GROUPING OF THE STRAINS OF MENINGOCOCCUS

ISOLATED DURING THE EPIDEMIC OF CEREBRO-
SPINAL MENINGITIS IN 1915.

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THE following account refers only to strains of meningococcus which were isolated from the cerebro-spinal fluid or meninges of cases of meningitis. All the strains conformed to the usual morphological and staining characters of the meningococcus. Culturally they did not make growth at the temperature of the laboratory and they fermented glucose but not cane sugar.

The formation of a uniform permanent emulsion in physiological salt solution was a constant characteristic of all the strains, except three, which sometimes showed agglutination in salt solution (NaCl 0.85 per cent.).

Several of the cultures gave a distinctly yoke-yellow growth on glucose agar; this was especially noticeable in the case of two strains, M. 13 and 4410, on some occasions. Many strains made a very mucilaginous growth on moist glucose agar. The culture medium found most convenient for applying the sugar tests was Hiss's serum water. After mixing serum one part with distilled water three parts, the mixture can be autoclaved at about 118° for twenty minutes without causing precipitation of the protein; 1 per cent. carbohydrate and litmus can then be added and the tubes steamed three times. Stock cultures were kept on 1 per cent. glucose agar at 37° in closed tin boxes.

Thirty-five strains were examined, which came from the following sources:

- Nine from cases occurring in the first Canadian contingent on Salisbury Plain, found February, 1915.
- Four other cases occurring in the first Canadian contingent on Salisbury Plain, found March, 1915.
- Eight cases occurring in the Herbert Hospital, Woolwich, received through the kindness of Mr. K. Goadby.
- Nine cases occurring among soldiers, kindly supplied to me by Major Gordon and Captain Hine, R.A.M.C.
- Two cases occurring in France, received from Major S. Rowland, R.A.M.C.
- Two cases occurring at Reading, received from Dr. Donaldson.
- One case occurring at Watford.

Agglutination tests have been performed with two main objects: (1) With polyvalent rabbit serum, with the object of obtaining a criterion for the diagnosis of the meningococci. (2) With serum prepared from rabbits with single strains, in order to determine whether these strains of meningococcus, isolated during the epidemic, fell into groups.

At the same time agglutination tests were made with antimeningococcus and antiparameningococcus serums obtained from the Pasteur Institute, through the kindness of Professor Roux.

Preparation of Serums.

In order to obtain agglutinating serums, rabbits were inoculated intravenously with unheated emulsion. Suitable serums, with a sufficiently high titre, were not obtained till the animal had received seven to ten injections; the maximum dose given was two glucose agar slopes. For the tests detailed below three rabbit serums were used:

- A. prepared from a single strain—Plank.
- B. prepared from a single strain—M. 13.
- C. prepared from six strains—Carter, Crisp, Plank, Palmer, M. 1, M. 13.

Agglutination Technique.

Cultures of one day's growth on glucose agar were emulsified in distilled water containing 0.5 per cent. phenol. The serum was diluted with salt solution (0.85 per cent. NaCl). The density of emulsion used was judged by eye, and was of about the consistence obtained by adding 10 c.cm. of water to one glucose agar slope. Several strains were tested on the same day, and two or four serums were used on each occasion; the results, therefore, to some extent controlled each other. A control

tube with salt solution was always included for each strain, and each strain was tested with normal horse or rabbit serum.

Agglutination was not obtained with $\frac{1}{2}$ normal serum with any strain unless agglutination also occurred in salt solution. It was found that rather higher specific agglutination was obtained when the tubes contained salt solution of half or one quarter the usual strength, than when both the emulsion and the serum dilutions were made up with physiological salt solution (NaCl 0.85 per cent.).

The agglutination tests were carried out macroscopically in small tubes which were put in the incubator at 37° C. for eighteen to twenty hours, and then kept on the bench at the temperature of the laboratory. When complete agglutination (C) occurred there was complete clearing of the supernatant fluid and marked deposit.

The least degree of agglutination (L) noted was shown by the absence of the small round collection of sedimented cocci, rather larger than a pin's head, seen in the control tubes, and by an irregular granular deposit scattered over the concave bottom of the tube.

