VARIETIES OF THE MENINGOCOCCUS WITH SPECIAL REFERENCE TO A COMPARISON OF STRAINS FROM EPIDEMIC AND SPORADIC SOURCES.

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The following paper is based on the results obtained by cultivating and experimenting with strains of Meningococcus from 45 different cases of Meningitis. Twenty-five of these occurred in Epidemic areas and 20 were Sporadic cases occurring in London. The Epidemic strains are referred to throughout this paper as E. 1, E. 2, E. 3, etc., and the London strains (Sporadic) as L. 1, L. 2, L. 3, etc. For most of the Epidemic strains I am indebted to Dr M. Macdonald of the Lightburn Hospital, Shettlestone, and to Dr C. B. Ker of the Fever Hospital, Edinburgh. Also I have used strains sent to the Lister Institute by Professor Symmers from Belfast and by Dr Buchanan from Glasgow.

The Sporadic strains are all from London sources and were chiefly isolated from specimens sent to the Lister Institute; for other strains I am indebted to Dr Graham Forbes, Dr F. E. Batten and Miss A. Taylor.

The points to which I have specially directed my attention are

(1) the variability of the Meningococcus and the usual directions which variation takes;

(2) the question of a difference between Sporadic and Epidemic strains;

(3) the occurrence in cases of Meningitis of certain other Gram-negative organisms which have some resemblance to the Meningococcus.

Variations.

The differences observed amongst the strains of Meningococcus which I have cultivated have not concerned the morphology nor the staining reactions.

All the strains have been consistently Gram-negative, and they have never grown in chains. With the one exception of a strain which I described in a previous paper (Arkwright, 1908), no growth has taken place at a temperature of 24° C. It has always been easy to make uniform suspensions of young agar cultures in salt solution (·85 %), and no spontaneous clumping has occurred in these suspensions. On the other hand the fermentation reactions in different sugars have not been quite uniform nor constant.

The sugars chiefly employed have been Glucose, Maltose, Laevulose and Cane sugar.

The fermentation of the sugars was tested in fluid media, either weak broth (25 % peptone broth and 75 % peptone water) or 2 % peptone water, ascitic fluid or serum being added to each tube.

On solid media with sugar and litmus added (method of v. Lingelsheim), the cultures were found to give usually a more rapid, but in many instances a very transient, reaction. The red colour changed again to blue in a few hours and the initial acid reaction varied much in its time of appearance with different strains, so that the results had to be noted at different times for different strains. Trautmann and Fromme (1908) also found that this medium for testing the fermentations of sugars gave irregular results of the same kind.

The reactions with sugar broth were not quite uniform, but were almost always complete by the 5th day.

The most constant features of the fermentations have been the formation of acid from Glucose and Maltose but never from Cane sugar. Laevulose was fermented by several strains, acid being produced when the medium contained broth but never when peptone water only was used. This is interesting, as some English observers have found Laevulose to be fermented, though German writers have usually considered the absence of acid-formation from Laevulose to be an important and distinctive characteristic of the Meningococcus.

With regard to Glucose and Maltose, when broth was used, Maltose was fermented most regularly and sometimes first, but when serum-peptone water only was used the Glucose tube most constantly showed the presence of acid and was generally the first to do so.

Several strains at some stage of their artificial culture lacked the power to produce acid from either Glucose or Maltose or (in a few cases) even from both. For instance Strain L. 5 at the earliest testing fermented no sugars and for months fermented only Maltose, but latterly after 10 months' artificial culture fermented both Glucose and Maltose. Strain

E. 2 on the other hand at first produced acid from both Glucose and Maltose but later fermented neither.

Strains L. 13 and E. 3 never fermented any of the sugars.

The change during long artificial culture in the power of the Meningococcus to ferment some particular sugar has been noted by Trautmann and Fromme (1908). The Meningococcus, therefore, shows evidence of mutation as regards its fermentation characters similar to that recorded by Twort (1907) for the *Bacillus typhosus* under special cultural conditions, and by Andrewes and Horder (1906) to a slight extent for Streptococci.

It may be mentioned here that a strain of Gonococcus, which was isolated from the urethral discharge in a case of acute gonorrhoea in an adult, fermented Glucose and Maltose and not Cane sugar, thus in its sugar reactions resembling most strains of Meningococcus. The fermentation of these two sugars has been found to be characteristic of the Gonococcus by Wollstein (1907) and Gurd (1908). Gordon however found that the Gonococcus fermented Glucose and Galactose but not Maltose and Cane sugar, and this I confirmed for one strain.

Table I shows the sugar reactions given by the various strains of Meningococcus, the Sporadic (L.) and Epidemic strains (E.) being placed in different series.

