IDENTIFICATION OF THE MENINGOCOCCUS IN THE NASO-PHARYNX WITH SPECIAL REFERENCE TO SEROLOGICAL REACTIONS.[1]

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In view of the apparent identity, so far as morphological and cultural characters are concerned, of meningococci from cases of cerebro-spinal meningitis with certain gram-negative cocci found in the naso-pharynx of non-contacts, it has been necessary to resort to serological tests to obtain further information on the question of their inter-relationship.

Monovalent agglutinating sera have been prepared from several varieties of each class of cocci and cross agglutination experiments have been made. The tests have been applied to cerebro-spinal strains and to naso-pharyngeal strains isolated during the recent epidemic. The strains used are those mentioned in Dr Eastwood's Report (pp. 405–445), which have been jointly investigated by us as regards cultural characters and fermentative capacities. I have submitted to agglutination tests the 34 cerebro-spinal strains and 30 of the naso-pharyngeal strains from patients at St Bartholomew's Hospital not known to have been in contact with cases of cerebro-spinal meningitis.

Before proceeding to the work on agglutination, I record additional observations on the cultural and fermentative capacities of the strains investigated, with special reference to differential features on various media. For assistance in the arrangement of the subject matter of this report, I am greatly indebted to Dr Eastwood.

Further Observations on Cultural Characters and Fermentation Tests.

In examining the primary cultures on placental serum agar plates, slight differences were noted between the meningococcus colonies from the cerebro-spinal fluid and the meningococcus-like organisms

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from the naso-pharynx. These differences, consisting mainly of slight variations in opacity, were eliminated when colonies of either class were plated in pure culture on fresh medium. Changes in shape and structure of single colonies were common to both classes of cocci as they increased in age. In fact, all slight variations in colour, consistency, ease or difficulty of growth, fermentative activity, or viability of the cerebro-spinal strains were also met with among the naso-pharyngeal strains, but none was found to be a feature distinguishing the one class from the other.

The fermentation tests of various sugars are next in order of importance to the appearances of the primary colonies as a means of identifying the meningococcus. Maltose and glucose only showed evidence of acid fermentation. Meningococci, recently isolated from cerebro-spinal fluid, rarely produced equal amounts of acidity from glucose and maltose contained in Lingelsheim's solid sugar media. They could, with a few exceptions, be placed in one or other of two groups, according as they fermented glucose or maltose more strongly, and, as will be seen in Table I, the two groups were correlated with the serological grouping. In the case of the naso-pharyngeal strains, on the other hand, both sugars were more often equally fermented (Table II). In fact, this feature was so noticeable that, in the early part of the work, it was considered as a possible means of distinguishing the so-called pseudo-meningococcus from the true meningococcus. But further observations showed that some strains of meningococci, recently obtained from cerebro-spinal fluid, fermented maltose and glucose equally. The acidity with either of the two sugars was sometimes extremely slight in degree and evanescent. The above observations, recorded in Tables I and II, refer to tests on strains recently isolated. On repeating the tests after prolonged sub-cultivation, the differences at first noted were in many cases found to have disappeared, and in one case even to be reversed.

On egg meningococci grow readily, producing a smooth, shiny layer slightly pink at the bottom of the tube, the same tint appearing in the growth when heaped up. Cultures remain alive in sealed tubes of this medium for many weeks and have been sub-cultivated after seven months in the incubator at 22° C.

Glucose agar was found to be a useful medium for producing considerable quantities of culture; it has the advantage of giving a firm surface which facilitates the removal of the growth with a platinum spatula. It was difficult to induce some strains to grow on

this medium, when first transferred from one containing serum; in this respect some of the naso-pharyngeal strains were found to be the most obstinate. With such strains the early growths produced on the glucose agar were very sticky. In preparing this medium, in order to avoid unfavourable changes in reaction, it is advisable to add the sugar, sterilised in distilled water, after the final sterilisation of the nutrient agar.

Technique of Agglutination Tests.

The sera were prepared by inoculating intravenously adult rabbits with increasing doses of living culture grown on glucose agar. The dose ranged from $\frac{1}{8}$ tube of culture to $1\frac{1}{2}$ tubes, which was found to be as much as could be given without killing the animal. The inoculations were usually made at intervals of 8–10 days, but no regular routine was followed in this respect. The progress of immunisation was followed by making frequent tests of small quantities of blood serum withdrawn from an ear-vein. Inoculations were repeated until a workable serum was produced, the time necessary to this end varying, with different rabbits, from 4 weeks to 14 weeks.

The culture suspensions were standardised in the following way. The moist growth on several glucose agar tubes of 24 hours' incubation was removed and weighed on a chemical balance. After emulsifying in ·5 % carbolic acid in normal salt solution, and heating to 65° C. for an hour, the suspensions were diluted to a uniform strength of 4 mg. per cubic centimetre, and were stored in rubber-capped tubes in a cool dark place.

