IDENTIFICATION OF THE MENINGOCOCCUS.

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(With 1 Text-figure.)

I. PRELIMINARY RESEARCHES.

During the years 1903 and 1904 while engaged upon an investigation of the micro-organisms present in saliva, I had frequent occasion to make a quantitative bacteriological examination of this material. The object then in view was to define the bacteria that are most numerous in the mouth, so as to see if they could be applied to detect particulate pollution of air by material derived from the upper respiratory passages in a way similar to that in which *B. coli* is used to detect and measure excremental pollution of water. While certain streptococci were found to provide the index in question, particularly *S. salivarius* (which is constantly present to the extent of 10 to 100 millions per cubic centimetre of normal saliva) it was observed that among other bacteria present in the cultures were certain gram-negative cocci which frequently exceeded 100,000 per c.c. of the saliva of normal individuals.

In 1905 in view of the recent manifestation of cerebro-spinal fever abroad in epidemic form and the discovery that this disease is spread by persons who carry the meningococcus in their nasopharynx, it appeared desirable to define the characters of these gram-negative cocci of normal saliva more closely, so that should occasion arise hereafter the information might be available for identifying carriers of the meningococcus.

Accordingly, an investigation of these gram-negative cocci was undertaken, and it was soon obvious that they were of several different kinds. As my object was to sort them, and previous experience with streptococci had shown that this was most likely to be achieved by a study of their fermentative characters, representative specimens of the gram-negative cocci were isolated and cultivated in a slightly

alkaline medium containing one or other of some sixty carbohydrates, polyatomic alcohols, or glucosides. As a result it was found that glucose, maltose, saccharose and galactose were of value for differentiating these salivary gram-negative cocci from one another, and by means of their ability to break up one or other of these carbohydrates 127 gram-negative cocci derived from human saliva were resolved into five separate groups. To make the investigation complete, the meningococcus and gonococcus were also examined with regard to their behaviour in these tests, and the points in which they differ from each other and from the gram-negative cocci of normal saliva defined.

In addition to the difference in respect of fermentative characters, it was found that the question of growth or no growth on agar or nasgar at 23° C. was a further point of practical value for the purpose of differentiating these gram-negative cocci from the meningococcus which, as Albrecht and Ghon first showed in 1901, does not grow on ordinary media below a temperature of 25° C.

While this investigation was at an early stage, I was invited by Dr R. A. Dunn, M.O.H. of East Herts, to co-operate with him in an investigation of a mysterious illness of an infectious nature which had appeared in the district for the health of which he was responsible. This illness was at one time suspected of being cerebro-spinal fever, and in course of the bacteriological investigation of material from the nasal passages of persons affected by it, my collection of gram-negative cocci became considerably augmented. The chief micro-organism found in the nasal secretion of the Herts case was *M. catarrhalis*, but among other gram-negative cocci encountered were some that resembled the meningococcus more closely than the majority of those which I had previously come across in cultures from normal saliva. On proceeding to compare certain of these cocci with the meningococcus it was discovered that even when the sugars previously mentioned failed to differentiate them, the majority were clearly distinguishable from the meningococcus by their ability to ferment mannose; moreover all of them were distinguished from it by the fact that they grew readily upon nasgar at 23° C.

Up to the time of this investigation, attempts had been made with variable success by several observers, to apply the agglutination test for the purpose of differentiating the meningococcus. My own efforts in this direction were not of an encouraging nature, and therefore I was unable to recommend the test at this stage of the research. Looking back, it is easy to see now that two of the chief reasons of this lack of

success were, first that I employed the microscopic method only, and secondly that the most satisfactory way to prepare agglutinin for the meningococcus had not then been determined.