Table II shows the frequency with which variations from the typical sugar reactions occurred among London and Epidemic strains respectively.

It will be apparent that there is not much difference between the Sporadic and Epidemic strains as regards their sugar reactions; Epidemic strains are rather more uniform than the Sporadic.

Summary of Fermentation Tests.

When the tests were made in weak broth with the addition of serum, of 14 Sporadic strains 5 fermented Glucose, Maltose and Laevulose, 6 fermented Glucose and Maltose, 1 Glucose and Laevulose, 1 Maltose only, and 1 no sugars. In three of the strains the reactions were not quite constant.

Of 22 Epidemic strains 12 fermented Glucose, Maltose and Laevulose, 9 Glucose and Maltose and 1 no sugars.

When 2 % peptone water with serum was used as a basis for the sugar media, of 7 Sporadic strains 6 fermented Glucose and Maltose and 1

TABLE I.

No. of sporadic strains (L.)	Source: Cerebro-spinal fluid, meninges or blood	Source: During life or P. M.	Growth at 24° C.	Sugar reactions: Broth+serum: Glucose	Broth+serum: Maltose	Broth+serum: Laevulose	Broth+serum: Saccharose	2 % peptone water+serum: Glucose	2 % peptone water+serum: Maltose	2 % peptone water+serum: Laevulose	2 % peptone water+serum: Saccharose
1	Men.	P. M.	–	+	+	+	–	+	+	–	–
2	Men.	P. M.	–	+	+		–				
3	C.-S. Fl.	Life	S	+	+		–				
4	Men.	P. M.	–	+	+		–				
5	C.-S. Fl.	Life	–	∓	∓	+	–	∓	∓	–	–
6*	,,	,,	+	–	–	–	–	–	–	–	–
7	,,	,,	–	+	+	+	–				
8	,,	,,	–	+	+	+S	–				
9 (1)	,,	,,	–	+	+	+	–				
9 (2)*	Blood	P. M.	+	–	–	–	–				
10	C.-S. Fl.	Life	–	+	+	–	–				
11	,,	,,	–	–	+	–	–				
12	,,	,,	–	+	+	–	–				
13	,,	,,	–	–	–	–	–				
14	,,	,,	–	+	+	+	–				
15*	,,	,,	+	–	–	–	–	–	–	–	–
16	,,	,,	–	±	±	±	–	±	±	–	–
17	,,	,,	–								
18	,,	,,	–					+	+	–	–
19	,,	,,	–	+	+	–	–	+	∓	–	–
20	,,	,,	–					+	+	–	–
21	,,	,,	–					+	–	–	–
22	,,	,,	–								
23*	C.-S. Fl.	Life	+	–	–	–	–	–	–	–	–
No. of epidemic strains (E.)											
1	C.-S. Fl.	Life	–	+	+	+	–				
2	,,	,,	–	∓	+	+	–				
3	,,	,,	–	–	–	–	–	–	–	–	–
4	,,	,,	–	+	+	+	–	–	–	–	–
5	,,	,,	–	+	+	–	–				
6	,,	,,	–	+	+	∓	–				
7	,,	,,	–	+	+	+	–	+	+	–	–
8	,,	,,	–	+	+		–				
9	,,	,,	–	+	+	+	–				
10	,,	,,	–	+	+	+	–				
11	,,	,,	–	+	+	+	–				
12	Men.	P. M.	–	+	±	±	–	+	+	–	–
13	C.-S. Fl.	Life	–	+	+	–	–				
14	,,	,,	–	+	+	+	–				
15	,,	,,	–	+	+	–	–				
16	,,	,,	–	+	+	–	–				
17	,,	,,	–					+	+	–	–
18	,,	,,	–	+	+	+	–	+	+	–	–
19	Men.	P. M.	–	+	+	–	–	+	∓	–	–
20	C.-S. Fl.	Life	–					+	–	–	–
21	,,	,,	–	+	+	+	–	+	–	–	–
22	,,	,,	–	+	+	–	–	+	–	–	–
23	,,	,,	–	+	+	–	–	+	∓	–	–
24	,,	,,	–					+	+	–	–
25	,,	,,	–	+	+	–	–	+	+	–	–

+ = Acid produced. – = No acid produced.
± = Acid produced on one occasion and not on another.
S = Slight.
* These strains of Gram-negative coccus are not Meningococci.

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Glucose only. Two of these strains at another time did not ferment any sugars.

Of 13 Epidemic strains 8 fermented Glucose and Maltose, 3 Glucose only and 2 did not ferment any sugars (*vide* Tables II and III).

Tables II and III show the number of strains giving each variety of fermentation.

TABLE II.

In Serum Broth.

