ON VARIATIONS OF THE MENINGOCOCCUS AND ITS DIFFERENTIATION FROM OTHER COCCI OCCURRING IN THE CEREBRO-SPINAL FLUID.

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Weichselbaum (1887) first described his *Diplococcus intracellularis meningitidis* (meningococcus) as having been cultivated within a few hours after death from the brain or meninges of six cases of acute cerebro-spinal meningitis. These cases occurred during the years 1885—1887 at Vienna, when no regular epidemic existed. Cases had however occurred from time to time, and there was an epidemic in part of Lower Austria in 1886. The youngest case was 14 years old. He described the micrococcus as being of a wide gonococcus-like shape, occurring in pairs or fours, not in chains, negative to Gram's stain. It failed to grow at 20° C., but grew well on agar at 37° C. almost exclusively on the surface. The growth was viscid, the colonies were often confluent and appeared finely granular when slightly magnified. The organisms required sub-culture every few days to ensure success, were easily killed by drying, and showed very little growth in broth.

Jaeger (1895) described a diplococcus associated with an epidemic of cerebro-spinal meningitis. He stated that it was similar to Weichselbaum's organism but that after a short period of cultivation in artificial media it changed its character, becoming Gram-positive, and occasionally grew in short chains and became resistant to drying.

Councilman, Mallory and Wright (1898) found Weichselbaum's diplococcus in an epidemic in Massachusetts, and confirmed the persistence of its Gram-negative characteristics and its susceptibility to drying.

Still (1898) cultivated the meningococcus from a series of cases of posterior basic meningitis, and found that it agreed very closely with Weichselbaum's description, but that it was a little longer-lived in cultures, and grew rather more readily on artificial media.

Albrecht and Ghon (1903) appear to have proved that Jaeger's meningococcus is another organism which appeared in his cultures, although he probably found the true meningococcus of Weichselbaum in his original preparations.

Various statements have been made about the resistance of the meningococcus to drying. Germano (1897) stated that meningococci mixed with dust and dried lived 80—90 days, but he was working with Jaeger's strain, which is not now regarded as the true meningococcus. Councilman, Mallory and Wright (1898) dried meningococci on sterile paper in Petri dishes and placed these in a dark drawer at room temperature. They succeeded in obtaining cultures from the dried material after 60 hours but failed to do so after 72 hours. Albrecht and Ghon (1903) dried a meningococcus emulsion on coverslips and kept them for 24 hours at room temperature in the dark. After this the cocci were dead. Bettencourt and França (1902) found meningococci alive after six hours, but not after nine hours' drying on cotton threads at room temperature in the dark.

v. Lingelsheim (1906), during the great epidemic in Upper Silesia, found that material obtained by lumbar puncture or post-mortem or from the nose, yielded meningococci more frequently if the specimen was received and cultures were made at the laboratory within a few hours of its removal from the body or the death of the patient.

In the case of 31 post-mortem specimens where special precautions were taken to promote rapidity of transit, the meningococcus was isolated from every one. This observer carried out experiments showing that the meningococcus is not affected by diffuse light though killed by direct sunlight. He is also of the opinion that cultures live a shorter time at low temperatures only because they do not grow and the individual life of the coccus is always short. He found that the meningococcus would survive a temperature of $-10°$ C. or $-20°$ C. for two hours.

It has been stated that the meningococcus is especially susceptible to cold, but the above experiments disprove this.

v. Lingelsheim expresses the opinion that the Gram-negative cocci found by him inside leucocytes in cerebro-spinal fluid in cases of meningitis were always true meningococci. He tested the reactions of cultures of various Gram-negative cocci grown on sugar-containing media, and found that the meningococcus produced acid on dextrose and maltose, but not on laevulose, galactose, mannite, dulcite, cane sugar, lactose or inulin. Indeed he makes the production of acid from laevulose a proof that the organism is not a true meningococcus, but as he used

solid media and took the reaction after 24 hours as his criterion his results are hardly comparable with that of Gordon. The different reactions of cultures and Gram-negative cocci in broth containing various carbohydrates as given by Dunn and Gordon (1905) are detailed in a paper by me (on the occurrence of *Micrococcus catarrhalis* and its differentiation from other Gram-negative cocci) in this *Journal* (Jan. 1907).