The results obtained with the multivalent serum C. are shown in Table I. Of 32 strains tested, 30 showed agglutination complete or almost complete in a dilution of 1 in 100. One strain (Llandrindod) agglutinated completely, 1 in 800; another strain (Carter) reached 1 in 400, and ten strains were completely agglutinated by a dilution of 1 in 200. Of the remaining two strains, one (Kennedy) only gave incomplete agglutination (I) in 1 in 100, and the other (McPhail) agglutinated slightly in salt solution, and only incompletely in 1 in 100. Tests with serum C. of strain of *Diplococcus flavus*, which did not agglutinate in the salt solution control, were negative.

The table also shows the agglutination results obtained with the two univalent rabbit serums A. and B., and also with antimeningococcus and antiparameningococcus serum from the Pasteur Institute.

By means of the two serums A. and B. it was possible to place 27 out of the 35 strains in one or other of two groups:

- 17 in Group A. were agglutinated best by Serum A. prepared with strain "Plank."
- 10 in Group B. were best agglutinated by Serum B. prepared with strain "M. 13."

By means of the two Pasteur Institute serums 27 out of the 35 strains could be divided into two groups:

- 15 into Group M. agglutinated best by antimeningococcus serum, and
- 12 in Group P. agglutinated by antiparameningococcus serum.

Whenever the strains could be grouped definitely by both pairs of serums—that is, A. and B. and M. and P. respectively—a strain falling into Group A. also fell into Group M., and those agglutinated by B. serum were also agglutinated by antiparameningococcus serum, except in the case of two strains.

It seems, therefore, justifiable to speak of two main groups, A.M. and B.P., corresponding to the meningococcus and parameningococcus groups of Dopter. Whenever a strain fell into the B.P. group it appeared to do so quite definitely; the A.M. strains varied more in their agglutinability, and were more influenced by the B.P. serums than the B.P. strain were affected by the A.M. serums.

By means of these four serums it was possible to definitely group 30 strains—18 in the A.M. group, and 12 in the B.P. group. One strain could not be classified. One strain (Chandler) agglutinated not very strongly, but equally with A. and B. serums. One strain (McPhail) agglutinated in salt solution. Two strains (Chase and Berkhamstead) fell in different groups on two occasions. Chase was agglutinated best by P. serum on June 25th, and by M. serum on August 18th. Berkhamstead was agglutinated by P. serum on July 20th, but not on August 30th, on which latter date it reacted better with A. serum than with B. serum. One strain (Littledale) gave no reaction with the Pasteur Institute serums, but agglutinated completely with serum C.

Some inquiries were made into the sources of the strains to find out (1) whether the different localities yielded more of one group of meningococcus than the other; (2) whether the clinical symptoms were different or more severe in cases infected with one group than with the other. No decisive answer could be given to either

TABLE I.