	Glucose, Maltose & Laevulose	Glucose & Maltose	Glucose	Maltose	Glucose & Laevulose	Maltose & Laevulose	No sugars fermented
14 London strains	5	6 (2)	(1)	1 (1)	1	(1)	1
22 Epidemic strains	12	9	—	—	—	—	1

TABLE III.

In Serum Peptone Water.

7 London strains	—	6	1	—	—	—	(2)
13 Epidemic strains	—	8	3 (1)	—	—	—	2

The figures in brackets denote strains which gave different results on other occasions and so appear twice in the Table.

Agglutination Experiments.

The agglutination experiments were all made by the microscopic method at the temperature of the laboratory and the observations were completed at the end of two hours.

Agglutination tests were made with the serum of a horse which was injected with killed, followed by living, cultures of Meningococcus by Dr MacConkey at Elstree. The injections were partly subcutaneous and partly intravenous. At first only one Sporadic strain (L. 1) was used and later after these injections had been made for a year, one strain of Epidemic Meningococcus (E. 12) was injected into the same horse for a further period of 10 months. Serum "1" was obtained by bleeding the horse after injections of strain L. 1 had been carried on for 12 months. This serum agglutinated the homologous strain L. 1 in dilutions up to 1—500, and several other Sporadic strains in dilutions of 1—100, but only three Epidemic strains were agglutinated by it in dilutions higher than 1—5 and none up to 1—100. It was found, however, that five Sporadic strains were likewise not agglutinated by Serum 1 in higher dilutions than 1—5. The strains which agglutinated best were among

those which had been longest isolated. As however, at least, one Sporadic strain (L. 8) which had been isolated for as long as one year was among those strains which were not agglutinated, the agglutinability cannot be simply the result of long artificial culture.

TABLE IV.

Microscopic Agglutination.

Serum 1 = Serum of horse injected with Sporadic strain L. 1 only.
Serum 2 = Serum of same horse after receiving in addition the Epidemic strain E. 12 for 10 months.

Date of exp.	Strains of Meningococcus (London strains)	Serum 1					Serum 2					Normal Serum					Normal salt solution	Date of isolation
		1/5	1/25	1/100	1/500	1/2000	1/5	1/25	1/100	1/500	1/2000	1/5	1/25	1/100	1/500	1/2000		
11/2/08	L. 1	‡‡	‡‡	‡‡	‡	–	‡‡	‡‡	‡‡	‡	+	–	–	–	–	–	–	1906
,,	L. 5	‡‡	‡‡	+	+	–	‡‡	‡‡	‡‡	‡	+	‡	+	–	–	–	–	3/4/06
15/2/08	L. 7	‡‡	‡‡	‡‡	+	–	‡‡	‡‡	‡‡	‡	+		–	–	–	–	–	–/2/07
11/2/08	L. 12	‡‡	‡‡	‡‡	‡	+	‡‡	‡‡	‡‡	‡‡	‡	‡	–	–	–	–	–	21/5/07
21/3/08	L. 15*	‡	–	–	–	–						–	–	–	–	–	–	24/1/08
25/3/08	L. 16	–	–	–	–	–						–	–	–	–	–		28/2/08
21/3/08	L. 18	‡	–	–	–	–						–	–	–	–	–		11/3/08
,,	L. 19	‡‡	–	–	–	–						–	–	–	–	–		17/3/08
,,	L. 20	‡‡	+	–	–	–						–	–	–	–	–		–/3/07
26/2/08	L. 21		–	–	–	–						–	–	–	–	–		
	L. 22	‡‡	+	–	–	–						‡	+	+	–	–		
15/2/08	L. 8	‡‡	–	–	–	–	‡	–	–	–	–	+	–	–	–	–		1907
	(Epidemic strains)																	
15/2/08	E. 2	+	–	–	–	–	‡‡	–	–	–	–	+	–	–	–	–	–	30/11/06
,,	E. 4	‡‡	‡	–	–	–	‡‡	‡‡	‡‡	+	+	‡	–	–	–	–	–	18/12/06
14/2/08	E. 5	+	–	–	–	–	‡	+	+	+s	+s	–	–	–	–	–	–	
,,	E. 7	+	–	–	–	–	‡‡	+	–	–	–	–	–	–	–	–	–	8/2/07
11/2/08	E. 12	‡	–	–	–	–	‡	+	–	–	–	–	–	–	–	–	–	8/3/07
,,	E. 16	–	–	–	–	–	‡‡	–	–	–	–	–	–	–	–	–	–	21/5/07
21/3/08	E. 17	‡‡	+		–	–						‡	+	–	–	–		
26/3/08	E. 18	‡‡	‡	–	–	–						+	–	–	–	–	–	
25/3/08	E. 19	–	–	–	–	–						–	–	–	–	–	–	
26/3/08	E. 21	–	–	–	–	–						–	–	–	–	–	–	
,,	E. 22	–	–	–	–	–						–	–	–	–	–	–	–/3/08
27/3/08	E. 23	–	–	–	–	–						–	–	–	–	–	–	–/2/08
26/3/08	E. 24	–	–	–	–	–						–	–	–	–	–	–	1907
27/3/08	E. 25	–	–	–	–	–						–	–	–	–	–		1907

* This strain of Gram-negative coccus is not Meningococcus.

