# A COMPARISON OF MENINGOCOCCI FOUND IN THE CEREBRO-SPINAL FLUID AND NASO-PHARYNX IN TWENTY-FIVE CASES.

By R. G. CANTI, M.B. (Cantab.).

*Demonstrator of Pathology at St Bartholomew's Hospital.*
*Pathologist to the Alexandra Hospital, London, E.C.*

This paper embodies the results of the routine bacteriological examination of patients suffering from cerebro-spinal fever at St Bartholomew's Hospital during the year 1916. The work was carried out in conjunction with Professor Andrewes who has already published abstracts of the first eleven cases (Andrewes, 1916).

A notable feature of the series of cases is the proportion which occurred in children under the age of two years.

Twenty-nine cases of the disease were treated in the hospital during the year. Two cases, bacteriologically proved, unfortunately died before nasopharyngeal cultures could be taken, and in two more cases meningococci could not be detected either in film or culture of the cerebro-spinal fluid, but the general characteristics of the fluid, the clinical condition of the patient, and the finding of copious growths of agglutinable meningococci in the nasopharynx, rendered the diagnosis all but certain. These four cases reduce the number under discussion to twenty-five.

## History.

v. Lingelsheim (1906), working during the epidemic in Upper Silesia in 1904–5, found that in the early stages of cerebro-spinal fever positive cultures could be obtained from the nasopharynx in 93·8 per cent. of the examinations performed, provided that the material to be cultivated was dealt with immediately.

Since then other workers have met with varying success but so many have obtained a high percentage of positive results that there seems little doubt that the organism is present in the nasopharynx in the early stages of the disease in all cases.

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The present outbreak which began in 1914 has again drawn attention to the organisms in the nasopharynx, one of the objects being to determine their relations with the meningococci in the cerebro-spinal fluid.

Early in 1916 Professor Andrewes (*loc. cit.*) published notes on eleven consecutive cases of cerebro-spinal fever showing that in each case the nasopharyngeal organisms were of the same agglutinable type as those in the cerebro-spinal fluid. These eleven cases are included with further details in the present series.

W. M. Scott (1916) isolated organisms of a similar agglutinable character in all but one of a series of seven cases. He further found that whereas the organisms first isolated from the nasopharynx in two of his cases were similar to the meningeal strains, those isolated at a later date did not agglutinate in the same way. He suggested that this may have been due to one of two causes, viz., either "modification may go on in the nasopharynx so that one type changes into the other" or "the later swabs were furnishing cultures of another, perhaps a normal, inhabitant of the nasopharynx which had been swamped by the infecting strain at the time of the first examination."

M. H. Gordon (1917) with the material forwarded to him from eight cases found that organisms of the same type were isolated from nasopharynx and meninges on every occasion.

M. Flack (1917) showed that similar meningococci were obtained from thirty cases in a series of thirty-two. Of the remaining two, one was found to yield organisms of different types from either site, and in the other no decision as to type was arrived at in the case of either strain.

### Cultural Technique.

The medium used for primary cultures both of cerebro-spinal fluid and nasopharyngeal swabs was legumin agar prepared according to Gordon's formula with the addition of a small quantity of sterile ascites fluid and fresh human blood. In sub-cultivations the blood was omitted. For storing purposes egg medium in tubes sealed with paraffin wax as recommended by Dr A. Eastwood was used. In one case the organism was found to be alive seven months after the culture had been made, this organism having been previously repeatedly sub-cultivated.

Nasopharyngeal cultures were made by means of West's post-nasal swab, the upturned end of the tube being greatly shortened for use on young children.

As a rule only one plate was used for nasopharyngeal cultivation, the swab being rubbed over a small area of the medium near its edge and the material so left behind distributed by means of a glass spreader, so that the intensity of the growth was graduated from one side of the plate to the other.

### CULTURAL APPEARANCE.

Meningococci were obtained from the nasopharynx of every patient, and in twenty-two out of the twenty-five cases at the first attempt. In children the cultures tended to be less pure than in adults and this was attributed to the frequent presence of vomit in the nasopharynx and to the mechanical difficulty of taking the swab without contamination by saliva, the organisms of which have been shown by Gordon (1917) to inhibit the growth of meningococci. The approximate percentages of meningococcus-like organisms present in the nasopharyngeal cultures is shown in the table.

No difficulty was experienced in obtaining a growth from the cerebro-spinal fluid.