The conclusion reached, therefore, was that the gram-negative cocci of normal saliva were of several different kinds, and the characters of most value for differentiating them from the meningococcus were the appearance of their growth on agar or nasgar at 37° C., their ability to grow on nasgar at 23° C. to 25° C., and their action upon glucose, maltose, galactose, and saccharose respectively and, in certain cases, mannose. The value of these fermentation tests for the purpose in view was subsequently confirmed by others, notably by von Lingelsheim whose results appeared in the year following that of the publication of the paper by Dunn and myself.

Shortly after this investigation had been completed, I was fortunate enough to secure the co-operation of Dr T. J. Horder in an experimental investigation of the protective value of various samples of antimeningococcus serum. It is unnecessary to describe this investigation further here than to remark that we found that whereas the total growth of as much as six young slope cultures on nasgar of the particular meningococcus with which we were working could be injected intravenously in a single dose into a rabbit without fatal effect, nevertheless a total of but four to six of such cultures was invariably fatal to the animal if given *seriatim*, and an hour's interval was allowed to elapse between the injection of each individual culture. The experience then gained with this method of saturation proved of very great value some eight years later.

II. MEASURES ADOPTED FOR IDENTIFICATION OF THE MENINGOCOCCUS ON THE MANIFESTATION OF CEREBRO-SPINAL FEVER AMONG TROOPS IN TRAINING DURING 1915.

In the early months of 1915 cerebro-spinal fever broke out among recruits in training, a large epidemic was threatened, and special measures became imperative for limiting the spread of this disease. The sanitary measures adopted for this purpose were directed by Colonel W. H. Horrocks, C.B., K.H.S., who was assisted by Surgeon-Colonel R. J. Reece, C.B., and I was invited by the Medical Research Committee to advise with regard to bacteriological matters, and also to carry out research. The following procedure was adopted. On occurrence of a case of cerebro-spinal fever, the patient was removed to an isolation hospital for treatment, and the contacts were segregated and swabbed.

Each contact whose nasopharynx yielded no meningococcus-like organisms was returned to duty with the smallest possible delay, while those whose nasopharynx was found to contain an organism resembling the meningococcus were kept in isolation until the characters of the suspect coccus had been further determined. Thus it was insured that contacts whose nasopharynx yielded an organism indistinguishable from the meningococcus were kept in isolation until two successive nasopharyngeal swabs taken at an interval of several days proved negative.

A Central Laboratory was set up at the R.A.M. College and District Laboratories were started or co-opted for the purpose of dealing with this disease in military districts throughout the country. A special department in the Central Laboratory was instituted for the manufacture and supply of media to the laboratories and placed under the charge of Major T. G. M. Hine. For the purpose of obtaining material from the nasopharynx for bacteriological examination, the covered swab introduced by Mr C. E. West. F.R.C.S. was adopted.

From the bacteriological point of view, the immediate need was for a practical and rapid method of identifying the meningococcus in the nasopharyngeal secretion, while it was essential that the procedure adopted should be of such a nature that the bacteriologists who were detailed or co-opted for this work—most of whom had little or no previous experience of identifying the meningococcus in nasopharyngeal secretion—could readily carry it out with the appliances of an ordinary bacteriological laboratory.

In the memorandum which was issued in February 1915 giving instructions as to the measures to be taken on the occurrence of a case of cerebro-spinal fever, and the mode of passing the nasopharyngeal swab, the following procedure was prescribed for identification of the meningococcus.

"Secretion from the nasopharynx. The stages of the investigation are as follows.

"(1) Examination of separate colonies on the cultures. Colonies of the meningococcus appear at 37° C. in 24—48 hours. They are larger than colonies of the accompanying pneumococci and streptococci, they are clear, smooth and transparent, have a firm outline, and are very characteristic. A portion of one of these colonies taken up on a platinum needle is found to emulsify readily in a drop of water on a glass slide.

"(2) Gram's Stain. A film made from one of these colonies shows gram-negative diplococci.

"Subcultures are made and placed at 37° C. and at 23° C. respectively.

The meningococcus does not grow at 23° C., whereas the vast majority of the gram-negative cocci of normal saliva grow readily at this temperature.