Strain.	Meningococcus Serum: Pasteur Institute (Horse).					Parameningococcus Serum: Pasteur Institute (Horse).					Multivalent Serum (Rabbit) C.					Univalent Serum (Rabbit) A. Strain: Plank.				Univalent Serum (Rabbit) B Strain: M. 13.				Salt Solution (0.42 per cent.).
	50	100	200	400	800	50	100	200	400	800	50	100	200	400	800	50	100	200	400	50	100	200	400	
Carter ...	0	0	0	0		C	C	C			C	C	C	C	I	I	0	0		C	C	0		0
Crisp ...	C	I	L	0	0	0	0	0	0	0	C	C	C	L	0	C	C	C		C	C	0		0
Palmer ...	C	C	L	0	0	0	0	0	0	0	C	C	I	L	0	I	C	I		C	I	0		0
Goodyear ...	L	I	C			0	0	0			C	C	L	0	0	I	C	C		C	C	0		0
Kennedy ...	0	I	0			0	0	0			0	I				C	C	C		C	0	0		0
Randall ...	0	0	0			C	C	L				C					0	0	0		I	0	0	0
Chase: (1) June 28th, 1915...	0	0	0	0	0	C	C	C	C	L		C												0
(2) Aug. 18th, 1915 ...	C	C	L			0	0	0								L	0	0		I	0	0		0
Murray ...		I	I	L			0	0	0		C	C	C	0	0		C	C	I		C	I	L	0
Chandler ...	0	0	0				0	0	0		C	C	L	0	0		C	L	0		C	L	0	0
Plank ...	L	I	I			0	0	0	0		C	C	I	I	L	C	C	C		C	I	0		0
Atkin ...	C	C	C	L	0		0	0	0			C	C	I	L		I	C	0		L	0	0	0
Somers ...	0	0	0			C	C	I			C	C	L	0	0	I	L	0		C	C	L		0
T. 167 ...	0	0	0				C	I	L		C	C	L	0	0		C	L	0		C	L	0	0
Lewis ...	L	C	I			0	0	0			C					I	C	C		C	I	0		0
Clifford ...	0	0	0			L	I	C			C	C	C	0	0	L	0	0		C	C	I		0
4378 ...	C	I	L			0	0	0			C	C	C	L	0	C	C	I		C	C	L		0
4409 ...	C	C	I	0	0	L	0	0	0	0		C	L	0	0									
4410 ...	0	0	0	0	0	C	I	0	0	0	I	0	0	0	0									
4411 ...	0	0	0			C	C	C				C				I	L	0		C	C	I		0
4412 ...	0	I	L			0	0	0			C	C	C	0	0	C	C	C		C	C	I		0
4442 ...		C	I	L			L	0	0		C	C	L	0	0		C	C	C		C	I	L	0
Llandrindod ...	L	0	0	0	0	L	I	C	C	I	C	C	C	C	C		L	0	0	I	I	0		0
Tring ...	0	0	0			0	0	0			C	C	0	0	0	C	C	C		C	0	0		0
Littledale ...	0	0	0	0	0	0	0	0	0	0	C	C	L	0	0									
Berkhampstead: (1) July 20th, 1915 ...	0	0	0	0		I	I	L	0	0	C	C	L	L										
(2) Aug. 20th, 1915 ...	0	0	0			0	0	0								C	C	L		I	0	0		0
Andrews ...	0	0	0			C	C	C			C	C	I	L		L	0	0		C	L	0		0
Turnpenny ...		L	L	0			0	0	0		C	C	0	0			C	C	C		C	I	L	0
Adamson ...	0	0	0			0	0	0			C	C	C	L		C	C	C		C	C	L		0
Jones ...	0	0	0			0	0	0			C	C	L	0		C	C	C		C	C	0		0
McPhail ...	L	I	I	L		I	I	I	I		I	I	L	L										L
King (Watford) ...	C	C	C	C	L	0	0	0			C	C	C	I	L				0		L	0	0	0
D. III ...	0	0	0			C	C	C				C				L	0	0		C	C	L		0
D. V ...	I	I	0	0	0	0	0	0	0	0	C	C	L	0	0		C	C	C		C	L	L	0
M. 7 ...	0	0	0	0			I	L	L	0							L	L	0		I	L	0	0
M. 13 ...	0	0	0			C	C	I								I	0	0		C	C	I		0

C = Complete agglutination.

I = Incomplete agglutination.

L = Least degree of agglutination.

0 = Negative result.

question, and neither inquiry supported the view that these two groups of meningococcus are permanently different stocks causing different types of disease, or were confined to different epidemiological outbreaks.

Table II (p. 887) shows the sources of the strains and their grouping by agglutinating serums.

The strains from Woolwich and those from Major Gordon and Captain Hine no doubt came from patients of various origin, but the Canadian strains were all isolated in January and February, 1915, and the four other strains from Salisbury Plain also came from cases in the same area in February and March. Of these thirteen strains from Salisbury Plain, seven fell in the A. and M. group and four in the B. and P. group. D. III and D. V, both coming from Reading in the spring of 1915, fell in different groups.

On analysis of nine cases occurring in the First Canadian Contingent, from which strains of meningococcus were isolated and tested, five cases were infected with meningococci of the A. and M. group; of these one recovered, the remainder died within four days of the onset of the disease. Two yielded cultures of the P. and B. group; one lived five months but eventually died, the other died within two days. The two strains from France—M. 7 and M. 13.—were both B. and P. strain, and both cases were fatal.

This small series shows that severe and fatal cases of the disease may be associated with either group of the two strains of meningococcus found in this epidemic.

