

AGGLUTINATION OF BACILLI OF THE ALKALIGENES,  
COLON AND TYPHOID GROUPS BY THE BLOOD  
SERUM OF CASES OF CEREBRO-SPINAL FEVER.

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BY means of a method introduced by one of us (Wilson, 1907), a bacillus closely resembling *B. faecalis alkaligenes* was recently isolated from Belfast tap water. This bacillus, for the purposes of this paper, will be spoken of as *B. Grosvenor*, it having been attained from a house in Grosvenor Road. We find that emulsions of this bacillus are agglutinated, often in high dilutions, by the blood serum from practically every case of the cerebro-spinal meningitis at present existing in epidemic form in Belfast.

That this epidemic is associated with Weichselbaum's *Meningococcus* we have pointed out in a former paper (22. vi. 1907).

Briefly the characters of the *B. Grosvenor* are as follows:—an actively motile bacillus; Gram-negative; gelatin not liquefied; no gas in glucose broth, nor with any other sugar or alcohol tested; milk not coagulated; very faint trace of indol in peptone water; neutral-red agar not fluorescent; Petruschky's litmus whey faintly alkalinised; Barsiekow's two media slightly alkalinised, then bleached, the blue returning on shaking; bluish colonies with faint reddish centre on Drigalski-Conradi plates; brown growth on potato, and abundant growth on agar.

A fairly highly agglutinating anti-typhoid serum clumped the bacillus in dilutions of 1 in 50, but in no higher dilution, whereas the same serum readily clumped *B. typhosus* in 1 in 1000 dilution in 15 minutes.

Our attention was strongly drawn to the *B. Grosvenor* by the following circumstances: six samples of blood were sent to us to be tested by the Grünbaum-Widal test for typhoid fever; these samples were found to be negative as regards agglutination of *B. typhosus* but one of them clumped *B. Grosvenor* in a dilution of 1 in 1000 and, on enquiry, this blood was found to have been taken from a case of epidemic cerebro-spinal meningitis, from the spinal fluid of which we isolated the specific *Meningococcus*. Moreover, this blood did not agglutinate *B. coli* in a dilution of 1 in 50.

Led by this hint, we tested the agglutinating power of normal human blood serum on *B. Grosvenor*, taking the blood from 31 healthy adults, with the result that three of the number, i.e. 9·6%, gave a positive reaction in dilutions of 1 in 50, but none gave clumping in 1 in 100. As a further control we tested the blood of 83 patients (non typhoid) from the wards of a general hospital, including such conditions as bronchitis, nephritis, tuberculosis, neuritis, acne, chronic rheumatism, dyspepsia, myocarditis and measles, and found that 56 were negative to 1 in 50, 25 were positive at this dilution, but negative to 1 in 100, while two only were positive to 1 in 100, and of these two one suffered from bronchitis, the other from furunculosis. As a still further control blood serum from 41 cases of enteric fever was tested and it was found that 24 were negative to *B. Grosvenor* in dilutions of 1 in 50, 10 were positive at this dilution, one was positive to 1 in 100 dilution; in the remaining six the reaction was doubtful.

Thus out of the above 155 cases used as controls there were three cases only in which the serum agglutinated *B. Grosvenor* in a dilution of 1 in 100; and 44 which gave a positive result in a 1 in 50 dilution; none of the controls clumped the bacillus in dilutions above 1 in 100.

In marked contrast to this result was the effect of serum from cases of cerebro-spinal fever upon *B. Grosvenor*, thus: blood serum from 135 of these cases acted as follows:—121 gave clumping (many of them instantaneously) in 1 in 50, 10 gave no clumping when first examined but when a fresh sample was tried on a subsequent occasion, the clumping occurred in some cases even in dilutions of 1 in 1000; lastly the remaining four were negative and the death of these patients prevented a subsequent re-testing.