"In view of the sharp distinction which this test provides, it will be sufficient for practical purposes to regard the suspicious cocci that have passed it as meningococci.

"Confirmatory tests should be employed for greater accuracy as follows:

"*Fermentation Tests.* The meningococcus ferments glucose with the production of an acid reaction, but fails to change saccharose. These tests are applied by making subcultures at 37° C. in media tinted with litmus and containing the above sugars respectively.

"*Agglutination.* The meningococcus shows positive agglutination when brought in contact with anti-meningococcus serum."

This scheme of identifying the meningococcus in nasopharyngeal secretion had the advantage of simplicity and speed; moreover it was found to work quite smoothly in practice. The majority of the contacts were found to harbour no meningococcus-like organisms, and were returned to duty within 48 hours.

It was plain, however, at the outset that the bacteriological procedure was of a provisional nature only, and that while it made practical application of the knowledge then available for the exclusion of the commoner gram-negative cocci with which confusion was likely to arise, there was reason to suspect the possible existence of a further group of these organisms indistinguishable from the meningococcus in the particular characters submitted to scrutiny, but without the same significance in regard to epidemic cerebro-spinal fever.

The history of bacteriology in its application to medicine shows repeated instances of the confusion of specific pathogenic bacteria with others closely resembling them in morphological, cultural, and sometimes even in fermentative characters also, but entirely devoid of the same pathogenic significance. The earliest instance in which this similarity led to error appears to have been brought about by the resemblance between the anthrax bacillus and *B. subtilis*. The similarity between the diphtheria bacillus and certain diphtheroid bacilli is another example that has certainly led to mistakes; and the resemblance in morphological and cultural characters between the cholera vibrio and some other vibrios found in nature has undoubtedly caused difficulty in the past. The history of the attempts to identify the typhoid bacillus in

water is a further and striking illustration of the need of caution before resting satisfied that a given micro-organism is identical with one of specific pathogenic importance solely because of a close resemblance in morphological, cultural, and fermentative characters.

Accordingly, it was realised at the outset that the procedure adopted for recognising the meningococcus in nasopharyngeal secretion merely represented the best that could be done in the circumstances, and that the only way to define and remedy its possible defects was by intensive research. This research was rendered still more urgent by the military necessity of holding up no man unless he carried a meningococcus of known epidemic significance.

III. DEFINITION OF THE MENINGOCOCCUS OF THE OUTBREAK BY MEANS OF THE AGGLUTINATION TEST.

In view of the work that had been done by Jochmann, Von Lingelsheim, Lieberknecht, Kutscher, Dopter, Netter and Debré, Elser and Huntoon, Raymond Koch and others, and the improvements in knowledge and technique that had resulted from their labours, it was anticipated that the agglutination test would prove of immediate use for the purpose of identifying the meningococcus. On trial, however, it was found that specimens of the anti-meningococcus serum then available failed to agglutinate meningococci isolated from the cerebro-spinal fluid of our cases.

ANALYSIS OF MENINGOCOCCI OCCURRING IN THE CEREBO-SPINAL FLUID OF THE CASES.

Before an agglutinating serum could be obtained, therefore, that would serve for the purpose of identifying the micro-organism of the outbreak, it was necessary to start *ab initio* and first of all to collect meningococci from the cerebro-spinal fluid of the cases and to define them by the agglutination test. It was clear also from the work of Lieberknecht, Dopter, and Elser and Huntoon that the absorption test should be applied as well in order to check the results of simple agglutination.