C. Fraenkel (1905) found that meningococci made a feeble growth on gelatin at 22° C., but not at 20° C.

Other cocci found in the cerebro-spinal fluid drawn off by lumbar puncture, are of special interest on account of the difficulty there may be in differentiating them from the meningococcus, and also because some of them occur associated with the meningococcus when this organism may nevertheless be considered the true or chief cause of the disease. The pneumococcus has long been known to be a cause of primary meningitis. Cases of this nature are described by Weichselbaum (1887). The pneumococcus is not infrequently associated with the meningococcus.

v. Lingelsheim (1906) enumerates the following organisms in addition to the *Pneumococcus*, as being found by him in the central nervous system in epidemic meningitis, *viz.*:—varieties of *Streptococcus*, *Staphylococcus*, *Diplococcus crassus*, *D. mucosus*, *D. pharyngis*, *flavus II*, and *Micrococcus cinereus*. The last three organisms are Gram-negative, but are distinguishable by the changes they produce in sugar-containing media, by their other cultural characters, and by agglutination tests.

Diplococcus crassus is of special interest, as v. Lingelsheim considers it identical with Jaeger's meningococcus. It is partly Gram-positive and partly Gram-negative; even cocci from adjacent colonies varying in the degree with which they retain the stain. It readily produces acid from several carbohydrates, including saccharose and lactose, and an antiserum obtained by injecting it into a rabbit does not agglutinate the meningococcus. v. Lingelsheim attributes certain symptoms in some cases of meningitis to an infection with *Diplococcus crassus*, added to that with the meningococcus.

Intracellular cocci in cerebro-spinal fluid may be the *Staphylococcus aureus*, as in a case recorded by Wright and Archibald (1906).

Agglutination experiments with the meningococcus have been recorded by many writers.

Bettencourt and França (1902) examined by the macroscopic method the agglutinating power of the blood serum of persons who were ill

with epidemic meningitis and of those who had recovered. As a control they tested the serum of 17 persons in health or suffering from other diseases. The serum from none of these latter agglutinated the meningococcus in dilutions of 1—10, and one doubtfully in those of 1—5.

In all the six patients suffering from meningitis whose serum they examined agglutination was obtained in dilutions varying from 1—10 to 1—100: in three cases as early as the 4th day, and in one as late as the 56th day. In a case examined on the 4th day the serum agglutinated at 1—50, whereas meningococci could not be found in the cerebro-spinal fluid till the 6th day. They tested the serum of 15 patients who had recovered and found that it still agglutinated in dilutions varying from 1—5 to 1—1000; 4 reaching 1—100 from 8 to 14 months after the onset of the disease.

v. Lingelsheim (1906) obtained agglutination of the meningococcus with the serum of patients in about 55 % of cases examined on the 6—20th day, and in about 25 % of those examined in the first 5 days or after the 20th day of the illness.

Both of these authors also obtained sera of high agglutinating values by injecting rabbits.

Goodwin and Sholly (1906) found that the agglutinating power of serum prepared by injecting meningococci varied much from day to day, and Dunham (1906) failed to obtain a serum of high agglutinating power.

Kutscher (1906) describes a strain of meningococcus agglutinated macroscopically by a specific horse serum, at 55° C., but not at 37° C.

The meningococcus may then be described as essentially a Gram-negative coccus, morphologically closely resembling the gonococcus and *M. catarrhalis*. It grows on agar at 37° C. after one or two cultures on serum-agar, as smooth or finely granular slightly milky translucent colonies, but does not grow on gelatin at 20° C. It forms acid in maltose and glucose broths, and as a rule in galactose and laevulose, but not in cane sugar. It is very easily killed by drying, and can be agglutinated by a specific serum prepared from a rabbit or horse.

I have cultivated the following strains:

Meningococcus XII, obtained from Dr Eyre.

Meningococcus XVII, kindly given me by Dr F. W. Andrewes. It was derived from a very rapidly fatal case of meningococcus septicaemia.