The agglutination tests were made in tubes measuring 3 in. by $\frac{1}{2}$ in. To each ·5 c.c. of serum dilution, ·5 c.c. of culture suspension was added and the tubes were placed in the incubator at a temperature of 55° C. for 24 hours, when the first readings were taken. They were then removed to the ice-chest and the final results were noted the following morning.

In order to present the results in a convenient manner, since it was impossible to give all the tables in full, it was necessary to select an end point. I have chosen as my end point the highest dilution in which the cocci had clumped and sedimented, leaving the supernatant fluid clear, or with the faintest suspicion of turbidity. This is represented in the tables by a figure giving the numerical value of the dilution. When the turbidity, though slight, was distinct and agglutination was

well marked, the result is represented by the symbols ± or ∓, according to the degree of turbidity, ± meaning agglutination well marked but incomplete in 1 in 50, ∓ meaning agglutination slight but definite. The negative sign indicates that the reaction was completely negative in 1 in 50, the highest concentration of serum considered, or showed no more than a trace of agglutination.

In order to compare agglutination with fermentative activity of individual strains, I have inserted in each of the tables a column showing the relative activity of each strain upon maltose and glucose.

In making orientation tests with a view to determining from which strains to prepare serum, I first selected two strains of cerebro-spinal origin, M. 8 and M. 23, the former because it fermented maltose more than glucose and the latter because its action on these two sugars was the reverse. On testing several strains of meningococci with these two sera I found obvious differences in agglutinability which appeared to coincide with differences in fermentative capacity. The subsequent selection of strains for the preparation of sera was determined by the differences in agglutinability already observed. At this stage it was found that the majority of the strains tested were agglutinated by one or other of the five sera, M. 8, M. 23, M. 9, M. 24, and M. 28. With a few of the strains, however, none of the sera gave good agglutination, and two of these last strains, M. 17 and M. 32A, were selected for the production of immune sera. On completing my results I re-arranged my strains in the order which exhibited most clearly their differences in agglutinability.

ANALYSIS OF TABLE I.

Table I gives the results of agglutination tests performed upon 34 strains of meningococci, with sera prepared from 7 of the same, and from 3 meningococcus-like organisms of naso-pharyngeal origin. The test with the first serum, M. 8, shows that this divides the meningococci into agglutinable and inagglutinable strains. That the inagglutinability is only relative to the particular serum employed will be seen on examination of the next column, where the series is tested with serum prepared from one of the strains, M. 23, not agglutinated by M. 8 serum. So far the results appear to justify the division of the meningococci into two main groups—Group I from M. 1 to M. 17, and Group II from M. 18 to M. 31. The remaining four strains, two of which, M. 32 and M. 32A, came from the same patient after an interval of 15 days, can be included in neither of the two groups, since in three, agglutination

TABLE I.

Cross-agglutination tests with 34 *strains of meningococci and monovalent sera prepared with* 7 *cerebro-spinal fluid strains and* 3 *naso-pharyngeal strains of Gram-negative cocci:*