The steps of this investigation have been described in detail in the *Journal* of the R.A.M.C., and also in a report to the Medical Research Committee. The first requirement was to obtain agglutinating serum of good quality in as short a time as possible. By means of the saturation method to which reference has been made, it was found that excellent agglutinating serum could be prepared from young rabbits within ten

days. Meningococci were collected from the cerebro-spinal fluid of thirty-two cases of the disease and systematically investigated by Captain E. G. Murray and myself in respect of their agglutination reactions and the results checked by the absorption test. In view of the work of Kutscher and Lieberknecht, the macroscopic method was used and the tubes examined after 24 hours at 55° C. From the first the method was standardised. Raymond Koch's discovery that a suspension of meningococci would keep for several months in saline and serve quite well for agglutination tests provided it is heated in the first place for half-an-hour to 65° C. and 0·5 % of phenol then added as a preservative, was made use of; and all suspensions standardised to contain the same number of meningococci by a turbidity test that was worked out for this purpose. In order that our results might be checked by others, full particulars have been given of the procedure employed.

As the outcome of this investigation the thirty-two meningococci were found to be resolved into four different groups as follows:

Type	1	2	3	4
Specimens	19	8	4	1

As a rule the results of simple agglutination were confirmed by those given by the absorption test. There were in some cases though not in all close affinities between members of Types 1 and 3, and 2 and 4; but absorption tests proved that these affinities were due to minor or co-agglutinins, and that the major agglutinin of each of the four types was univalent and specific. Complete cross tests and controls were carried out with all thirty-two cocci against each of the four univalent sera.

IV. APPLICATION OF THIS INFORMATION FOR THE PURPOSE OF IDENTIFYING THE MENINGOCOCCUS IN NASOPHARYNGEAL SECRETION.

Gram-negative cocci from the nasopharynx of nine contacts and one doubtful case of cerebro-spinal fever were now submitted to investigation with the four univalent agglutinating sera that had been proved to include all of the serological types of meningococcus occurring in the cerebro-spinal fluid of the thirty-two cases. Each of these ten nasopharyngeal cocci was indistinguishable from the meningococcus in morphological, cultural, and fermentative characters. As a result of the test, six of these nasopharyngeal cocci were found to be serologically identical with the meningococcus—five being specimens of Type 2 and

one of Type 1. The remaining four nasopharyngeal cocci could not be identified serologically with any of the four types of meningococcus. In order to test the matter further, two specimens of these four pharyngococci were injected into rabbits and an agglutinating serum prepared against each of them. The specific agglutinin of each of the pharyngococci while readily removed by the homologous coccus, was quite unaffected by any of the four types of meningococcus obtained from the cerebro-spinal fluid of the cases. The two pharyngococci in question also appeared to be serologically distinct from one another. Thus the suspicion mentioned previously was confirmed and the existence established of a group of pharyngococci indistinguishable from the meningococcus in the morphological, cultural and fermentative characters submitted to examination, but nevertheless distinct serologically from any of the types of meningococcus present in the cerebro-spinal fluid of the thirty-two cases of cerebro-spinal fever.

V. ROUTINE ADOPTION OF THE AGGLUTINATION TEST FOR THE PURPOSE OF IDENTIFYING THE MENINGOCOCCUS IN CASES OR IN CONTACTS.

During the autumn of 1915 further specimens of meningococcus from the cerebro-spinal fluid of cases were submitted to scrutiny with the four univalent sera. For this purpose Major Arkwright generously supplied me with cultures from the collection which he had made during preceding stages of the outbreak, and Dr O'Brien did the same. As a result, by the end of 1915 over sixty specimens of meningococcus had been examined with all four agglutinating sera, and found to be identical with one or other of the four serological types.

As soon as the types of meningococcus present in the outbreak had been defined, cultures of them were forwarded to those who prepare anti-meningococcus serum with a note as to their frequency. In order also to make the identification of the meningococcus more accurate it was decided to provide all District Laboratories with the necessary materials for determining the type present either in the cases or in contacts. Major Hine carried out a research in which he defined the relative value of various modes of dosage for the purpose of obtaining agglutinating serum for the meningococcus in the shortest possible time, and by this means elaborated an intensive method which has now been in continual use at the Central Laboratory for over two years with uniform success. The manufacture and supply of univalent agglutinating sera and emulsions was taken over by him and these were

supplied to District Laboratories in outfits, the expense of which was defrayed by the Medical Research Committee. With each outfit the following directions were issued:

CEREBRO-SPINAL FEVER.