‡‡ = Complete agglutination. ‡ = Incomplete agglutination.
+ = Slight ,, +s = Very slight ,,

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The injections of strain E. 12 were carried out on the same horse during the months following the cessation of the strain L. 1 injections. The animal was then bled a second time yielding Serum "2."

This bivalent serum possessed agglutinins for some Epidemic strains, but these could be entirely removed by absorption with the homologous Epidemic strain (E. 12) and partly by absorption with the homologous Sporadic strain (L. 1). The agglutination was only partial however in dilutions above 1—100 for any Epidemic strain.

Serum "3," which came from the same horse at a later date after the injections with E. 12 had been carried on for 10 months, did not give high agglutination with six Epidemic strains, but agglutinated E. 12 (homologous strain) and E. 19 in dilution of 1—500.

Kolle's Anti-Meningococcus serum was used to test the agglutination of 22 strains. This serum agglutinated some Epidemic strains better than any of the Sporadic strains. Of 11 Epidemic strains tested, six were agglutinated in a dilution of the serum of 1—100 or 1—200, whilst of the Sporadic strains tested, only one was agglutinated in a higher dilution than 1—20.

TABLE V.

Microscopic Agglutination.

Serum 3 = Serum of same horse which produced Serums 1 and 2, after it had received injections of Epidemic strain E. 12 for 10 months.

Meningococcus London strains	Serum 3				Normal Serum		Normal salt solution
	1/20	1/100	1/500	1/2000	1/20	1/100	
L. 1	++++	++++	+++	++	++++	–	
L. 15*	+++	–	–	–	+++		
L. 16	–	–	–	–	–		–
L. 19	–	–	–	–	–		–
L. 23*	++++	+++	++		++++		+++
Epidemic strains							
E. 2	++++	–	–	–	++	–	
E. 7	–	–	–	–	–	–	
E. 12	++++	++++	++++	–	++	–	
E. 18	–	–	–	–	–		–
E. 19	++++	++++	++++	–	–		–
E. 21	–	–	–	–	–		–
E. 22	++++	++	–	–	++		–
E. 23	+++	–	–	–	–		–
E. 25	++	–	–	–	–		–

* These strains are not Meningococci.

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TABLE VI.

Summary of agglutination tests, showing number of strains agglutinated by different sera in various dilutions. The highest dilution at which each strain agglutinates is considered the dilution appropriate to that strain.

	1/2000	1/500	1/100	1/20	1/5	None
Immune Serum 1.						
11 London strains	—	4	—	2	4	1
14 Epidemic ,,	—	—	—	3	4	7
Immune Serum 2.						
5 London strains	4	—	—	—	1	—
6 Epidemic ,,	1	—	1	2	2	—
Immune Serum 3.						
3 London strains	1	—	—	—	—	2
9 Epidemic ,,	—	2	1	3	—	3
Normal Serum.						
10 London strains	—	—	1	1	3	5
14 Epidemic ,,	—	—	—	1	3	10

Normal horse serum agglutinated one strain (Sporadic) in dilution of 1—100, two strains at 1—20, six at 1—5 and 17 not at all in dilution of 1—5.

Experiments on the power of four Sporadic and two Epidemic strains to absorb agglutinins from Serum 1, showed a power of absorption in the different strains which was to a certain extent proportional to their agglutinability.

Of the Sporadic strains two (L. 1 and L. 5) which were agglutinated about equally and in high dilution (1—500) absorbed their own and each others agglutinins, but absorbed to a much less extent the agglutinins of the other two Sporadic strains.

Strains L. 7 and L. 12 were not agglutinated so well as L. 1 and L. 5 by Serum 1. They were capable of absorbing to a certain extent their own and each other's agglutinins, but hardly removed any of those for L. 1 and L. 5.

The two Epidemic strains, which were only partially agglutinated in dilutions of 1—20 and 1—50, absorbed none of the agglutinins for themselves nor for the other strains.

This experiment then indicates a grouping of these six strains into three groups, viz.:

Group 1. Two Epidemic strains.
Group 2. Two Sporadic strains.
Group 3. Two Sporadic strains of intermediate character.

TABLE VII.