Strain	Fermentation of glucose and maltose	M. 8 Serum	M. 23 Serum	M. 9 Serum	M. 24 Serum	M. 28 Serum	M. 17 Serum	M. 32A Serum	N.P. 11 Serum	N.P. 10 Serum	N.P. 26 Serum	Normal Rabbit Serum
M 1	M.>G.	1000	–	1000	–	–	100	±	400	±	±	–
M. 2	M.>G.	1000	±	1000	∓	∓	200	–	500	50	±	–
M. 3	M.>G.	800	50	1000	±	–	200	–	500	±	±	–
M. 4	M.>G.	800	50	800	∓	±	200	–	400	50	±	–
M. 5	M.>G.	800	±	800	–	∓	100	–	400	±	–	–
M. 6	M.>G.	600	±	800	–	–	100	∓	400	±	∓	–
M. 7	M.>G.	500	50	500	50	–	100	–	300	–	∓	–
M. 8	M.>G.	400	±	400	50	±	100	±	200	±	±	–
M. 9	M.>G.	400	∓	400	±	±	100	–	400	–	±	–
M. 10	M.>G.	200	–	300	–	–	200	–	400	–	–	–
M. 11	M.>G.	200	±	400	100	±	100	±	600	±	–	–
M. 12	Equal	100	–	400	±	–	800	–	400	–	–	–
M. 13	M.>G.	100	50	400	±	∓	400	∓	400	–	±	–
M. 14	M.>G.	100	∓	400	50	–	100	–	400	±	–	–
M. 15	M.>G.	100	–	400	50	–	100	∓	400	–	±	–
M. 16	M.>G.	100	–	100	50	100	–	50	50	±	∓	–
M. 17	Equal	50	∓	100	∓	∓	400	∓	400	–	–	–
M. 18	Equal	–	1000	–	800	500	–	50	100	400	50	–
M. 19	G.>M.	∓	1000	±	800	400	200	400	∓	400	±	–
M. 20	G.>M.	∓	800	–•	800	300	–	100	50	800	100	–
M. 21	Equal	100	800	∓	1000	500	–	300	∓	500	100	–
M. 22	G.>M.	∓	600	–	400	500	–	400	300	200	200	–
M. 23	G.>M.	–	500	–	100	400	–	400	±	200	∓	–
M. 24	G.>M.	±	500	–	300	500	–	200	±	200	±	–
M. 25	G.>M.	±	400	±	800	200	–	±	±	200	±	–
M. 26	G.>M.	–	400	–	800	300	–	400	100	100	–	–
M. 27	G.>M.	–	400	–	800	500	100	50	±	400	–	–
M. 28	G.>M.	–	400	–	100	500	–	–	±	200	∓	–
M. 29	G.>M.	–	400	∓	800	500	–	50	±	200	50	–
M. 30	G.>M.	–	400	–	800	300	–	100	100	300	±	–
M. 31	G.>M.	–	200	–	100	200	100	50	100	50	±	–
M. 32	Equal	–	±	–	50	∓	±	800	±	–	±	–
M. 32A	Equal	–	±	–	50	∓	–	800	±	±	50	–
M. 33	G.>M.	±	∓	–	100	50	–	50	100	±	100	–
*M. 34	G.>M.	+	+	+	+	+	+	+	+	+	+	+

The symbols ±, ∓, –, refer to reactions at 1 : 50.
± = Well marked but incomplete agglutination.
∓ = Slight but definite agglutination.
– = No agglutination.

* This strain possessed the property of auto-agglutination.

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is insufficiently marked, and in the fourth, M. 34, is not specific, since agglutination took place in normal salt solution, and with normal rabbit serum.

On examination of the individual strains in Group I, one finds marked differences in the degree to which they are agglutinated by M. 8 serum. Several show no more agglutination than M. 21, which has been included in Group II. In Group II likewise the various strains are unequally agglutinated by M. 23 serum. With M. 17, the least agglutinable of Group I, a serum was prepared and tested on the whole series. It was found to agglutinate, with a single exception, all the individuals in Group I, and, in addition, three in Group II. This shows that, in respect of its agglutinogenic capacity, M. 17 belongs to Group I.

In addition to the above three sera, I have prepared also sera from M. 9, a member of the first group, and from M. 24 and M. 28, members of the second group. In column five, M. 9 serum is markedly specific in its action upon strains in Group I. M. 24 and M. 28 sera agglutinate mainly strains in Group II[1]. These three sera, therefore, corroborate the above division of the strains into Groups I and II.

The two strains of M. 32 and the strain M. 33 may now be considered. From the agglutination tests with the sera prepared with M. 8, M. 23, M. 9 and M. 17, there is no evidence that they are related to one group more than the other, and, indeed, no serological evidence that they are meningococci. But the serum M. 24 was found to agglutinate them slightly. A serum was then prepared from M. 32, which shows that this strain has agglutinogenic capacities establishing its relationship to meningococci of Group II. In preparing a serum from M. 33 (not included in the table), more than usual difficulty was experienced, and up to the present no higher titre than 1 in 100 for the homologous strain has been reached. With this serum several strains in Group II only were agglutinated, in dilutions of 1 in 50 and 1 in 100.

The auto-agglutinating strain, M. 34, was heated to 80° C. for an hour. This treatment apparently destroyed the auto-agglutinating property and a permanent suspension in salt solution could be made, which showed no trace of agglutination in normal rabbit serum in a dilution of 1–10. This suspension of M. 34, tested subsequently with immune sera, gave an agglutination titre of 1 in 1000 with M. 23 serum, 1 in 800

[1] M. 28 was a more active serum than would appear, but owing to an accident to a centrifuge tube only sufficient serum was preserved to make the test on the whole series in dilutions from 1 in 50 to 1 in 500.

with M. 32A serum, and only a slight agglutination in 1 in 200 with M. 8, results which indicate that the strain probably belongs to Group II.

The agglutination tests with the three sera N.P. 11, N.P. 10, N.P. 26, can best be considered in conjunction with the tests given in Table II on the naso-pharyngeal strains.

The salient facts brought out in Table I are (1) that a series of meningococci tested upon a monovalent meningococcus agglutinating serum can be arranged to show a progressive diminution in agglutinability, ending in a number of completely inagglutinable strains; (2) a second monovalent serum will give a similar result, but in inverse order, the inagglutinable becoming the agglutinable.