AGGLUTINATION TESTS.

Directions for applying the agglutination test to meningococci and meningococcus-like organisms with the outfit supplied for this purpose from the Central Cerebro-Spinal Fever Laboratory, R.A.M. College, S.W.

20th December, 1915.

Investigation of meningococci occurring in the recent outbreak of cerebro-spinal fever among the troops in this country has shown that the majority of specimens of this micro-organism isolated from the cerebro-spinal fluid of the cases, although alike in cultural and fermentative characters, are differentiated by the agglutination test into one or other of three main types.

The relative abundance of each of these types up to the present stage of investigation of the recent outbreak is seen from the following figures. The number of specimens of meningococci from cerebro-spinal fluid examined was sixty-one:

Type	Specimens	Percentage
1	31	50
2	20	32
3	10	16

In addition to these three predominant types, several other types of meningococcus have been differentiated by the same means, but the latter, owing to their comparative rarity so far, appear to be relatively unimportant from the point of view of controlling the epidemic among troops.

The above facts have a practical bearing both on treatment, and also on identification of the meningococcus in the nasopharynx of carriers.

SERUM TREATMENT OF THE PATIENT.

In order to ensure that the correct specific serum is given, it is desirable to prepare a suspension of the particular meningococcus occurring in the cerebro-spinal fluid of the case, and to determine its type by the agglutination test.

IDENTIFICATION OF THE MENINGOCOCCUS IN THE NASOPHARYNX OF CARRIERS.

According to present evidence, cerebro-spinal fever is chiefly spread by carriers. In order to check the spread of this disease, therefore, it is desirable to detect and isolate any persons carrying in their nasopharynx known epidemic strains. Recognition of such strains of this micro-organism is now possible by means of the agglutination test.

METHOD RECOMMENDED FOR APPLYING THE TEST.

The following articles are required:

1. Specific agglutinating sera univalent for each of the three chief types of meningococcus occurring in the cerebro-spinal fluid of cases during the present outbreak, and a sample of normal serum for use as control.
2. Four sterile test-tubes for making dilutions of these sera.
3. A fine calibre pipette holding 0·5 c.c. and graduated to 0·1 c.c.
4. A 5 c.c. pipette graduated in 1 c.c. and 0·1 c.c. divisions up to the point.
5. Small test tubes 3″ by ½″, sterilized and plugged with sterile wool.
6. A stand to hold these tubes. (See below.)
7. Fresh sterile saline (0·85 per cent.).
8. Standardized and phenolated suspensions of each of the meningococci homologous to the three specific sera, and similar suspensions of the several cocci to be tested.

When identifying the meningococcus in cultures from the nasopharynx, the first stage is the selection of meningococcus-like colonies in the plates after twenty-four hours growth at 37° C. A colony sufficiently typical for further test having been carefully selected, the steps for carrying out the agglutination test are as follows:

PREPARATION OF STANDARD SUSPENSION OF SELECTED COCCUS.

As the method recommended is the macroscopic one, it is necessary in the first place to obtain enough growth of the coccus to make a fair quantity of the suspension. The coccus, therefore, should be spread over the surface of two or more legumin agar plates or plates of other suitable medium. After twenty-four hours incubation at 37° C., the growth on each plate is washed off in 5 c.c. of saline with the aid of a sterile wire or a glass rod bent to an angle, then poured into a sterile test tube and well shaken. Film preparations are next made of these suspensions, stained with Gram, and examined for purity. Meantime the tubes have been placed in a water bath at 65° C., at which temperature they are allowed to remain for thirty minutes in order to kill the coccus and to destroy its autolysin.

STANDARDIZATION OF THE SUSPENSION.