Microscopic agglutination of six strains by Serum 1, *before and after it has been absorbed by each strain. In each case* 0·25 *c.c. of serum after dilution* 1/10 *was used to emulsify one* (24 *hour*) *agar slope of the Meningococcus. The emulsion was incubated at* 37° C. *for two hours and the cocci removed by centrifugalisation.*

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by L. 1 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	⁜	+	−	−	−	−
L. 5	⁜	⁜	−	−	−	+	+	−	−	−
E. 5	+	+	−	−	−	⁜	−	−	−	−
E. 7	+	−	−	−	−	+	−	−	−	−
L. 7	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	−	−
L. 12	⁜	⁜	+	−	−	+	+	+	−	−

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by L. 5 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	⁜	−	−	−	−	−
L. 5	⁜	⁜	⁜	⁜	⁜	−	−	−	−	−
L. 7	⁜	⁜	⁜	−	−	⁜	⁜	⁜	⁜	−
L. 12	⁜	⁜	⁜	−	−	⁜	⁜	+	−	−

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by E. 5 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	+	⁜	⁜	⁜	⁜	⁜
L. 5	⁜	⁜	⁜	⁜	+	⁜	⁜	⁜	⁜	⁜
L. 7	⁜	⁜	⁜	+	+	⁜	⁜	⁜	+	+
L. 12	⁜	⁜	⁜	⁜	+	⁜	⁜	⁜	−	−

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by E. 7 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜
L. 5	⁜		⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜
E. 5	+	−	−	−	−	+	−	−	−	−
E. 7	−	−	−	−	−	−	−	−	−	−
L. 7	⁜		⁜	⁜	⁜	⁜	⁜	⁜	+	+
L. 12	⁜	⁜	+	−	−	⁜	+	+	−	−

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by L. 7 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜
L. 5	⁜	⁜	⁜	⁜	⁜	⁜		⁜	⁜	⁜
L. 7	⁜	⁜	⁜	⁜	⁜	⁜	⁜	+	+	+
L. 12	⁜	⁜	⁜	⁜	+	⁜	−	−	−	−

Strain of Meningococcus	Serum 1 1/20	1/50	1/100	1/200	1/500	Serum 1 absorbed by L. 12 1/20	1/50	1/100	1/200	1/500
L. 1	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	+
L. 5	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	⁜	+
L. 7	⁜	⁜	⁜	⁜	⁜	⁜	+	+	−	−
L. 12	⁜	⁜	⁜	⁜	+	⁜	−	−	−	−

The Epidemic strains E. 5 and E. 7, which are not themselves agglutinated, absorb no agglutinins.

The agglutination experiments shew that numerous injections, extending over a long period, of two strains of Meningococci produced a serum which agglutinated in high dilutions the two homologous and a few other strains, but failed to agglutinate considerably more than half the strains tested.

The experiments also indicate an arrangement of the strains into groups. Each group would consist almost entirely of Epidemic or Sporadic strains. This is shown both by the agglutinations with the sera prepared at the Lister Institute and also by the use of Kolle's serum, assuming that this serum was prepared with strains all of which originated from Epidemic cases.

The variations in the agglutinability which occur among different strains of Meningococcus recorded here have a specific character and are not necessarily correlated with variations in their agglutinability by normal serum.

By the results of absorption experiments with Serum 1 the strains can be arranged into groups, the members of which are apparently closely related whilst having few if any agglutinins in common with the remainder, e.g., a group of the London strains containing L. 1, L. 5, L. 7, and L. 12 of which L. 1 and L. 5 form one sub-group and L. 7 and L. 12 another.

Amongst the Epidemic strains the results of agglutination and absorption closely connect E. 12 and E. 19 whilst shewing no relationship of these to the rest. The agglutination results do not enable us to differentiate accurately the Epidemic strains from the Sporadic strains since there are as great differences between the individual Epidemic and the individual Sporadic strains as between the members of the two groups. For instance, some Epidemic strains were agglutinated (1—25) by Serum 1 (Sporadic) while several Sporadic strains were not agglutinated at all.

The differences between various strains of Meningococci are then very great as regards agglutination. They are not, however, greater than those shown by Torrey (1907) to exist between different strains of Gonococci in respect of agglutination and also of power of fixing complement in the presence of an immune serum (Bordet's method).

FIXATION OF COMPLEMENT (BORDET'S METHOD).

A series of experiments made to test the power of Serum 1 (prepared with one Sporadic strain) to fix complement in a haemolytic system in

the presence of different strains of Meningococcus tended, on the whole, to confirm the differences and affinities between the strains as shewn by agglutination.

For these experiments on Fixation of Complement suspensions of agar cultures of Meningococci were used. A trial was made with extracts of the cocci, but the results did not appear to be so satisfactory, perhaps, because certain strains autolysed more readily than others.