But when a larger number of sera is employed, this parallelism between (1) and (2) is found not to be absolute, since certain strains which were inagglutinable in (1), but agglutinable in (2), do not always correspond in regard to agglutinability or inagglutinability, respectively, when tested with other sera prepared with members of Groups I and II.

Analysis of Table II.

The 30 naso-pharyngeal strains have been tested with 9 of the sera, the agglutinating properties of which have been studied in relation to meningococci of cerebro-spinal origin.

In consequence of the difficulty in obtaining good growths of many of the naso-pharyngeal strains upon glucose agar, a sufficient quantity of culture suspension was not made at one time for the whole series of tests, as was the case with the majority of the cerebro-spinal fluid strains. The different suspensions of the same strain have not been equally agglutinable, and in repeated tests the agglutination results, though in general conformity, have not been always identical.

Applying the same method of analysis as in the consideration of Table I, I find that the two sera, M. 8 and M. 23, each divide the series into an agglutinable and an inagglutinable class. Whereas in the case of the cerebro-spinal meningococci, the strains relatively inagglutinable to both sera were few in number, in this series they comprise the majority. As with the cerebro-spinal meningococci, the agglutinable naso-pharyngeal strains are divided into two groups by the sera M. 8 and M. 23. M. 9 serum also agglutinates only the same four strains as M. 8 serum, thus confirming the almost complete identity of agglutinogenic function already exhibited by the strains M. 8 and M. 9 in relation to the cerebro-spinal fluid strains. Again, M. 24 serum agglu-

tinates the same strains as M. 23 serum, but the agglutinogenic capacity of M. 24 has a wider range, since the serum agglutinates in addition a number of other strains. Still greater agglutinating capacity towards the naso-pharyngeal strains is shown by M. 32A serum. M. 32A is a meningococcus strain, which was agglutinated, to any considerable

TABLE II.

Cross-agglutination tests with 30 *strains of naso-pharyngeal Gram-negative cocci and monovalent sera prepared with* 6 *cerebro-spinal fluid strains and* 3 *naso-pharyngeal strains:*

Strain	Fermentation of glucose and maltose	M. 8 Serum	M. 23 Serum	M. 9 Serum	M. 24 Serum	M. 17 Serum	M. 32A Serum	N.P. 11 Serum	N.P. 10 Serum	N.P. 26 Serum	Normal Rabbit Serum
N.P. 1	M.>G.	800	–	1000	∓	100	±	400	–	±	–
N.P. 2	Equal	200	±	300	200	100	50	400	100	200	–
N.P. 2A	M.>G.	100	–	50	50	50	±	300	±	50	–
N.P. 3	Equal	50	∓	100	±	∓	50	200	±	100	–
N.P. 4	M.>G.	50	–	50	300	±	400	100	–	100	–
N.P. 5	Equal	±	±	±	50	100	200	400	–	100	–
N.P. 6	G.>M.	–	800	∓	300	–	50	100	200	100	–
N.P. 7	G.>M.	–	400	–	100	–	100	50	100	100	–
N.P. 8	G.>M.	–	400	±	100	–	100	300	200	±	–
N.P. 9	Equal	±	400	±	300	±	400	300	200	200	–
N.P. 10	G.>M.	–	500	–	800	–	±	50	500	50	–
N.P. 11	M.>G.	∓	400	∓	300	50	50	300	100	±	–
N.P. 12	Equal	±	50	∓	100	–	100	100	∓	100	–
N.P. 13	M.>G.	∓	50	∓	50	∓	200	400	–	±	–
N.P. 14	G.>M.	50	∓	±	200	±	100	400	100	100	–
N.P. 15	Equal	±	∓	±	100	±	100	100	–	±	–
N.P. 16	Equal	±	±	±	200	50	200	800	200	400	–
N.P. 17	M.>G.	±	–	...	100	–	200	400	100	400	–
N.P. 17A	Equal	±	–	±	±	–	200	400	±	300	–
N.P. 18	Equal	±	–	±	50	–	200	400	–	300	–
N.P. 19	Equal	±	±	±	50	±	50	300	±	100	–
N.P. 20	Equal	–	–	–	±	–	50	400	–	50	–
N.P. 21	Equal	±	–	±	±	∓	±	400	±	100	–
N.P. 22	M.>G.	∓	∓	±	±	–	100	400	±	100	–
N.P. 23	Equal	±	–	∓	±	–	50	400	±	100	–
N.P. 24	Equal	±	–	±	±	–	±	400	–	±	–
N.P. 25	M.>G.	±	–	±	±	±	±	400	–	100	–
N.P. 26	Equal	∓	∓	±	±	–	200	200	±	400	–
N.P. 27	M.>G.	–	–	±	∓	–	±	400	–	100	–
N.P. 28	Equal	–	–	∓	100	–	–	±	–	–	–
N.P. 29	Equal	–	–	...	±	–	±	50	...	±	–
N.P. 30	...	–	–	–	–	–	–	–	–	–	–

The symbols ±, ∓, –, have the same significance as in Table I.
N.P. = Non-contact naso-pharyngeal strain.