This is effected in the following way. With a pipette delivering 0·1 c.c., this amount of the suspension is transferred to an ordinary clean ½-in. test tube specially kept for this purpose. Clear tap water is then run in from the 5 c.c. graduated pipette until the contents of the tube are only just—but still definitely—turbid by daylight when compared with the control tube of tap water. This end-point is taken to represent a content of 100 millions of the coccus per cubic centimetre. A simple calculation then gives the number of cocci contained by the suspension. For example if 0·1 c.c. of the suspension requires to be diluted to 8 c.c. with tap water to reach the end point, 0·1 c.c. of the suspension, therefore, contains 800 million cocci; and 1 c.c. of it 8,000 million. Supposing there are 5 c.c. of the suspension; this contains, therefore, 40,000 million cocci altogether. Now it has been found by experiment that a suspension of meningococcus containing 2,000 million per c.c. gives excellent results as regards macroscopic agglutination. In the present example, then, there are sufficient

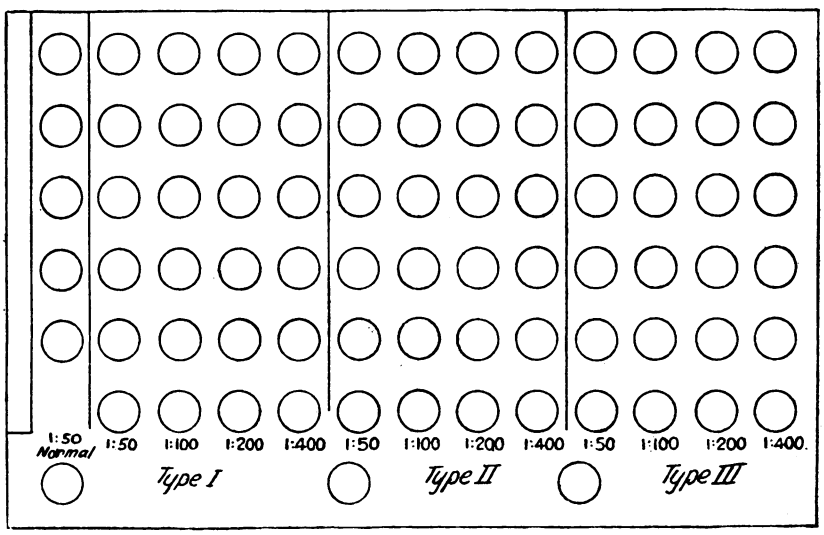
cocci to make 20 c.c. of such a standard suspension. Accordingly the 5 c.c. of suspension is poured into a sterile measuring glass, diluted with saline up to 18 c.c., and then 2 c.c. of a 5 per cent. solution of phenol in distilled water is added to it as a preservative. The suspension, heated, standardized, and phenolated in this way is poured into a bottle, labelled and dated. It has been found that such suspensions of meningococci keep for several months.

The standardized suspensions having been prepared in this way, and the other articles being at hand, procedure is as follows:—Let us imagine, for example, that five gram-negative cocci are to be tested with the agglutinating sera prepared against the three chief types of meningococcus that have been obtained from the cerebro-spinal fluid of epidemic cases.

ARRANGEMENT OF TUBES IN THE STAND.

This stand is designed to hold enough tubes to test five different cocci at the same time against the three specific sera with the necessary controls.

In order to determine the degree of agglutination, the coccus is put up against each of these three sera in four dilutions. To prove that the three agglutinating sera are active, a control is also put up of each of them against its homologous coccus in the same dilutions. Thus eight cocci in all are tested, three known and five unknown. As an additional control, each coccus is also put up against a sample of normal serum.



Plan of Agglutination Board. ⅛ *Scale.*

The stand consists of a wooden block with six rows of thirteen cylindrical holes to contain the agglutination tubes. On the left is a xylonite strip for labelling each row. The holes are marked out in three blocks, stained amber, white, and blue respectively; each one of these coloured blocks contains twenty-four holes, and is

assigned to a separate specific serum. In addition there is a vertical row of five holes labelled "Normal," and there are also three single holes at the bottom for controls of normal serum against the three "standard" cocci.