The haemolytic serum used was the heated serum of a rabbit which had been immunised with ox corpuscles, and the red corpuscles were those of the ox, washed and suspended in a concentration which corresponded to 2 per cent. of the original blood.

The complement used was that of the guinea-pig diluted 1—10.

0·1 c.c. of the diluted complement was added to 0·3 c.c. of the emulsion of cocci and 0·3 c.c. of Serum 1 (from the horse injected with the Sporadic Meningococcus L. 1). This serum was used in varying dilutions, 1/1, 1/5, 1/25, 1/100 and 1/500.

The degree of correspondence between the particular strain of Meningococcus and the anti-Meningococcic serum was measured by the dilution of serum by which the complement was fixed as indicated by the absence of haemolysis, when the haemolytic serum and corpuscles were added.

The anti-Meningococcic serum in various dilutions, the cocci and the complement were mixed and put in the incubator at 37° C. for 40 minutes. To each tube were then added 1 c.c. of the emulsion of ox corpuscles and 0·1 c.c. of inactivated haemolytic serum diluted 1—10. The whole was then returned to the incubator and left at 37° C. for 1½ hours, after which the tubes were placed at the laboratory temperature for about 20 hours. At the final examination of the tubes it was found that Serum 1 in dilutions of 1—25 had completely prevented haemolysis with some strains of Meningococci, but with other strains the haemolysis had taken place as readily as when cocci or Serum 1 were absent.

The Sporadic Meningococci L. 1, L. 7, L. 12 and L. 8 prevented haemolysis completely or very nearly completely, while L. 5 prevented haemolysis in some experiments, but not in others. These were the only Sporadic strains used. On the contrary, five Epidemic strains did not prevent haemolysis.

A number of controls testing the powers of the individual constituents of the test tubes were made simultaneously and also a control made by substituting normal horse serum for Serum 1. These controls

in each instance gave a satisfactory result as shewn in Table IV. When other bacteria were used instead of Meningococci with Serum 1, either haemolysis took place as if no bacteria or serum were present or else the presence of these bacteria prevented haemolysis even in the absence of serum. The bacteria used as controls in this way were Staphylococcus, *Micrococcus catarrhalis* and *B. diphtheriae.*

SUMMARY OF FIXATION OF COMPLEMENT EXPERIMENTS.

Of five Sporadic strains of Meningococcus tested with Serum 1 (prepared with one Sporadic strain) one (homologous) strain prevented haemolysis when the serum was diluted to 1—100, three strains when it

TABLE VIII.

Fixation of Complement in a Haemolytic System by different strains of Meningococci in presence of Serum 1.

Complement = Fresh guinea-pig's serum.
H. S. = Rabbit's serum (heated) Immune v. Ox corpuscles.

Complement	Serum 1	Cocci	Time at 37° C. mins.	H. S.	Ox C.	Time at 37° C. hours	Time at 20° C. hours	Haemolysis with different strains: No cocci	L. 1	L. 12	E. 5	L. 8	E. 2	E. 4	E. 12	E. 7	L. 7	L. 5
0·1	0·0	0·0	40	0·1	1·0	1½	20	‡	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0·1	0·0	0·0	,,	0·0	,,	,,	,,	–	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0·0	0·0	0·0	,,	0·1	,,	,,	,,	–	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0·1	0·3	0·0	,,	0·1	,,	,,	,,	‡	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0·1	0·3	0·0	,,	0·0	,,	,,	,,	–	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
0·1	0·0	0·3	,,	0·1	,,	,,	,,		‡	⋮	⋮	‡	⋮	⋮	‡	+	‡	‡
0·1	0·0	0·3	,,	0·0	,,	,,	,,		–	⋮	⋮	–	⋮	⋮	⋮	⋮	+sl.	⋮
*0·1	0·3	0·3	,,	0·1	,,	,,	,,		‡	‡	‡	+	+	‡	⋮	⋮	‡	⋮
*0·1	0·3	0·3	,,	0·0	,,	,,	,,		–	⋮	⋮	–	–	–	⋮	⋮	–	⋮
0·1	0·3	0·3	,,	0·1	,,	,,	,,		–	–	–	–	+	+	–	–	–	–
0·1	$0·\frac{3}{5}$	0·3	,,	0·1	,,	,,	,,		–	⋮	⋮	–	+	+	+	+	–	+
0·1	$0·\frac{3}{25}$	0·3	,,	0·1	,,	,,	,,		–	–	‡	–	‡	‡	+	+	+sl.	+
0·1	$0·\frac{3}{125}$	0·3	,,	0·1	,,	,,	,,		–	⋮	⋮	+	‡	‡	+	‡	+	‡
0·1	$0·\frac{3}{625}$	0·3	,,	0·1	,,	,,	,,		–	⋮	⋮	‡	‡	‡	+	‡	+sl.	‡

‡ = Complete haemolysis. + = Marked haemolysis.
– = No haemolysis. + = Slight haemolysis.