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extent, by the homologous serum alone, though the serum prepared from it agglutinated several cerebro-spinal strains of Group II. Thus agglutinogenic capacity demonstrates its relationship alike to the cerebro-spinal meningococci and to the naso-pharyngeal cocci.

N.P. 11 produces a serum which has marked agglutinating properties for almost all the naso-pharyngeal strains, as well as for cerebro-spinal strains in both of the groups. With this strain, however, agglutinability and agglutinogenic capacity are not in correspondence. N.P. 11 is agglutinated by the sera of M. 23 and M. 24, members of Group II, but the serum produced by N.P. 11 agglutinates mainly members of Group I, giving only incomplete reactions in 1 : 50 with M. 23 and M. 24.

N.P. 10 which was agglutinated by M. 23 serum, produces a serum which agglutinates with a few additions the same naso-pharyngeal strains as M. 23 serum, and reference to Table I shows that N.P. 10 serum acts mainly upon the group of meningococci to which M. 23 belongs.

From the above considerations it is clear that there is a definite relationship between the cerebro-spinal meningococci and many of the naso-pharyngeal cocci. Some, however, of the latter strains were agglutinated only to a very slight extent by the six immune sera produced by meningococci of cerebro-spinal origin. The question arises whether it is possible by serological tests to justify a classification of these cocci as meningococci. It has already been pointed out, in analysing Table I, that two strains from cases of meningitis, M. 32 and M. 33, agglutinated only to a minor degree with five meningococcus immune sera, but their identity with meningococci has been confirmed by demonstration of their specific agglutinogenic capacity. Experiments on similar lines have been begun with the naso-pharyngeal strains which are apparently inagglutinable when tested by some of the above meningococcus immune sera. Some progress in this direction has already been attained. With one of these strains, N.P. 26, an agglutinating serum was prepared. This serum agglutinated to some extent the majority of the naso-pharyngeal strains, and (Table I) a number of the meningococci in Group II.

The two strains which differed in certain cultural features from meningococci, N.P. 29 and N.P. 30, have been included as controls. N.P. 29 produced slight yellow pigmentation on glucose ascitic agar, but fermented only maltose and glucose. N.P. 30 was markedly pigmented and fermented in addition laevulose.

The points of importance in the analysis of this table of agglutination tests are (1) that the majority of the naso-pharyngeal strains, fermenting

only glucose and maltose, show some degree of correspondence with meningococci of cerebro-spinal origin as regards agglutinability; (2) a certain number can definitely be pronounced as identical with standard strains of meningococci; (3) when agglutinogenic capacity is included as proof of relationship, further evidence is provided of correspondence between cerebro-spinal meningococci and naso-pharyngeal strains of non-contacts.

AGGLUTININ ABSORPTION EXPERIMENTS.

The following experiments have been made with the object of comparing absorptive capacity with agglutinability.

Experiment 1.

M. 8 serum absorbed with cerebro-spinal and naso-pharyngeal strains.

Each absorbing strain was suspended in 1 c.c. of 1 in 10 dilution of serum. In Table III A the amount of culture added was the whole

TABLE III*.

A.

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain					Titre of absorbing strain for M. 8 serum before absorption
	40	80	160	320	640	
(Control serum)	+	+	+	+	trace	...
M. 8	trace	–	–	–	–	400
M. 9	±	–	–	–	–	400
M. 3	trace	–	–	–	–	800
M. 4	trace	–	–	–	–	800
M. 16	+	+	±	–	–	100
M. 17	+	+	+	±	trace	50
M. 32	+	+	+	+	trace	–
M. 23	+	+	+	+	trace	–
M. 32A	+	+	+	±	trace	–
M. 33	+	+	+	+	trace	±
M. 24	+	+	+	+	trace	±
N.P. 1	trace	–	–	–	–	800
N.P. 14	+	+	±	trace	–	50
N.P. 4	+	+	±	±	–	50
N.P. 15	+	+	+	+	trace	±
N.P. 13	+	+	+	+	trace	∓
N.P. 9	+	+	+	+	trace	±
N.P. 27	+	+	+	+	trace	–
N.P. 17	+	+	+	+	trace	±

* In this and the following tables—

\+ Complete agglutination. ± Marked, but incomplete agglutination.