Three strains of meningococcus isolated from the throats of persons in contact with cases of cerebro-spinal meningitis have been so far examined with the same

TABLE II.—Classifications of Strains into Groups A. and B. by Serums A. and B. and into Groups M. and P. by Antimeningococcus and Antiparameningococcus Serums.

Strains from Cases on Salisbury Plain, First Canadian Contingent.		Civilian.	
Strains.	Groups.	Strains.	Groups.
Crisp ...	M. and A.	Plank ...	M. and A.
Palmer ...	M. and A.	Strain from Major S. Rowland (France).	
Goodyear ...	M. and A.	M. 7 ...	P. and B.
Kennedy ...	M. and A.	M. 13 ...	P. and B.
Murray ...	M. and A.	Strains from Major Gordon and Captain Hine.	
Chase ...	?	Tring ...	A.
Chandler ...	?	Turnpenny ...	M. and A.
Carter ...	P. and B.	Adamson ...	A.
Randall ...	P. and B.	Jones ...	A.
Strains from Cases, Mr. K. Goadby (Woolrich).		Islandrindod ...	P. and B.
Lewis ...	M. and A.	Andrews ...	P. and B.
4378 ...	M.	Berkhampstead ...	P. and A.
4409 ...	M.	Littledale ...	?
4410 ...	P.	McPhail ...	?
4411 ...	P. and B.	Strain from Dr. Donaldson (Reading).	
4442 ...	M. and A.	D. III ...	P. and B.
Clifford ...	P. and B.	D. V ...	M. and A.
4412 ...	M. and A.	Strain from Watford.	
From Lieut. Treadgold.		Watford ...	M. and A.
Atkin ...	M. and A.		
Somers ...	P. and B.		
167 ...	P.		
Total 35:	17 Group A. 15 Group M. 18 Group A. and M.	10 Group B. 12 Group P. 12 Group B. and P.	

serums. Two of these strains fall definitely into the B. and P. group; the third was feebly agglutinated by both M. and P. serums, but more by M. serum.

REMARKS.

In the first twenty years after the discovery of the meningococcus by Weichselbaum (1887) the tendency to accentuate the variability of this organism was carried by Jaeger (1895) to the point of claiming for it an ability to change into a Gram-positive diplococcus, which grow readily at 20° C., and was very resistant to drying. The observations of Councilman, Mallory and Wright (1898), Albrecht and Ghon (1903), and others confirmed the description of Weichselbaum, and von Lingelsheim (1906) claimed a very high degree of uniformity for the strains isolated by him during the Silesian epidemic, and stated that all these strains of meningococcus agglutinated with a univalent serum obtained by injecting a rabbit with one strain.

Many other writers have reported on the value of agglutinating serum for identifying the meningococcus. Several workers, however, especially Eberle (1908), Elser and Huntoon (1909), Traubmann and Fromme (1908), Arkwright (1909), described undoubted strains of meningococcus which were inagglutinable with antimeningococcus serum, and pointed out the irregularity with which univalent serums affected strains which had not been used in their preparation. Eberle, and Elser and Huntoon, moreover, showed that the serum made with one strain might have a far higher agglutination titre for some strains than they had for others, even if the homologous strain remained relatively inagglutinable. Most of these writers, especially Eberle, and Elser and Huntoon, attributed the irregularities observed to the varied agglutinability of different strains and to their different value as antigen (agglutinin). Houston and Rankin (1907) claimed to be able to differentiate strains of meningococcus obtained from cases of infantile posterior basic meningitis from strains derived from epidemic cases.

In 1909 and 1912 I was able to divide the sporadic and epidemic strains of meningococcus with which I was working into groups which did not inter-react, by agglutination and complement fixation tests. The groups appeared to overlap somewhat. By absorption of agglutinins the groups could be further subdivided. My results led me to the conclusion that epidemic and sporadic strains were both divisible into an uncertain number of groups which were not closely related serologically, but which were connected by intermediate strains.