Except in one case the colonies both in primary culture and sub-culture exhibited the same appearances as are usually described and these need no comment. In one case, however, No. 19, the colonies from the cerebro-spinal fluid on both of the only two occasions on which it was cultivated, appeared after twenty-four hours' incubation, as minute translucent points easily overlooked at a casual glance. Subsequently a few of the colonies increased in size till they appeared like average normal colonies, but the bulk, though slightly increasing in size, remained minute. Sub-cultivations from small colonies through a large number of generations behaved in exactly the same way as the original cultures, while sub-cultivations from the large colonies yielded large colonies only.

A fuller description of this organism will be published at an early date.

### AGGLUTINATION.

Pure twenty-four hour cultures of the organisms grown on legumin agar with the addition of ascites fluid but without blood were emulsified in a small quantity of physiological saline solution. These emulsions were heated to 65° C. in a water bath for half-an-hour and then diluted to a concentration of about 2000 millions per c.c., one-tenth of the volume of a 5 per cent. solution of phenol in distilled water being afterwards added.

The macroscopic method of agglutination was adopted. The dilutions of 1 in 50, 1 in 100, 1 in 200 and 1 in 400, were made in quarter inch Durham's tubes, the concentration of organisms in each tube being 1000 million per c.c. The results were recorded after heating for twenty-four hours in an oven at 55° C.

With the exception of Type II S.B.H. serum, which was prepared at St Bartholomew's Hospital partly from the cerebro-spinal and partly from the nasopharyngeal coccus of the same patient (No. 2), all the sera employed, which were kindly supplied by Lt-Col. Gordon and Dr F. Griffith, were monovalent preparations from spinal strains. Gordon's sera were supplied having a titre of 1 in 400 with the homologous coccus, whilst Griffith's sera and Type II S.B.H. serum were of somewhat higher potency.

### Time after onset of illness at which the meningococcus was first isolated from the pharynx.

Patients were swabbed as soon as possible after diagnosis. Meningococci were found in the first week of the disease in sixteen cases, in the second week in four cases, in the third week in three cases, and in the fourth week in two cases. These figures include the three cases in which meningococci were not obtained at the first attempt; they were eventually obtained from two of them in the third week and from one in the fourth week.

### Carbohydrate Reactions.

The carbohydrate reactions were carried out in 1 per cent. glucose and in 1 per cent. saccharose ascites litmus broth on both the meningeal and nasopharyngeal cocci in sixteen cases. Fermentation was always obtained in glucose and never in saccharose. The time taken for the red colour of the glucose tubes to reach its greatest intensity was from three to six days. It was observed that in ten cases the nasopharyngeal organism took the same time to ferment glucose as the meningeal, and in all but one of the remaining cases the nasopharyngeal organism formed acid more rapidly.

In one case (No. 7) the glucose tubes both of the meningeal and nasopharyngeal coccus were first turned red and then bleached yellow by the fifth day, the red colour returning in each case on the seventh or eighth day. This investigation was performed on two occasions and controlled by other tubes of the same batch.

### TYPES OF MENINGOCOCCI OBTAINED FROM THE CEREBRO-SPINAL FLUID.

Reference to the table shows that all organisms obtained from the cerebro-spinal fluid were agglutinated with at least one of the sera employed, and they are thereby divided into types as represented by Type I, Gordon, and Group I, Griffith on the one hand, and Type II, Gordon, Group II, Griffith, and Type II, S.B.H. on the other. Most of the organisms which were agglutinated by sera of the first type, were also agglutinated by Type III serum, Gordon, but usually to a less degree. In the absence of further proof they have been considered as belonging to Type I.

The proportion of the types found is as follows:

| | | |
|---|---|---|
| Type I | 7 cases | 28 per cent. |
| Type II | 18 cases | 72 per cent. |

### CHILDREN.

The number of children examined under two years of age was ten.

No appreciable differences in the proportion of types among them and among older patients were shown. The total number of cases examined however is too small to permit of an accurate conclusion being drawn on this point. Type I was yielded by three children (30 per cent.) and seven older patients (27 per cent.) and Type II by four children (70 per cent.) and eleven older patients (73 per cent.)

### COMPARISON OF AGGLUTINABILITY OF THE MENINGOCOCCUS ISOLATED FROM THE CEREBRO-SPINAL FLUID AND NASOPHARYNX OF THE CASE.

Referring again to the table a striking similarity of agglutinability of the cerebro-spinal and nasopharyngeal cocci is seen. Firstly, the two are without exception shown to be of the same agglutinable type. Secondly, in a large number of instances a serum is found to agglutinate the nasopharyngeal coccus to the same titre as the cerebro-spinal coccus although that titre may not be the same as with the homologous coccus. This point is particularly well brought out by certain of the poorly agglutinable organisms which are entirely untouched by one or more of the sera of a particular type and yet are agglutinated by others. Examples of this are furnished by Nos. 8, 11 and 22.

