serum were kept constant and falling doses of extract were used as in Exp. II.

TABLE III.

Sera		Extracts						
M.M.R.S.141	Dose	*M. 119*	*M. 135*	*M. 141*	*M. 162*	*M. 164*	*M. 165*	*G. 1*
0·5	0·5	+	–	–	–	–	–	–
0·5	0·25	+ +	+	–	+ +	+	+	–
0·5	0·125	+ + +	+ +	+	+ +	+ +	+ +	+
0·5	0·06	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +
0·5	0·03	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
M.G.R.S. 1								
0·5	0·5	+	–	–	+	–	–	–
0·5	0·25	+ +	–	–	+ +	+ +	+	–
0·5	0·125	+ + +	+ +	+ +	+ + +	+ +	+ +	+
0·5	0·06	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
0·5	0·03	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

The results of Exp. III recorded in Table III show that the degree of complement fixation produced by the monovalent gonococcal serum and by the monovalent meningococcal serum in the presence of the same gonococcal and meningococcal extracts was almost the same. Also the meningococcal serum caused fixation of complement to the same extent whether the homologous meningococcal extract or a gonococcal extract was used. On the other hand in the presence of each of five heterologous meningococcal extracts this meningococcal serum bound complement less than when in association with the gonococcal extract.

Experiment IV.

Sera prepared with the same two cocci were used in Exp. IV as in Exp. III and in addition a gonococcal serum was employed.

M.M.R.S. 141 = Monovalent meningococcal rabbit serum prepared with *M. 141.*
M.G.R.S. 1 = Monovalent gonococcal rabbit serum prepared with *G. 1.*
M.G.R.S. 2 = Monovalent gonococcal rabbit serum prepared with *G. 2.*
M.E. 135 = Extract of *M. 135.*
M.E. 141 = Extract of *M. 141.*
G.E. 1 = Extract of *G. 1.*

The dose of extract was kept constant in this experiment and falling doses of serum were used.

TABLE IV.

Sera	Extracts			
M.M.R.S. 141	Dose	*M. 135*	*M. 141*	*G. 1*
0·5	0·5	–	–	–
0·25	0·5	–	–	–
0·125	0·5	+	–	+
0·06	0·5	+ +	+	+ + +
0·03	0·5	+ + +	+	+ + +
M.G.R.S. 1				
0·5	0·5	–	...	–
0·25	0·5	–	...	–
0·125	0·5	+	...	–
0·06	0·5	+ +	...	–
0·03	0·5	+ + +	...	+
M.G.R.S. 2				
0·5	0·5	–	...	–
0·25	0·5	+	...	–
0·125	0·5	+ +	...	–
0·06	0·5	+ +	...	–
0·03	0·5	+ + +	...	–

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The results obtained in Exp. IV and shown in Table IV appear at first sight to suggest that a strain of Gonococcus can be distinguished from a meningococcal strain by means of a gonococcal serum used in falling doses, but that this is not a constant result is shown by Exps. V and VI. In Table IV is again shown the very uniform degree of fixation of complement obtained with a monovalent meningococcal serum, and meningococcal and gonococcal extracts.

Experiment V.

Three sera were tested in this experiment and three extracts of cocci. Falling doses of serum and constant doses of extract were used.

M.M.R.S. 162 = Monovalent meningococcal rabbit serum prepared with *M. 162*.
M.G.R.S. 3 = Monovalent gonococcal rabbit serum prepared with *G. 3*.
M.G.R.S. 4 = Monovalent gonococcal rabbit serum prepared with *G. 4*.

TABLE V.

Sera *M.M.R.S. 162*	Dose	Extracts *M. 135*	*M. 162*	*G. 2*
0·5	0·5	+	–	–
0·25	0·5	–	–	–
0·125	0·5	+	–	–
0·06	0·5	+ +	+	–
0·03	0·5	+ + +	+ +	–
M.G.R.S. 3				
0·5	0·5	+ +	+ +	–
0·25	0·5	+ +	+ + +	–
0·125	0·5	+ + +	+ + +	–
0·06	0·5	+ + +	+ + +	–
0·03	0·5	+ + +	+ + +	+
M.G.R.S. 4				
0·5	0·5	–	–	–
0·25	0·5	–	–	–
0·125	0·5	+ +	+ +	+
0·06	0·5	+ + +	+ +	+ +
0·03	0·5	+ + +	+ + +	+ + +

Table V shows the results of complement-fixation obtained in Exp. V. The most noticeable fact is that the gonococcal extract in the presence of either meningococcal (*162*) or gonococcal (*3*) serum produced a greater degree of complement-fixation than either of the meningococcal extracts. When, however, Gonococcus serum (*4*) was used, the three coccal extracts (*M. 135*, *M. 162* and *G. 2*) all produced nearly the same degree of fixation.

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Experiment VI.

In Experiment VI two meningococcal sera were used, three meningococcal extracts and one gonococcal extract. Falling doses of serum and constant doses of extract were again employed.

TABLE VI.

Sera		Extracts			
M.M.R.S. 141	Dose	*M. 141*	*M. 162*	*M. 164*	*G. 6*
0·5	0·5	−	+	+	−
0·25	0·5	+	+	+ +	−
0·125	0·5	+	+ +	+ +	−
0·06	0·5	+ +	+ +	+ +	+
0·03	0·5	+ + +	+ + +	+ + +	+ +
M.M.R.S. 162					
0·5	0·5	−	−	+	−
0·25	0·5	−	−	−	−
0·125	0·5	−	−	−	−
0·06	0·5	−	−	+	−
0·03	0·5	+	−	+	−

Table VI shows the results of Experiment VI. Here again the gonococcal extract was more efficient for complement-fixation than the meningococcal extracts, even although the sera used were monovalent sera. This superiority is especially well shown in the upper part of the table where the results of fixation with a rather weak serum (*M.M.R.S. 141*) are recorded.