As mentioned, many of the cases gave instantaneous agglutination in dilutions of 1 in 50, so that we were encouraged to push the dilutions to a higher degree, and found that 12 of these cases gave marked clumping within an hour to dilutions of 1 in 1000, and further, we

TABLE I. Shows agglutinating effect of blood serum of Belfast cerebro-spinal fever cases on *Bac. Grosveneri*.

(The sign + indicates a positive reaction, ++ indicates marked agglutination, - indicates no clumping.)

Name of patient	Date	Dilutions employed																Remarks			
		1 in 50	100	200	300	400	500	600	800	1000	1200	1400	1600	2000							
Cr-n	1/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	Post-mortem showed typical lesions of cerebro-spinal fever and meningococcus was cultivated from cerebro-spinal fluid. <i>B. typhosus</i> 1 in 50 negative. <i>B. coli communis</i> 1 in 50 negative.
	16/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Meningococcus isolated from the blood on 11th day of disease. Patient died on the 78th day of his illness.
	19/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	27/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	4/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
W-n	19/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	20/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
M-n	23/3/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>B. typhosus</i> 1 in 50 negative. <i>B. coli communis</i> 1 in 50 negative.
	10/4/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Patient convalescent.
Ga-le	12/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	<i>B. typhosus</i> 1 in 50 and 1 in 100 positive, 1 in 200 feebly positive.
	15/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
T. R-y	5/4/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	10/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
For-e	20/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	19/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Mur-y	10/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	27/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
M-ns	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Patient convalescent. <i>B. typhosus</i> in 50 negative. <i>B. coli communis</i> 1 in 50 negative.
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Patient convalescent.
McJ-y	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	" "
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	" "
Fr-er	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	" "
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	" "
Sk-y	22/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	24/4/07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Set-e	25/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	30/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
M-s	4/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Rich-d	4/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	9/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Dong-s	16/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	16/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Ward-w	16/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
St-rt	23/5/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	29/4/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
McM-n	7/8/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	28/8/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bel-r	28/8/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	5/9/07	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

found four of the twelve agglutinated in dilutions of 1 in 1600, and four others in dilutions of 1 in 1200, 1 in 1400, and 1 in 2000 respectively.

These higher dilutions were practised with blood taken a second and third time and oftener at intervals from the same patient, it being apparent that the agglutinins increase more and more as the patient progresses in convalescence, or rather in chronicity, seeing that death has ultimately occurred in some of these cases.

In the next place the agglutinative effect of blood serum from undoubted cases of cerebro-spinal meningitis was proven upon a flaginac colon bacillus which had been isolated from the urine of a cerebro-spinal case. Eighteen different samples of blood were so tested and it was found that 16 of them gave no clumping in dilutions of 1 in 50 while one of these bloods, in dilutions of 1 in 1000, clumped *B. Grosvenor*. One sample gave feeble clumping at 1 in 50. The remaining sample clumped the *B. coli* at 1 in 400, but although agglutinating *B. Grosvenor* at 1 in 50 was quite inactive at 1 in 400; it also clumped *B. typhosus* at 1 in 50 dilution, but not at 1 in 100.

Similarly the serum from 21 cases of cerebro-spinal fever was tried with *B. typhosus*. Fourteen of these did not clump Eberth's bacillus in dilutions of 1 in 50, although several of them agglutinated *B. Grosvenor* when diluted 1000 times. The other seven samples gave excellent clumping with *B. typhosus* as shown in Table II.

TABLE II. Shows agglutinating effect of blood serum of cerebro-spinal fever cases on *B. typhosus*.

(The sign + indicates a positive reaction, ++ marked agglutination, - indicates no clumping.)