Meningococcus XIX, kindly given to me by Dr Graham Forbes, who isolated it from a very acute and rapidly fatal case of meningitis in a child.

I have also examined several specimens of cerebro-spinal fluid drawn off during life from patients suffering from meningitis. The cerebro-spinal fluid was poured on to sloped mixtures of agar and blood in tubes, and any deposit after centrifugalising was smeared on similar tubes. One post-mortem specimen of exudation on the meninges is included here.

Specimen 1. (From which strain XVIII was cultivated.) Cerebro-spinal fluid obtained by tapping the lateral ventricles of a child of 6 months old, who had been ill for 6 weeks. This child had a typical form of posterior basic meningitis and was an in-patient at St Bartholomew's Hospital under the care of Dr Morley Fletcher, who kindly gave me permission to examine the fluid. The diagnosis was confirmed by the post-mortem. The fluid was colourless not at all turbid and there was no deposit after centrifugalising. No cells or cocci were seen in the smear. Typical colonies appeared on blood-agar. The first cultures also contained streptococci.

Specimen 2. (From which strain XXIX was derived.) Cerebro-spinal fluid drawn off by lumbar puncture on the 5th day of illness from a child aged 6 months, under the care of Dr W. Carr at the Victoria Hospital, Chelsea, to whom I am indebted for the material. This was a case of posterior basic meningitis, confirmed post-mortem. The fluid was clear with small fibrin clots: these smeared on a slide showed microscopically polymorphonuclear cells containing Gram-negative cocci, resembling meningococci. Inoculation of this material on blood-agar yielded colonies of (1) Meningococci, (2) Gram-positive staphylococci, (3) Streptococci resembling Pneumococci.

Specimen 3. (Which yielded strain 4*M*.) Lumbar puncture fluid from a child aged 4½ years, under the care of Dr W. Carr at the Victoria Hospital. The child died of meningitis after about three weeks' illness: no post-mortem. The fluid was examined by Dr W. E. Marshall. A single colony appeared on the cultures which resembled a meningococcus colony, and consisted of Gram-negative cocci.

Specimen 4. (*M*. 8.) Cerebro-spinal fluid obtained by lumbar puncture after an indefinite illness of some weeks' duration from a child aged 4 months under the care of Dr W. Carr, at the Victoria Hospital. The fluid was clear and contained some leucocytes, mostly polymorphonuclear, and streptococci. Cultures yielded a Gram-negative small oval bacillus which liquefied gelatin. Post-mortem no macroscopic meningitis.

Specimen 5. (*M*. 10.) Post-mortem material from the meninges of a child aged 3 months under the care of Dr Montague Murray at the Victoria Hospital, who kindly allowed me to make cultures. There was a history of only 26 hours' illness. Post-mortem tough yellowish exudation was found under the pia mater. A smear showed a pneumococcus-like diplococcus. Cultures from the meninges gave almost pure Gram-positive lancet-shaped cocci. The pneumococci still showed capsules in agar culture after the second generation.

Specimen 6. (*M*. 11.) Cerebro-spinal fluid removed by lumbar puncture from a child aged 9 months with symptoms of posterior basic meningitis, under the care of Dr W. Carr at the Victoria Hospital. A smear of the deposit in the fluid showed many polymorphonuclear leucocytes and a few lymphocytes and large mononuclears,

and cocci which morphologically resembled meningococci. Culturally (1) meningococci found but not isolated, (2) Gram-positive cocci, and (3) a Gram-negative short bacillus.

Specimen 7. (*M.* 27.) Cerebro-spinal fluid, from a case of meningitis, which was sent by post and was almost 48 hours on the journey. Broken up cells and no cocci were seen microscopically. Only Gram-positive streptococci and staphylococci were isolated, probably contaminations.

Specimen 8. (*M.* 34.) Cerebro-spinal fluid which was about 20 hours in the post, from a typical case of cerebro-spinal meningitis on 4th day of disease. The fluid was watery in appearance and gave a very small deposit on centrifugalising. Microscopically many polymorphonuclears were seen but no cocci.

In the cultures only Gram-positive staphylococci appeared.