∓ Slight, but definite agglutination.

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B.

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain					Titre of absorbing strain for M. 8 serum before absorption
	40	80	160	320	640	
(Control serum)	+	+	+	+	±	...
N.P. 2	+	+	+	±	−	200
N.P. 2A	+	+	±	±	−	100
N.P. 17	+	+	+	+	±	±
N.P. 18	+	+	+	+	±	±
N.P. 20	+	+	+	±	±	−
N.P. 21	+	+	+	±	∓	±
N.P. 22	+	+	+	±	∓	∓
N.P. 16	+	+	+	+	±	±
N.P. 25	+	+	+	+	±	±
N.P. 11	+	+	+	+	±	∓

of the living 24-hour growth from one glucose ascitic agar culture, in Table III B the growth from two tubes. The tubes together with the control serum dilution were incubated at 55° C. for 2 hours and placed over night in the ice-chest. After centrifuging, the agglutination tests were made upon the homologous strain (the culture suspension of the homologous strain in A was a little less agglutinable than that used in B). Almost complete absorption occurred with four cerebro-spinal strains, including the homologous, and with one naso-pharyngeal strain; of the remaining some showed slight absorption, others none.

Experiment 2.

M. 23 serum absorbed with cerebro-spinal and naso-pharyngeal strains.

In Table IV A the method of absorption was similar to that in Experiment I. Table IV B shows the effect of absorption on two successive occasions, each time with the growth from a single glucose ascitic agar tube.

The homologous strain alone absorbed the specific agglutinin completely with a single treatment. Different degrees of absorption were shown by several naso-pharyngeal strains, complete with N.P. 8, after the second treatment.

Experiment 3.

In absorption experiments 1 and 2, where sera M. 8 and M. 23 have been treated on a single occasion with culture N.P. 11, the agglutinating capacity of these sera towards the homologous strains has

TABLE IV*.

A.

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain 40	80	160	320	640	Titre of absorbing strain for M. 23 serum before absorption
(Control serum)	+	+	+	+	±	...
M. 23	–	–	–	–	–	500
M. 24	+	±	trace	trace	–	500
M. 22	+	+	±	±	trace	600
M. 20	+	+	±	±	trace	800
M. 32A	+	+	+	±	±	±
M. 33	+	+	+	+	±	∓
M. 32	+	+	+	+	±	±
M. 8	+	+	+	+	±	±
M. 9	+	+	+	+	±	∓
M. 16	+	+	+	+	±	–
N.P. 6	+	+	±	±	trace	800
N.P. 8	+	±	±	∓	–	400
N.P. 9	+	+	±	±	±	400
N.P. 10	+	+	±	±	–	500
N.P. 11	+	+	+	±	±	400
N.P. 12	+	+	+	±	±	50
N.P. 13	+	+	+	+	±	50
N.P. 14	+	+	+	+	±	±
N.P. 3	+	+	+	+	±	∓

* In this and the following tables N.P. = non-contact naso-pharyngeal strain.

B.

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain 60	120	240	480	960	Titre of absorbing strain for M. 23 serum before absorption
(Control serum)	+	+	+	+	±	...
N.P. 8	–	–	–	–	–	400
N.P. 10	±	±	trace	–	–	500
N.P. 6	+	∓	trace	–	–	800
N.P. 7	+	+	±	trace	–	400
N.P. 24	+	+	±	∓	trace	–
N.P. 9	±	±	±	trace	trace	400
N.P. 4	+	+	+	±	∓	–
N.P. 17	+	+	+	+	±	–
N.P. 18	+	+	+	+	±	–
N.P. 29	+	+	+	+	±	–
N.P. 26	+	+	+	+	±	∓
N.P. 16	+	+	+	+	±	∓
N.P. 12	+	+	+	+	±	50

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not been appreciably diminished. In the following experiment sera M. 8 and M. 23 were treated on four successive occasions with culture N.P. 11. The concentration of serum was 1 in 20, and in each case 4 c.c. were exhausted with the growth from 7 glucose agar tubes divided into four portions. After each addition of culture the tubes were incubated for 2 hours at 55° C. and centrifuged; tubes containing serum dilution alone were incubated and centrifuged at the same time. The sera were treated in identical manner with the other cultures included in the tables.

Tables V and VI show that N.P. 11 absorbs agglutinin from both sera, the absorption of M. 23 serum being the more marked. N.P. 29 and 30, both pigmented strains, the latter a laevulose fermenter, were used as controls; they have not absorbed any of the specific agglutinin. N.P. 10 has already been shown (Table IV A) capable of absorbing M. 23 agglutinin.

TABLE V.