* Normal serum used instead of Serum 1 in these tubes.

The dilution 1—25 is the series which best shews difference of strains.

The Serum 1 of horse immunised with one Sporadic strain (L. 1) prevents haemolysis in presence of an emulsion of Strains L. 1, L. 12, L. 8, L. 7 (all Sporadic strains), but not in the presence of E. 2, E. 4, E. 7, and E. 12 (Epidemic strains).

was diluted to 1—25 and one strain gave a variable reaction. Of five Epidemic strains used none did more than partially prevent haemolysis when the dilution was 1—25.

TABLE IX.

Summary of Fixation of Complement Experiments.

	Dilution of Serum	Amount of Serum undiluted c.c.	Emulsions of Meningococcus strains 0·3 c.c. of each									
			L. 1	L. 5	L. 7	L. 8	L. 12	E. 2	E. 4	E. 5	E. 7	E. 12
Anti-Meningococcic Serum 1	1/5	·06	–	±	–	–	–	ǂ	ǂ	+	ǂ	ǂ
	1/25	·012	–	±	–	–	–	ǂǂ	ǂǂ	ǂǂ	ǂ	ǂ
	1/100	·003	–	ǂ	+	ǂ	+	ǂǂ	ǂǂ	ǂǂ	ǂǂ	ǂ
Normal Serum	1/1	·3	ǂǂ	ǂǂ	ǂǂ	ǂǂ	ǂǂ	ǂ	ǂ	ǂǂ		

ǂǂ = Complete haemolysis. ǂ = Marked haemolysis.
\+ = Slight haemolysis. – = No haemolysis; complement completely fixed.

Importance of the Difference between Strains.

In assessing the value to be put on the differences in sugar fermentation and serum reactions between different strains of Meningococcus, as indications for division of the group into sub-species, the similar variations occurring amongst other groups of bacteria and especially amongst the members of the closely allied species of Gonococcus must be borne in mind.

As mentioned above, Dunn and Gordon (1905) state that the Gonococcus ferments Glucose but not Maltose, whereas Wollstein (1907) and Gurd (1908) found that Glucose and Maltose were both fermented by a large number of strains of Gonococcus. Of two strains of Gonococcus which I isolated from different cases of acute urethritis in male patients the one produced acid from Glucose and Maltose, the other from Glucose but not from Maltose. The sugar reactions of the Gonococcus are therefore not constant.

As regards agglutination, fixation of complement and the formation of antibodies, great similarity has been shown to exist between some strains of Meningococcus and some strains of Gonococcus by Bruckner and Cristéanu (1906), Wollstein (1907), Teague and Torrey (1907), Gurd (1908), and Dopter and Koch (1908).

The differences between the individual races of Gonococcus, or of Gonococcus as tested by these methods, appear to be almost as great as between the two species.

It is, therefore, unlikely that well-marked constant differences along these lines will be found between the groups and sub-divisions of the whole class to which the Gonococcus and Meningococcus both belong.

If specific differences between the two groups of Meningococci exist, other methods must be found than those detailed above by which to demonstrate them. Of the Opsonic test as described by Houston and Rankin (1907) and also used by Taylor (1907) I have no experience.

One feature of the group of Meningococci from Sporadic cases taken as a whole appears to be that its members are more frequently found to deviate from the type to which most strains conform, than is the case with the Epidemic group.

The great variability among the strains shown above points strongly to the desirability of employing a polyvalent serum for therapeutic purposes.

ABERRANT STRAINS OF THE MENINGOCOCCUS, AND ORGANISMS LIABLE TO BE CONFUSED WITH THE MENINGOCOCCUS.

Apart from its morphology the Meningococcus possesses certain characteristics which are almost invariable, viz. (1) its inability to grow at 24° C. and (2) the property which allows a young agar culture to be easily made into a uniform suspension which does not shew spontaneous agglutination.

The other characters are not quite constant and some strains occur which deviate further than usual from the accepted type of the species. For instance, strains L. 13, E. 3 (and E. 4 in peptone water) did not ferment any of the sugars used, though they resembled typical Meningococci in every other way and E. 3 and E. 4 were agglutinated by Kolle's anti-Meningococcic serum.