Specimen 9. (*M.* 33.) Lumbar puncture fluid from a typical case of cerebro-spinal meningitis at the 6th day of the disease. The specimen was about 20 hours in the post; no leucocytes nor cocci were seen after centrifugalising. Cultures yielded a Gram-positive staphylococcus and a bacillus resembling the *Bacillus coryzae segmentosus.*

Specimen 10. (Yielding strain *M.* 38.) Lumbar puncture fluid from a child aged 5 suffering from meningitis, which was about 16 hours in post, had a deposit of thick pus which was composed chiefly of polymorphonuclear leucocytes some of which contained a few (1 to 3) large Gram-negative cocci of typical appearance. Meningococci grew freely in cultures and one or two colonies of a *Staphylococcus albus* appeared.

The strains of meningococcus XII, XVII, XVIII, XIX, XXIX, and *M.* 38, when grown on blood agar, formed small faintly milky and raised colonies, which after two or three generations grew on ordinary agar at 37° C. as translucent, very slightly whitish colonies, which were confluent when much crowded, and were slightly brownish or amber as seen by transmitted light. Under a 1-inch objective the colonies were seen to be very finely granular or quite smooth. The growth was rather sticky and slimy. The earlier generations died if not sub-cultured every three days, but later generations if protected from drying by tight plugging of the tube, sometimes lived for one month at 37° C. At room temperature sub-culture was never successful after three days. In ordinary broth at 37° C. growth took place (though very slowly when first isolated), a general turbidity being caused, and later a whitish muddy deposit.

When 1% of various carbohydrates was added to peptone water and broth (75% of the former, 25% of the latter) acid was formed in three days from maltose and glucose, and later from galactose and laevulose in the case of XII, XVII, XVIII and XIX; but XXIX, only produced acid from glucose after prolonged artificial culture and not constantly from laevulose. XXIX never formed acid from galactose. None of

these strains produced acid from cane sugar or lactose. The medium became turbid and a whitish ring, adherent to the glass, formed at the upper level of the liquid in seven days.

In no instance was the result altered by adding ascitic fluid or serum to the sugar-broth, though the change was sometimes hastened.

These strains did not grow on gelatin at 22° C., but on one occasion when the incubator was raised to 24° C. for a few hours, a very slow growth of XVIII began and continued at 20° C., liquefying the gelatin at the surface. The purity of this culture was repeatedly proved by sub-culture and microscopic examination. Accordingly I made cultures on gelatin of four (XII, XXIX, XVIII, and XIX) of the strains of meningococcus, strain 4*M*. and several strains of *M. catarrhalis* obtained from the nose, and incubated them at 37° C.; after 10 days the gelatin containing strains XVIII, XXIX and XII remained liquid, but was of a thick syrupy consistency, on cooling to 15° C., whereas those tubes containing XIX, 4*M*. and the different strains of *M. catarrhalis* became quite solid.

It, therefore, appears that some difference exists among strains of meningococcus, in the power of producing acid from sugars and in that of liquefying gelatin.

Those strains isolated from typical posterior basic cases showed the most aberrant characters.

The strain 4*M*., which was found in pure culture and a single colony isolated from the cerebro-spinal fluid of a case of meningitis, is particularly interesting, in that it agreed in some particulars with the meningococcus, but was certainly not a member of this species, and also because Gram-negative intracellular cocci of very similar shape and appearance to meningococci were seen by Dr W. E. Marshall in polymorphonuclear leucocytes in a smear of the same specimen of cerebro-spinal fluid. Of course these may have been meningococci which did not grow in the culture tubes. Only one colony had grown on the blood agar at the end of 48 hours, and this was indistinguishable from a colony of meningococci in appearance. On sub-culture, however, it grew well on agar, but always discretely, and the colonies were whiter than those of meningococcus. It grew in broth rendering the medium uniformly turbid, and the colonies on agar were not coarsely granular and were moist and rather sticky. An emulsion was easily made and did not agglutinate spontaneously. In these respects 4*M*. resembled the meningococcus very closely, but it grew on gelatin at 22° C. without liquefaction; it failed to produce acid from glucose,

maltose or galactose, and an emulsion was not agglutinated by meningococcus serum in dilutions of 1—2 and 1—10. Moreover in a film from the original colony the cocci were rather larger and stained better with methylene blue than it is usual for meningococci to do. It is, therefore, clear that this organism is not the meningococcus. It resembles *Micrococcus cinereus* of v. Lingelsheim in most particulars. (See my paper on *Micrococcus catarrhalis* &c. in this *Journal*, Jan. 1907, Table III shewing cultural differences.)