M. 8 *serum.*

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain				
	80	160	320	640	1280
(Serum control)	+	+	+	∓	–
N.P. 29	+	+	+	∓	–
N.P. 30	+	+	+	∓	–
N.P. 11	+	±	∓	–	–

TABLE VI.

M. 23 *serum.*

Absorbing strain	Dilutions of absorbed serum tested upon homologous strain				
	80	160	320	640	1280
(Serum control)	+	+	+	∓	trace
N.P. 30	+	+	+	∓	trace
N.P. 10	±	±	trace	–	–
N.P. 11	±	∓	trace	–	–

Experiment 4.

M. 8 serum absorbed on three successive occasions with N.P. 11 culture (Table VII): 10 c.c. of 1 : 10 dilution of serum treated with the growth from 10 glucose agar tubes. The serum after absorption was tested as to its agglutinating capacity upon the homologous strain and upon several cerebro-spinal and naso-pharyngeal strains. The results show that N.P. 11 culture has absorbed from M. 8 serum the agglutinin that had, before the absorption, acted upon eight strains of meningococci.

TABLE VII.

Strain	50	100	200	300	400	500	600	Titre of tested strain for the unabsorbed serum
M. 8	+	+	+	±	±	±	±	400
M. 3	+	+	+	+	±	±	±	800
M. 9	–	–	–	–	–	–	–	400
M. 11	+	∓	–	–	–	–	–	200
M. 15	trace	–	–	–	–	–	–	100
M. 16	trace	–	–	–	–	–	–	100
M. 14	∓	–	–	–	–	–	–	100
M. 21	–	–	–	–	–	–	–	100
M. 13	–	–	–	–	–	–	–	100
M. 12	–	–	–	–	–	–	–	100
M. 17	–	–	–	–	–	–	–	50
N.P. 1	+	+	+	+	±	...	...	800
N.P. 2A	trace	–	–	–	–	...	...	100
N.P. 2	∓	∓	∓	trace	trace	...	...	200
N.P. 11	–	...	...	...	...	...	...	50

Experiment 5.

M. 23 serum absorbed on three successive occasions with N.P. 11 culture and tested upon the homologous strain, in addition to several cerebro-spinal and naso-pharyngeal strains: 15 c.c. of 1 : 10 dilution of serum treated with the growth from 12 glucose agar tubes. The results show (Table VIII) that the agglutinins for several of the

TABLE VIII.

Strain	50	100	200	300	400	Titre of tested strain for un-absorbed serum
M. 23	+	+	±	∓	trace	500
M. 18	+	+	±	∓	–	1000
M. 22	–	–	–	–	–	600
M. 26	±	∓	–	–	–	400
M. 28	+	±	∓	trace	...	400
M. 19	+	±	±	trace	trace	1000
M. 20	+	±	trace	trace	...	800
M. 21	+	±	trace	...	...	800
M. 24	+	+	±	±	–	500
M. 29	+	±	trace	trace	...	400
M. 25	±	±	–	–	–	400
M. 30	±	±	–	–	–	400
M. 27	+	±	*trace*	*trace*	–	400
N.P. 11	–	–	–	–	–	400
N.P. 6	±	±	∓	trace	–	800
N.P. 10	+	±	∓	trace	–	500
N.P. 9	∓	–	–	–	–	400
N.P. 8	±	∓	trace	–	–	400
N.P. 7	+	±	∓	trace	–	400

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cerebro-spinal meningococcal strains are reduced, and in one case, M. 22, completely absorbed.

Experiment 6.

Absorption experiment with M. 9 serum (Table IX). In each case 1·5 c.c. of 1 : 10 dilution of serum were treated with the growth from a single glucose ascitic agar tube.

M. 8 and M. 9 have absorbed almost completely the specific agglutinin from M. 9 serum. In Experiment 1 M. 9 was shown to absorb the specific agglutinin from M. 8 serum.

TABLE IX.

	Dilutions of absorbed serum tested upon homologous strain				
Absorbing strain	80	160	320	640	1280
(Control serum)	+	+	+	±	trace
M. 8	trace	–	–	–	–
M. 9	trace	–	–	–	–
M. 16	+	+	+	trace	–
M. 11	+	+	+	trace	–
M. 13	+	+	+	trace	–
M. 14	+	+	+	trace	–
M. 17	+	+	+	trace	–
M. 15	+	+	±	∓	–
M. 12	+	+	±	∓	–
N.P. 11	+	+	+	∓	–

Analysis of Absorption Experiments.

Absorption experiments 1 and 2 demonstrate that strains which on the score of agglutinogenic capacities or agglutinability might be regarded as belonging to the same group do not necessarily coincide in absorptive capacities. In Experiment 1 there is a correspondence between agglutinability and absorptive capacity, but not in Experiment 2.