Dopter (1909) described a strain which he called parameningococcus. It was isolated from the naso-pharynx, and resembled the meningococcus except in serum reaction. Antimeningococcus serum did not agglutinate the parameningococcus. Subsequently (1911) he recorded the isolation of the parameningococcus from the cerebro-spinal fluid of severe cases of sporadic meningitis, all of which were fatal. He considered that cerebro-spinal meningitis might be caused by either the meningococcus or the parameningococcus. He also began preparing therapeutic serum from horses for the two classes of cases. In 1914 Dopfer described three groups of parameningococci differentiated by absorbing agglutinin from antimeningococcus and from antiparameningococcus horse serums, and also by univalent rabbit serums. He stated that antimeningococcus serum sometimes agglutinates parameningococcus strains as well as the meningococcus, but that parameningococcus serum usually agglutinates both varieties.

The large majority of the strains examined in the present paper appear to fall into two main groups which are identical with or closely allied to the meningococcus and parameningococcus main groups described by Dopfer. This has been shown by using Pasteur Institute therapeutic M. and P. serum for agglutination tests. These serums, however, are no doubt made by the inoculation of several strains, since Dopfer (1914) stated that three parameningococcus strains were used for the horse.

Of the five strains which I have been unable to classify, two (Chase and Berkhampstead) appear to occupy an intermediate position between the two groups, and perhaps contain individual cocci which react with different serums. One (Littledale) was not agglutinated with the serums used. One (Chandler) was feebly and equally agglutinated by serum for both groups, and therefore perhaps belongs to the M. rather than to the P. group.

The strains belonging to the B. and P. parameningococcus group appeared to give sharper differentiating reaction than those of the A. and M. group. This corresponds with Dopfer's observation that parameningococcus serum usually agglutinates meningococci, but that meningococcus serum as a rule has no action on the member of the other group.

Effect of Therapeutic Serums.

The fact that meningococci are not all alike serologically, but fall into different groups, provides a reason why the therapeutic serums used during the 1914-15 epidemic in this country appeared to have so little value. It is probable that the horses from which the serum was derived had not received the same groups or subgroups as occurred in this epidemic. However, the strains isolated in 1915 appear to fall for the most part into the same main groups as Dopfer's meningococci and parameningococci which have been present in France for some years.

A. Netter (1915) had very successful results in France with the Pasteur Institute serum. His cases showed a mortality of only 22 per cent. without excluding fulminant cases and deaths from other causes.

It has been demonstrated by Elser and Huntoon and other workers that the antigenic properties of different strains of meningococcus are very different in value. It is therefore very probable that where large numbers of strains of meningococcus have not been available for immunizing the horses, those strains which offered the best antigen have not been used.

CONCLUSIONS.

1. Thirty out of thirty-two strains of meningococci tested agglutinated with a polyvalent rabbit serum prepared with six strains isolated in the same epidemic.
2. Thirty strains isolated during the present epidemic fall into two main groups according to agglutination tests. Five other strains presented difficulties in their classification.
3. These two main groups correspond to Dopfer's classification into meningococcic and parameningococcic.
4. The numbers falling into the two groups—meningococcic and parameningococcic—were 18 and 12 respectively—that is, 3 to 2.
5. There is some evidence that two of the five unclassified strains were intermediate in character between the two main groups.

6. Neither group was more frequently found among cases from one district than from another.

7. Rapidly fatal cases of meningitis occurred with infection with either type of meningococci.

I must express my thanks to those who have kindly given me cultures, and especially to Captain A. W. M. Ellis, C.A.M.C., who isolated many Canadian strains, and to Major Gordon and Captain Hine, R.A.M.C. To these and other workers I am indebted for cultures and for help. I look forward with great interest to the publication of the results obtained from their work during the present year.

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Remarks

ON

SOME OF THE EFFECTS OF EXPOSURE TO WET COLD AND THEIR PREVENTION.

(PREVENTION OF "FROST-BITE," "TRENCH-FOOT," "WATER-BITE.")

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I. FRIGORISM.

In a recent communication¹ I recorded some experiments showing that when the temperature of the skin is reduced by exposure to an external cold medium serious sensory disturbances; cramps, and anaesthesia are produced when the temperature of the skin is still several degrees above 0° C.