I made four experiments with regard to the power which the meningococcus has to withstand drying.

Experiment 1. Impressions of blood agar colonies of XXIX and XVIII on coverslips were put into dry sterile tubes and left at room temperature for 2 days, and then transferred to broth and incubated at 37° C. The meningococcus could not be recovered from them.

Experiment 2. Portions of broth cultures of strains XVIII and XIX were dried in sterile Petri dishes (*a*) mixed with sterile sand, (*b*) mixed with sterile violet powder, (*c*) without admixture. The dishes were then placed in an air-pump receiver over strong sulphuric acid and the air exhausted. Samples taken at the end of 24 hours did not contain living meningococci.

Experiment 3. Some of a broth culture of strain XXIX was dried on strips of sterile blotting paper or on sand, over sulphuric acid in the receiver of an air-pump; after three hours the sand looked dry but was still damp; from a sample put into broth and incubated at 37° C. meningococci grew. From a strip of blotting paper taken at the same time and put into broth at 37° C. no meningococci grew. After 24 hours and complete drying of the sand no meningococci could be recovered.

Experiment 4. A broth culture of strain XII poured on dry sterile garden soil in a Petri dish and left at room temperature yielded no meningococci after 20 hours.

To test resistance to cold, agar cultures of four strains of the meningococcus (XII, XVII, XVIII, XIX) were put in the cold room at 1° C. They could be sub-cultured after 72 hours.

In order to further test the susceptibility of this organism to cold, the cerebro-spinal fluid of specimen 10 was placed in the cold room at 4° C., and successful cultures were made after 48 hours, but not after 72 hours. Before this specimen reached me 15 hours had already elapsed since its removal from the body.

This high degree of viability is in marked contrast to that observed in the case of some strains such as those contained in specimens 7 and 8, which performed the same journey but could not be grown when received though similar care and media were used. It seems probable therefore that the rapid death of meningococci in lumbar puncture fluid, which has often been observed, is not due to cold but to some other

cause, such as the presence of other bacteria or of harmful substances in the cerebro-spinal fluid as suggested by Flexner (1906).

Agglutination Experiments.

To prepare an agglutinating serum from a rabbit it is necessary to give large and frequent doses of meningococci intravenously for a considerable time, and the serum appears to quickly lose its agglutinating properties if the injections are discontinued.

Agglutinating serum was obtained for me by Dr W. E. Marshall by injecting large quantities of meningococci intravenously into three rabbits; the doses chiefly used were from 2—4 agar tubes, and the injections had to be given for 4—5 weeks before the serum showed much agglutinating power. I used the microscopic method of examination for agglutination.

Rabbit I was injected intravenously with 2-day agar cultures of meningococcus XII, emulsified in a 0·9 % sodium chloride solution and killed at 65° C. for 30 minutes.

TABLE I.

Agglutination with serum from Rabbit I.

		1/2	1/10	1/25	1/50	1/100	1/200	1/500	1/1000	Normal 1/2
March 30	Microscopic									
	XII & XVIII 1½ hrs.	–		–	–					
	Macroscopic									
	XII 24 hrs.			–	–	–				
April 26	Microscopic									
	XII 1 hr.	+	+	sl.		–				
May 1	Microscopic									
	XII 1 hr.		+ + +	+ +	+	+ +	+	sl.	–	
	XIX ,,		+ + +	+ +	+	+ +	+ +	+	–	
	XVIII ,,	+ + +	+ + +	+ +	+	+	+	+		
	XVII ,,	+	+ +	+ +	+	+	sl.			
	XXIX ,,	+	–	sl.	sl.	–	–			
May 19	XII ,,	+	sl.							
	XXIX ,,			+	sl.					
				1/20						1/2000
May 25	XII ,,	+	+	+ +		+	+ +		+	
	XVIII ,,	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +			
	XXIX ,,	+ + +	+ + +	+ +	+ +					
	4*M.* ,,	–	–	–						