Name of patient	Dilutions				Remarks
	1 in 50	1 in 100	1 in 200	1 in 400	
1. L—s	++	-			<i>B. Grosvenor</i> 1: 50 positive, 1 in 200 negative. <i>B. coli</i> 1 in 50, 100, 200, and 400 positive.
2. M <sup>a</sup> A—er	++	-			<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.
3. G—ble	++	++	+	-	<i>B. Grosvenor</i> 1 in 200 positive.
4. M <sup>c</sup> G—n	++	++	++	++	<i>B. coli</i> 1 in 50 negative.
5. M <sup>c</sup> G—re	++	++	++	-	<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.
6. W—son	++	-			
7. Cl—son	++	++	++	-	<i>B. Grosvenor</i> 1 in 200 positive. <i>B. coli</i> 1 in 50 negative.

The last case (number 7) in the above table was particularly interesting. The patient was a strongly built woman, 27 years of age.

Clinically her symptoms were typically those of meningitis, and yet her blood on three different occasions, when tested against *B. typhosus*, gave a marked agglutinative effect in dilutions of 1 in 200, both the microscopic and macroscopic methods being employed, as well as two separate strains of *B. typhosus*. An autopsy was permitted by her father, who informed us that she had never had any illness since an attack of scarlet fever in her ninth year. The post-mortem showed an advanced purulent inflammation of the meninges of the brain and spinal cord, from the pus of which the *Meningococcus* was recovered in pure culture. Moreover, the thoracic and abdominal organs presented the appearances so often found in cases of epidemic cerebro-spinal fever (see Symmers, Radmann, Westenhoefer) particularly the gastro-enteritis with enlarged solitary follicles and swollen and hyperaemic mesenteric glands. Peyer's patches were normal, the spleen was about a third larger than normal. The spleen, mesenteric glands and urine were exhaustively examined by cultural methods for *B. typhosus*, but none were obtained. This case is in various particulars similar to one obtained by Cowie (1907) who, relying, very naturally, on the positive Widal reaction, regarded his case as one of enteric fever with meningeal symptoms. Dr Cowie's report of the post-mortem lesions in his case are also very interesting in their similarity to those mentioned above.

In addition to the cases of cerebro-spinal fever occurring in Belfast we have been able, through the kindness of Dr Darling, of Lurgan, Dr Johnston, of Glasgow and Dr Ker, of Edinburgh, to examine blood coming from these three centres respectively. A glance at Table III will show that the results, judging from the few cases available, are along the same lines as mentioned in regard to the Belfast cases.

We thus see that there exist in some normal bloods substances that agglutinate the *B. Grosvenor*, that these substances are increased in some individuals suffering from general diseases, that in many cases of typhoid fever they undergo a further increase, but that it is in cerebro-spinal fever that the increase is constant, striking and progressive. We may remark that *B. Grosvenor* on agar furnishes a perfect emulsion that shows no tendency to spontaneous clumping, but that a specimen of *B. faecalis alkaligenes* (kindly sent to us by Dr A. C. Houston) was, owing to spontaneous clump formation, unsuitable for agglutinative work.

As far as our experiments have gone the blood of rabbits immunised with the *Meningococcus* does not agglutinate the *B. Grosvenor*. In view of the fact that we have never succeeded in isolating *B. Grosvenor* from

the bodies of cerebro-spinal fever cases, although a number of such attempts has been made, we content ourselves with merely stating the facts and refrain from all attempts at explanation.

TABLE III. Shows the agglutinating effect of blood serum from cerebro-spinal cases from Lurgan, Glasgow and Edinburgh on *B. Grosvenor*.

(+ indicates a positive reaction, ++ marked agglutination, -- indicates no clumping.)