Another strain L. 5 at one period of its culture fermented no sugars though at a later period it produced acid from Glucose and Maltose. L. 3, obtained in pure culture during life from the cerebro-spinal fluid of a case of posterior basic meningitis, gave the usual sugar reactions and agreed with typical Meningococci in every way except that it grew very slowly at 24° C. This strain was mentioned by me in a previous paper (Arkwright, 1907).

Strain L. 9 (2) may be mentioned here though it is not implied that

it was a Meningococcus. This strain closely resembled *Micrococcus catarrhalis*. It grew at 20° C., fermented no sugars, resisted attempts to make a uniform suspension of the cocci, clumped spontaneously, and was of characteristic appearance in cultures and microscopically. It was obtained post-mortem from the heart-blood of a case of Sporadic meningitis from the cerebro-spinal fluid of which during life a typical Meningococcus (L. 9 (1)) was isolated.

It remains to mention some interesting micro-organisms which were, perhaps, all of the same species and which were isolated from the cerebro-spinal fluid of three cases of Sporadic meningitis.

These organisms, in stained films, from the cerebro-spinal fluid or from young agar cultures appear as gram-negative cocci which might well be taken for Meningococci. Two of these strains were isolated by Dr W. E. Marshall, to whom I am indebted for them, and one strain was isolated by myself. These strains are denoted in the tables as L. 6, L. 15 and L. 23. One of them (L. 6) was recorded in a former paper as a Gram-negative coccus resembling the description of *M. cinereus* given by v. Lingelsheim (Arkwright, 1907). I think, however, that it probably belongs to the present group. The characters of L. 15 and L. 23 have been more particularly examined and present the following features:—a growth on agar or gelatine at 22° C. is quite distinct in 24 hours; it is transparent and soon becomes confluent. The colonies appear bluish grey by reflected light and yellowish by transmitted light. Glucose, Maltose, Laevulose, and Cane sugar are not fermented. In broth the growth causes uniform turbidity. On MacConkey's bile salt agar there is fair growth. Uniform suspensions of an agar culture in salt solution are easily made and in the case of two strains no spontaneous clumping occurred, but in a suspension of the third strain some clumping occurred without the addition of serum.

Morphology:—A film made from a 24-hours' agar culture and stained by Gram's method with neutral red as a counterstain shews coccal forms in coherent masses or in short lines of 4 or 5. These cocci are rather smaller than most Meningococci, are of uniform size and mostly round, sometimes with an unstained dividing line across the middle. Some double forms like typical Meningococci are present but not in great numbers. In some fields, one or two long bacillary forms are seen which stain rather more deeply with the neutral red than the cocci, and which often shew indications of division into three or four segments. These bacilli are always present though sometimes in very small numbers and are more frequent in older cultures.

In broth cultures the morphology is more that of a short oval bacillus with some long forms.

At first the bacillary forms were regarded as due to a contamination, but microscopical preparations from isolated colonies after plating always presented the same appearance.

Similar organisms were isolated from cases of meningitis by W. James Wilson (1908).

As to the pathogenicity of these Gram-negative organisms and of others more closely resembling *M. catarrhalis*, which have been isolated from the cerebro-spinal fluid or blood in cases of meningitis it would be rash to give too positive an opinion at present. They apparently have no relationship to the Meningococcus of Weichselbaum. They may however be the causal organism in some cases of meningitis, or may play a secondary part.

CONCLUSIONS.

1. Whilst most Meningococci produce acid from Glucose and Maltose, strains of undoubted Meningococci are met with which at some stage of their artificial culture ferment only one or neither of these sugars.

2. Other minor differences in the fermentation reactions also occur amongst undoubted Meningococci.

3. These atypical varieties are found amongst strains from both Epidemic and Sporadic cases of meningitis, but more frequently come from Sporadic cases.

4. Specific agglutination in high dilutions (1—500 to 1—2000) obtained with the serum of a horse injected with two strains of Meningococcus was limited to a very few strains besides those used for the inoculation.

5. Agglutination experiments after the absorption of agglutinins tended to further mark the division of the strains of the Meningococcus used into sub-groups.

6. Experiments on the fixation of complement by the specific serum in the presence of different strains of Meningococcus confirmed on the whole the grouping indicated by the agglutination experiments.

7. The variations observed in the sugar and serum reactions were not such as to indicate a specific difference between the Epidemic and Sporadic strains, for the differences between individual members of each group were as great as any found between the two groups.

8. A Gram-negative coccus resembling more nearly the *Micrococcus catarrhalis* than the Meningococcus was found in the blood post-mortem of a case of Meningococcal meningitis.

9. A Gram-negative bacillus, whose morphology was chiefly that of a micrococcus and closely resembled that of the Meningococcus in the cerebro-spinal fluid and in young cultures, was found in pure or almost pure culture, in the cerebro-spinal fluid of three cases of Sporadic meningitis.

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