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March 9th. 4 agar tubes injected intravenously.
„ 16th. 7 agar tubes injected intravenously.
„ 30th. Bled: the serum did not agglutinate meningococcus XII nor XVII in a dilution of 1—2.
April 6th. 4 agar tubes injected intravenously.
„ 12th. 3 agar tubes injected intravenously
„ 17th. 3½ agar tubes injected intravenously
„ 21st. 4 agar tubes injected intravenously
„ 24th. Bled: agglutination of meningococci in 1—2 and 1—10 dilutions.
May 1st. Bled: agglutination good 1—200 to 1—500.
„ 9th. 3 agar tubes injected.
„ 18th. 3 agar tubes injected.
Bled.
„ 23rd. Bled.

Rabbit II. Injected intravenously with 2-days' old agar cultures, killed at 65° C. for 30 minutes.

March 9th. 4 agar tubes injected.
„ 16th. 7 agar tubes injected.
April 3rd. Bled: no agglutination.
„ 6th. 4 agar tubes injected.
„ 12th. 3 agar tubes injected.
„ 17th. 3½ agar tubes injected.
„ 21st. 4 agar tubes injected.
„ 24th. Bled: agglutination 1—10.

TABLE II.

Agglutination with serum of Rabbit II.

		1/2	1/10	1/25	1/50
	Microscopic				
April 3	XII 1 hr.	–		–	–
	XVII „	–		–	–
April 24	XII	+	+		sl.
	XII with normal serum from rabbit.	–	–		

This rabbit shows the difficulty sometimes encountered in obtaining an agglutinating serum.

Rabbit III. Injected with agar cultures of meningococcus XXIX killed at 60° C.

April 9th. 3½ agar tubes of 48 hours' growth injected.
„ 12th. 3 agar tubes of 48 hours' growth injected.
„ 17th. 4 agar tubes of 3 days' growth injected.
„ 24th. Bled.
„ 30th. 3 tubes of 48 hours' growth injected.
May 8th. 3 tubes of 24 hours' growth injected.
„ 18th. 3 tubes of 24 hours' growth injected.
Bled.

TABLE III.

Agglutination with serum of Rabbit III.

Dilution	1/2	1/10	1/50	1/100	1/200	1/1000	1/2000
9 April 1906							
XXIX	–	–	–				
26 April 1906		+ +	+ +	+ +	+		
19 May 1906			+ +	+ +	+ +	+	

One sample of cerebro-spinal fluid which I examined, and which was drawn by lumbar puncture from a child who had been ill about 13 days, agglutinated the corresponding strain (XXIX) in dilution of 1—5, and other strains (XVII and XVIII) in dilutions of 1—100 and 1—50 respectively.

TABLE IV.

Agglutination with cerebro-spinal fluid from the same case from which Strain XXIX was isolated.

Dilution	1/2	1/20	1/50	1/100
XXIX	+	–	–	–
XVII	+	+ + +	+ +	+
XVIII	+	+ +	+	–

The serum of rabbits repeatedly injected with cultures of *M. catarrhalis* did not agglutinate the *meningococcus*, and could not be tested on *M. catarrhalis* on account of the constant spontaneous agglutination of this organism.

The agglutinating power of *M. catarrhalis* serum has been tried by v. Lingelsheim (1906). He found that it never agglutinated the *meningococcus* in higher dilution than 1—50 macroscopically.

Conclusions.

1. Gram-negative cocci obtained from the cerebro-spinal fluid are not always true meningococci, even in cases of meningitis.

2. Slight differences between different races of meningococci occur, especially as regards their growth and activity in sugar media and on gelatin.

3. The meningococcus is not easily killed by cold, therefore its rapid death in lumbar puncture fluid and post-mortem material must be due to some other cause.

4. The means by which the meningococcus is carried from the diseased to the healthy can hardly be such as to involve drying.

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