In the exceptional instances there is no indication as to whether the nasopharyngeal cocci are on the whole more or less agglutinable than the cerebro-spinal.

| Case No. | Age Sex | Source of organism | Day of isolation of organism | Rough °/₀ of meningococcus like organisms in cult. of Ph. | SERA: Littledale or Mitchell Type I Gordon 1:200 | Littledale or Mitchell Type I Gordon Highest titre | M 17 Group I Griffith 1:200 | M 17 Group I Griffith Highest titre | Chase Type III Gordon 1:200 | Chase Type III Gordon Highest titre | Gliddon Type II Gordon 1:200 | Gliddon Type II Gordon Highest titre | Waterman Type II Gordon 1:200 | Waterman Type II Gordon Highest titre | M 24 Group II Griffith 1:200 | M 24 Group II Griffith Highest titre | Thomson Type II S.B.H. 1:200 | Thomson Type II S.B.H. Highest titre |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 44♀ | Sp. | 4 | . | + | >400 | . | . | − | 50 | − | − | . | . | . | . | . | . |
| | | Ph. | 8 | 99 | + | >400 | . | . | + | >400 | − | − | . | . | . | . | . | . |
| 2 | 21♀ | Sp. | 2 | . | − | − | . | . | − | − | − | − | . | . | . | . | + | >400 |
| | | Ph. | 7 | 5 | − | − | . | . | − | − | − | − | . | . | + | >200 | . | . |
| 3 | 11♂ | Sp. | 4 | . | + | >400 | . | . | + | >400 | − | − | . | . | . | . | . | . |
| | | Ph. | 9 | 10 | + | >400 | . | . | − | − | − | − | . | . | . | . | . | . |
| 4† | 33♀ | Sp. | 2 | . | − | − | . | . | − | − | − | − | . | . | . | . | . | . |
| | | Ph. | 3 | 90 | − | − | . | . | − | − | − | − | . | . | . | . | . | . |
| 5 | 3♀ | Sp. | 2 | . | − | − | . | . | − | − | . | . | . | . | . | . | + | 50 |
| | | Ph. | 7 | 5 | − | − | . | . | − | − | . | . | . | . | + | >400 | + | 50 |
| 6 | 24♂ | Sp. | 1 | . | − | − | . | . | − | . | . | . | . | . | . | . | + | 200 |
| | | Ph. | 2 | 1 | . | . | . | . | . | − | . | . | . | . | . | . | + | 200 |
| 7 | 5/12♂ | Sp. | 2 | . | − | − | . | . | − | − | . | . | . | . | . | . | + | 100 |
| | | Ph. | 2 | 5 | − | − | . | . | − | − | . | . | . | . | . | . | + | 100 |
| 8 | 7♂ | Sp. | 2 | . | − | − | . | . | . | . | − | 100 | − | − | + | >400 | − | − |
| | | Ph. | 2 | 60 | − | − | . | . | . | . | − | 50 | − | − | + | >400 | − | − |
| 9 | 17♀ | Sp. | 2 | . | − | − | . | . | − | − | + | 200 | + | 200 | + | >200 | + | 200 |
| | | Ph. | 3 | 50 | − | − | . | . | − | − | + | 200 | + | 200 | . | . | + | 200 |
| 10 | 7/12♀ | Sp. | 10 | . | − | − | . | . | − | − | + | >400 | + | >400 | . | . | + | >400 |
| | | Ph. | 20 | 5 | − | − | . | . | − | − | + | >400 | . | . | . | . | . | . |
| 11 | 5/12♂ | Sp. | 17 | . | − | − | . | . | − | − | − | − | − | − | + | >400 | − | − |
| | | Ph. | 17 | 30 | − | − | . | . | − | − | − | − | − | − | + | >400 | − | − |
| 12 | 1½♂ | Sp. | 4 | . | − | − | . | . | − | − | + | >400 | . | . | . | . | . | . |
| | | Ph. | 4 | 75 | − | − | . | . | − | − | + | 200 | . | . | . | . | . | . |
| 13 | 1½♀ | Sp. | 3 | . | + | >400 | . | . | + | 200 | − | − | . | . | . | . | . | . |
| | | Ph. | 3 | 50 | + | >400 | . | . | − | 100 | − | − | . | . | . | . | . | . |