Name of patient	Date	Dilutions							Remarks
		1 in 50	1 in 100	1 in 200	1 in 300	1 in 400	1 in 600	1 in 800	
<i>Lurgan cases.</i>									
1. Fr—s	20/3/07	++	++						} From the spinal fluid of all these cases the <i>Diplococcus intracellularis meningitidis</i> was isolated.
2. B. G.	20/3/07	++	++						
3. M <sup>c</sup> J—y	19/5/07	++	-						
	23/5/07	++	++	+					
<i>Glasgow cases.</i>									
1. P—er	15/5/07	++	++	++		-	-	-	} From the spinal fluid of all these cases the <i>Diplococcus intracellularis meningitidis</i> was isolated.
2. W—r	15/5/07	++	++	++	++	+	-	-	
3. F—lay	15/5/07	++	++	++	++	++	++	+	
4. L—y	15/5/07	++	++	++		-			
5. K—y	15/5/07	-	-						
<i>Edinburgh cases.</i>									
1. A case of posterior } basic meningitis)	19/5/07	++	-						} From the spinal fluid of all these cases the <i>Diplococcus intracellularis meningitidis</i> was isolated.
2. Ed. I. c. s. f.	23/5/07	++	+						
3. Ed. II. c. s. f.	23/5/07	++	+						

Having thus determined that the serum of cerebro-spinal cases has an agglutinative effect on *B. Grosvenor* and that the serum of rabbits infected with the *Meningococcus* has no effect on this bacillus we proceeded to show that the Grosvenor-agglutinins in the blood of patients suffering from cerebro-spinal meningitis are separate and distinct from the meningococcus-agglutinins present in the patients. This distinction between these two agglutinins is evident from the following experiment:—from a certain patient affected with cerebro-spinal meningitis, a serum was obtained that agglutinated *B. Grosvenor* in dilutions up to 1 in 2000. One drop of this serum was added to 19 drops of 0.8% saline solution, to this was added a loopful of an agar culture of *B. Grosvenor*, the mixture was allowed to stand at room temperature for two hours (or incubate at 37° C.), was then centrifuged and the supernatant fluid was found to be completely devoid

of agglutinating effect on *B. Grosvenor*. Whereas, the serum similarly diluted and treated, but with the addition of the *Meningococcus* in place of *B. Grosvenor*, retained its full agglutinative effect on *B. Grosvenor*. It is thus evident that saturation with meningococci has no effect on the agglutinin that acts on *B. Grosvenor*. Similarly it was found that saturation with *B. typhosus*, *B. coli*, or *B. faecalis alkaligenes* did not remove the agglutinin that acts on *B. Grosvenor*.

Conversely, we found that saturation with *B. Grosvenor* did not in any way alter the agglutinative effect of the serum as regards the *Meningococcus*.

Further Dr Houston determined for us the agglutinative and opsonic power of this serum after saturation respectively with (1) *Meningococci* and (2) *B. Grosvenor*;—the result being that saturation with *B. Grosvenor* did not diminish the opsonic power of the serum as regards *Meningococci*, neither did it lessen the degree of agglutination. Whereas saturation with *Meningococci* abolished the opsonic and agglutinative effect of the serum for *Meningococci*. This result is apparent in the following table:—

	Opsonic effect on <i>Meningococci</i>	Agglutination of <i>Meningococci</i>
Untreated serum ... ..	383 cocci in 40 leucocytes	++
Serum saturated with <i>B. Grosvenor</i>	401 " " "	++
" " " <i>Meningococci</i>	27 " " "	-

Note: ++ means agglutination; the sign - means no agglutination.

In the next place we found that as a general rule the serum of cerebro-spinal cases when possessed of a high opsonic index as to the *Meningococcus* also showed high agglutinative power towards *B. Grosvenor*,—this relation of the two properties was however not invariable.

That the mere fact of suppuration did not explain the presence of agglutinin active toward *B. Grosvenor* was evident from the complete absence of such agglutination in three cases of suppurative meningitis that were respectively associated with *B. typhosus*, *B. enteritidis* Gaertner, and a pure streptococcal infection.

#### CONCLUSION.

1. The blood of patients suffering from epidemic cerebro-spinal meningitis is in practically all cases agglutinative to *B. Grosvenor*.
2. If the patient lives long enough, the reaction can be obtained in dilutions of 1 in 2000.

3. Occasionally the blood is agglutinative to *B. typhosus* and *B. coli* in comparatively high dilutions.

4. The opsonin and agglutinin acting on *B. Grosvenor* are quite distinct from those acting on *Meningococcus*.

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