Experiment 3 shows that a naso-pharyngeal strain has absorbed specific agglutinin from sera of both groups of cerebro-spinal meningococci and that absorption was more marked with that serum which agglutinated the absorbing strain.

Experiment 4 shows that a naso-pharyngeal strain has removed from a cerebro-spinal meningococcus serum a certain amount of the agglutinin for the strain homologous to that serum, but has removed

all the agglutinin for a second strain, which (Experiments 1 and 6) has been shown to be identical with the homologous strain.

Experiment 5 shows that a naso-pharyngeal strain has removed from a meningococcus serum, to an unequal extent, the agglutinin for other strains belonging to the same group.

Experiment 6 demonstrates equal absorptive capacity in M. 8 and M. 9, members of the same group.

SUMMARY OF RESULTS.

Simple agglutination tests divided into two main groups a series of 34 meningococci obtained from the cerebro-spinal fluid of cases of meningitis during the recent epidemic.

Certain of these strains of meningococci, which were either not agglutinated or only slightly by any of the sera employed, could be placed in one or other of the groups by the demonstration of their agglutinogenic capacity.

Certain individual strains in each of the groups were agglutinated to a less degree than the homologous strain, and certain strains were agglutinated by sera of both groups.

For further evidence as to specific relationship resort was made to agglutinin absorption experiments.

While the question, as to whether meningococci can be divided into independent groups by means of agglutinin absorption tests, must, at this stage of the investigation, remain open, the experiments detailed above indicate that variations in absorptive capacity between individual strains of meningococci are analogous to, though not in actual correspondence with, variations in agglutinability.

The non-contact naso-pharyngeal strains, culturally identical with meningococci, exhibited in relation to monovalent agglutinating sera prepared with cerebro-spinal meningococci, the same tendency to grouping as the cerebro-spinal strains and similar variations in agglutinability. The 28 non-contact strains, which have been investigated serologically, reacted to the following extent with one or other of the above-mentioned meningococcus immune sera:—5 showed complete agglutination in 1 : 400 or over, 10 in 1 : 200 or over, 6 in 1 : 100; 7 were not completely agglutinated in dilutions higher than 1 : 50. The first-mentioned 5 absorbed, from the respective agglutinating sera, the agglutinins for the homologous strains. From one of these 5, N.P. 10,

a serum was prepared which was found to agglutinate strains in Group II. Of the 16 strains which agglutinated with meningococcus sera between 1 : 100 and 1 : 400, a few were tested as to their absorptive capacity in relation to two sera and, as will be seen from the absorption tables, showed evidence of agglutinin absorption. The absorption, though slight in amount, was equal to that occurring with the same sera treated with certain cerebro-spinal strains. The remaining 7 strains, which were agglutinated feebly by the meningococcus sera employed, are being subjected to further investigation (1) as to their agglutinability in relation to other cerebro-spinal meningococcus sera, (2) as to their capacity for producing agglutinating sera for cerebro-spinal meningococci. Taken as a whole, the serological results afford indication of a division of meningococci into two groups with some overlapping of each.

The theoretical explanation may be that the antigenic substance of the meningococcus contains one or other of two specific components, A and B, and sometimes contains both components, one of the two then being present in greater amount than the other. Consequently some strains produce sera with agglutinins of the A class alone; others create agglutinins of the B class alone; others produce both A and B agglutinins, with preponderance in some cases of A and in other cases of B. As regards agglutinability, again, some strains are capable of combining with A alone, others with B alone, and others with both A and B, but to a greater degree with the one than with the other.

Comparing the capacity of an individual strain for producing agglutinin with its capacity for combining with agglutinin, I find that in some cases these two capacities appear to coincide. But this is not a general rule. For example, a strain may have limited capacity for combining with agglutinin, but much greater capacity for producing agglutinin; again, it may combine with A alone, or mainly with A, but produce agglutinins in which B preponderates over A.

Absorption experiments, again, show that whilst there is sometimes a correspondence between capacity to absorb and capacity to create, or combine with, specific agglutinin, this correspondence is not a general rule.

These last two considerations show that the characteristics of different strains of meningococci, while affording a basis for division into groups, are closely inter-related, and, in fact, are connected by inseparable links, which appear to make it impossible to effect a definite cleavage between the one group and the other.

The above observations have been suggested by the results so far obtained, but further work on this subject is in progress, with special reference to the identification of the meningococcus in the naso-pharynx.

CONCLUSION.

All strains of Gram-negative cocci, obtained from the naso-pharynx, identical microscopically, culturally and in fermentation tests with meningococci, must, in default of a specific test for virulence and until the serological relationships have provided definite evidence to the contrary, be considered to be meningococci.

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