These experiments appear to show that though a monovalent serum produces generally a more marked complement-fixing reaction with its homologous strain of coccus than with heterologous strains, nevertheless such a serum has not necessarily any greater affinity for the other strains of the same group (Meningococcus or Gonococcus) than for strains of the other group.

It was, however, noticed (and this appears in the tables) that extracts prepared from strains of Gonococcus were on the whole more efficient for producing complement-fixation with a meningococcal or gonococcal serum than extracts made from strains of Meningococcus. Colombo (1911) arrived at a similar conclusion, both as regards the non-specificity of the complement-fixation reaction and the greater effect produced by gonococcal extract.

SUMMARY AND DISCUSSION.

The results which were obtained in the foregoing six experiments and are detailed in Tables I to VI may be summarised as follows.

Statement of complement-fixation reactions combined from all the Tables.*

Reaction of *M.M.H.S.* (*XII*) was	...	Positive with *E.M. 141* and *E.G. 1.* Very slight with *E.M. 162.* Negative with *E.M. 119* and *E.M. 162.*
Reaction of *P.M.H.S.* (20 strains) was		Positive with *E.M. 141*, *E.M. 162* and *E.G. 1.* Negative with *E.M. 119.*
Reaction of *M.M.R.S. 141* was	...	Positive with *E.M. 135*, *E.M. 141*, *E.M. 162*, *E.M. 164*, *E.M. 165*, *E.G. 1* and *E.G. 6.* Very slight with *E.M. 162* and *E.M. 164.*
Reaction of *M.M.R.S. 162* was	...	Positive with *E.M. 135*, *E.M. 162*, *E.M. 164*, *E.G. 2* and *E.G. 6.*
Reaction of *M.G.R.S. 1* was	...	Positive with *E.M. 135*, *E.M. 141*, *E.M. 164*, *E.M. 165* and *E.G. 1.* Negative with *E.M. 119* and *E.M. 162.*
Reaction of *M.G.R.S. 2* was	...	Positive with *E.M. 135* and *E.G. 1.*
Reaction of *M.G.R.S. 3* was	...	Positive with *E.G. 2.* Negative with *E.M. 135* and *E.M. 162.*
Reaction of *M.G.R.S. 4* was	...	Positive with *E.M. 135*, *E.M. 162* and *E.G. 2.*

* In some cases slightly different results were obtained on different occasions.

It is seen then that any attempt to classify these two groups of cocci by means of complement-binding reactions would arrange them into more or less well marked sub-groups, some of which would contain both meningococcal and gonococcal strains, and some perhaps strains from only one of these groups.

It will be noticed that extracts of three strains of Meningococcus (*119*, *162* and *164*) showed an especial tendency to give negative or feeble complement-fixation reactions with heterologous meningococcal sera, and two of these strains (*119* and *162*) also gave negative reactions with some of the gonococcal sera. Though the complement-fixation reactions of the strains used in this research were not fully worked out on account of the difficulty in obtaining satisfactory sera, nevertheless the following classification appears to be indicated having regard to complement-fixation alone.

Sub-group	I.	*M.* (*XII*), *M. 141*, *M. 165*, *G. 1*, *G. 3.*
	II.	*M. 135*, *G. 2.*
	III.	*M. 162*, *M. 164*, *G. 4*, *G. 6.*
	IV.	*M. 119.*

Sub-group II has affinities for sub-groups I and III, but there is little, if any, affinity shown between I and III directly. *M. 119* shows slight affinity for *M. 141*, but for no other strains.

The sub-groups are not clearly defined, but overlap and are connected with each other in various directions. For instance *G. 1*, *M. 135* and *G. 2* all appear to have common receptors, and *M. 135* and *G. 2* also have receptors in common with *G. 4* and *M. 162*, but *G. 1* shows no affinity for these two latter strains.

The explanation of these facts is not quite simple but they may be explained by assuming, (1) that several group antigens occur which are common to the Meningococcus and the Gonococcus, but only some of which are present in any given strain of coccus, and (2) that specific antigens which are peculiar to the Meningococcus on the one hand or to the Gonococcus on the other hand do occur, but are often absent in the case of any given strain.

The second assumption is perhaps unnecessary, and the first is almost equivalent to affirming the occurrence of special antigens peculiar to certain sub-groups which contain strains of both Meningococcus and Gonococcus.

The evidence, then, from complement-fixation experiments as also from other serum tests as far as they are of any value, appears to point to a closer relationship between some strains of Gonococcus and some strains of Meningococcus than between different sub-groups of Meningococcus. In fact rather the unity of these two groups than any essential difference between them, is suggested by these facts.

These considerations lend further support to the view that the most constant bacteriological characters available for differentiating the Meningococcus from the Gonococcus are the cultural characters seen when the organisms are grown on agar of different degrees of alkalinity.

Conclusions.

(1) Meningococcal sera produce complement-fixation as readily with some gonococcal extracts as with extracts of some strains of Meningococcus; whereas no reaction is obtained with some heterologous meningococcal extracts.

(2) A monovalent serum usually reacts better with an extract of its homologous coccus than with extracts of other strains of Meningococcus or Gonococcus, but a gonococcal extract sometimes gives a

better reaction with a meningococcal serum than the homologous extract does.

(3) Gonococcal sera and extracts are on the whole more potent than those prepared from Meningococci as regards complement-fixation.

(4) No satisfactory distinction between Meningococci and Gonococci can be demonstrated by means of complement-fixation tests.

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