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| 14 | 1¼♀ | Sp. | 22 | . | + | >400 | . | . | + | 200 | − | − | . | . | . | . | . | . | |
| | | Ph. | 22 | 20 | + | >400 | . | . | + | >400 | − | − | . | . | . | . | . | . | |
| 15 | 10♀ | Sp. | 6 | . | − | − | . | . | − | − | + | 200 | − | 50 | + | >400 | + | 200 | |
| | | Ph. | 7 | 60 | − | − | . | . | − | − | − | − | − | 50 | + | >400 | − | − | |
| 16 | 18♂ | Sp. | 6 | . | − | − | . | . | − | − | − | 100 | . | . | + | >400 | . | . | |
| | | Ph. | 7 | <1 | − | 100 | . | . | − | − | + | 200 | . | . | . | . | . | . | |
| 17 | 2♀ | Sp. | 4 | . | − | − | . | . | − | − | + | 200 | . | . | . | . | . | . | |
| | | Ph. | 4 | 25 | − | − | . | . | − | − | + | 200 | . | . | . | . | . | . | |
| 18 | 2¾♀ | Sp. | 4 | . | − | − | . | . | − | − | − | 100 | . | . | − | 100 | . | . | |
| | | Ph. | 6 | 50 | − | − | . | . | − | 100 | − | 50 | . | . | − | 100 | . | . | |
| 19 | ⅝♀ | Sp. | 23 | . | − | − | . | . | − | − | . | . | . | . | + | 200 | . | . | |
| | | Ph. | 23 | 50 | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| 20 | ⅝♀ | Sp. | 7 | . | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| | | Ph. | 7 | 10 | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| 21 | 3♂ | Sp. | 19 | . | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| | | Ph. | 19 | 10 | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| 22 | 36♂ | Sp. | 4 | . | − | − | + | >400 | − | 100 | . | . | . | . | − | 50 | . | . | |
| | | Ph. | 18 | 2 | − | − | + | >400 | − | 100 | . | . | . | . | − | 50 | . | . | |
| 23 | 4♀ | Sp. | 4 | . | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| | | Ph. | 4 | 20 | − | − | . | . | − | − | . | . | . | . | + | >400 | . | . | |
| 24 | 1♂ | Sp. | 10 | . | + | >400 | + | >400 | − | 100 | . | . | . | . | − | 50 | . | . | |
| | | Ph. | 21 | 20 | + | >400 | + | >400 | − | − | . | . | . | . | − | − | . | . | |
| 25 | 1¾♀ | Sp. | 11 | . | . | . | − | − | . | . | . | . | − | − | + | >400 | . | . | |
| | | Ph. | 12 | 10 | . | . | − | − | . | . | . | . | − | 50 | + | >400 | . | . | |

The cases are arranged in chronological order.

\+ indicates the presence of agglutination as evidenced by marked flocculi when viewed with the hand lens.

> indicates that agglutination was present at the titre indicated but that this does not necessarily represent the highest titre.

Sp. indicates that the organism was isolated from the cerebro-spinal fluid.

Ph. indicates that the organism was isolated from the nasopharynx.

† These organisms have been shown by Lt-Col. Gordon to belong to Type II.

SUMMARY.

In twenty-five consecutive cases in which nasopharyngeal cultures have been made from patients suffering from bacteriologically proved cerebro-spinal meningitis, meningococci have been obtained.

A striking similarity between the organisms from the two sites has been shown to exist for not only are they of the same agglutinable type but in the majority of instances individual peculiarities of agglutination have corresponded. The closeness of relation has been further demonstrated by the carbohydrate reaction in one case in which the unusual property of bleaching glucose litmus broth to a yellow colour was possessed by both organisms. Nevertheless, in a certain number of instances, though organisms of a different agglutinable type have not been isolated, as by other workers, there have been found certain minor variations of agglutination. That most if not all of these variations are due to alteration of the agglutinable capacity of one of the organisms seems probable, as in the six cases where most marked difference existed (Nos. 1, 3 and 24 of Type I and Nos. 15, 16 and 18 of Type II) the chances of the other alternative, namely that a second infection had happened in each case to be of the same agglutinable type, are remote.

In the twenty-five cases the proportion of the types present has been shown to agree with that found by other workers during the same period. Seven of the infections (28 per cent.) have been due to Type I and eighteen (72 per cent.) to Type II.

Ten of the cases have occurred in children under the age of two years, and the proportion of the two types among them has been approximately the same as among older patients.

The writer is indebted to the members of the staff of St Bartholomew's Hospital for permission to publish their cases, and gratefully acknowledges the encouragement and assistance of Professor Andrewes at whose instigation these investigations were conducted.

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