In experiments of this kind it is difficult to ascertain the cutaneous temperature absolutely because the recording instrument cannot be completely protected against the influence of the external medium without causing part of the skin to escape the same influence, but in comparative experiments it is possible to show that a reduction from 30° C. to below 10° C. in the indicated skin temperature is gradually and successively followed by sensations indicating irritation of the cutaneous sense organs, reflex contraction of the vessels, cramps, to which may be added after a variable time more or less complete anaesthesia and motor paralysis. These phenomena are not associated with permanent lesions when the exposure is not prolonged. They are produced in a few minutes when one of the extremities is plunged into salt water at 1° or 2° below 0° C.

They occur more slowly when the temperature of the water is a few degrees above 0° C., but when the water is unlimited in amount and circulating freely, very severe symptoms may be produced even when the temperature of the fluid is as high as 7° C. or even 10° C., more especially when the individual is ill nourished, the general or local circulation is bad, and the exposed part attenuated or protected by an insufficient amount of subcutaneous fat. The latter condition, which is the least important, is not negligible, as is shown by a well-known experiment. A

block of ice being applied to the external, and a thermometer to the internal, surface of one cheek, the thermometer may after one hour indicate a fall of 8° C. in a thin subject, while in a stout person the fall may be 3° C. only.

All these phenomena occur independently of any freezing of the parts. They undoubtedly indicate that cold has a powerful action upon the peripheral nerves and end organs, the vasomotor mechanism, the voluntary muscles, etc. These effects are, however, quite different from the more permanent lesions caused by freezing, and occurring in the blood, blood and lymph vessels, nerves, muscles, etc., which have been described by Pouchet, Tillaux and Grancher, Jamain and Terrier, Cohnheim, and others, in connexion with frost-bite.

Repeated or prolonged cooling of the skin to a temperature a few degrees only above 0° C. is, however, also liable to produce permanent effects, and in the course of my investigations I have observed in a foot which had been cooled several times evidences of permanent increase of irritability of some cutaneous nerves, probably indicating some slight localized peripheral neuritis, and a tendency to disturbances of circulation predisposing to excessive liability to coldness of the extremity.

These effects are not properly covered by the names "frost-bite," "trench-foot," "water-bite," but correspond to a state which is better defined by the term "froidure," used by some French writers, or its English equivalent "frigorism," suggested by me.

This state is produced when the cooling of the body or any part of it is progressive—that is, when it cannot be compensated by the production in the tissues, and the distribution by the circulating blood, of a sufficient amount of heat. Frigorism may be general or local.

When the cooling of a part reaches a certain point, the functions of the tissues are interfered with to an extent determined by the nature of the tissue, the temperature and the duration of the exposure. When the freezing-point is reached certain mechanical complications connected with the change of state are superadded to the effects of lowering of temperature.

When a body is surrounded by a medium at a lower temperature than itself, heat passes from the body into the medium until the temperature of both is the same.

The rapidity of this exchange is determined by many well-known factors. The loss of heat from any part of the body of an animal is governed mostly by the following conditions:

1. The temperature, the thermo-conductivity, the volume, and the movements of the external medium.
2. The duration of the exposure.
3. The bulk, movements, and superficial area of the part exposed.
4. The thermo-conductivity of the tissues and more particularly of the integuments.
5. The amount and velocity of the flow of blood through the part.
6. The state of nutrition, activity and soundness of the tissues locally and generally (the kind and amount of food available being included under nutrition).

The importance of all these factors is a matter of general experience and is capable of experimental proof. Some experiments bearing upon this aspect of the question have been related in my previous communication.

With regard to the share taken by the nature of the external media, it is sufficient to refer to some well-known facts. Exposure to dry air at 0° C. does not materially affect a healthy, active, well-clothed person. Arctic explorers have been able to bear very much lower temperatures without serious consequences on account of the dryness of the air in the polar regions. Exposure to damp air at 0° C. or several degrees higher produces a very marked sensation of cold which is distinctly uncomfortable.

Immersion in water at 0° C. is immediately followed by a sensation of intense cold, and within a few minutes loss of sensation and paralysis occur.

Complete contact of the skin with bars of metal at 0° is immediately painful, and if the temperature of the metal is a few degrees below 0°, the tissues in contact with it are rapidly frozen.

With the object of determining to what extent the low