The dilutions of the several sera are first of all filled into the tubes in vertical rows; then the suspensions of the different cocci are added in turn to the horizontal rows.

Thus each of the three sera has a block of four vertical rows, and a single row is reserved for the normal serum on the left. Each coccus of test has one horizontal row which runs through all three blocks of specific sera starting with the row devoted to normal serum.

The standard suspensions and anti-sera issued are put up in amber, white and blue bottles and capsules for Types I, II and III respectively, corresponding to the three blocks of holes in the board. There is also a green capsule containing the normal serum.

EXAMPLE OF TEST.

The following example will illustrate the procedure employed in further detail. The titre of all the three specific sera used is supposed to be 1 : 400. It is proposed to test the cocci against them in a dilution of 1 : 50, 1 : 100, 1 : 200, and 1 : 400 respectively.

SERUM DILUTION.

(*a*) Normal serum (control).

0·2 c.c. of normal serum is placed in a test tube by means of the small 0·5 c.c. pipette and 4·8 c.c. of saline added. From the 5 c.c. of 1 : 25 dilution of normal serum thus obtained, half cubic centimetre amounts are distributed in the first left-hand vertical row of five tubes, and the three single tubes below them.

(*b*) Agglutinating serum for "Type I" meningococcus.

A 1 : 25 dilution is made of this serum in the same way as in the case of the normal serum, but as more of it will be required in order to furnish the larger number of dilutions necessary, 0·3 c.c. of this serum is taken in the first place and diluted with 7·2 c.c. of saline. Half cubic centimetre amounts of this 1 : 25 dilution are then run into the six tubes of the first left-hand vertical row of Type I block (amber). In order to convert the remainder of this dilution in the test tube into a 1 : 50 dilution, 4·5 c.c. of saline are added to it, and half cubic centimetre amounts of this are then distributed in the second vertical row of six tubes. The procedure is repeated in the same way for the next two rows, but as the volume of the dilution that is left in the tube steadily increases it is advisable to reject some of it after row two, so that the quantity can be easily contained in a test tube of ordinary size, when diluted with an equal volume of saline. As the result of this series of dilutions, the four vertical rows of block I contain half cubic centimetre amounts of Type I serum in dilutions of 1 : 25, 50, 100 and 200 respectively.

The procedure is then repeated with the agglutinating sera for meningococcus Types II and III, and successive dilutions of these distributed as before in half cubic centimetre amounts in the tubes of blocks 2 (white) and 3 (blue). All the tubes have now a dilution of the required serum in half the final dilution decided upon.

The next step is to fill in from the standardized suspension bottles half cubic centimetre amounts of each of these respectively into each tube in the horizontal

rows with the 5 c.c. pipette including the control tubes in the left-hand row containing normal serum. Finally four half cubic centimetre amounts of each of the control cocci homologous to the specific sera are filled into groups of four tubes in the lowest horizontal row of the stand. All the tubes are then plugged and incubated for twenty-four hours at 37° C., or 55° C.

RESULTS.

In reading off the results it is advisable to always examine the tubes in the same order. Thus the controls of each coccus with normal serum in the left-hand vertical row and three bottom single tubes are examined first, and then the controls of homologous cocci in the lowest horizontal row. The former being negative and the latter positive; the degree of agglutination of each coccus to the three specific sera respectively is next noted. It is convenient to have one's own signs for indicating the degree of agglutination. Thus "‡" signifies absolutely clear fluid and the cocci clumped in flocculi at the bottom of the tube; "+" indicates obvious flocculi of fair size floating in the fluid, but the latter still somewhat turbid; "(+)" indicates turbid fluid with small flocculi only.

If the controls are not satisfactory no inference can be drawn. Thus unless agglutination of the control type coccus takes place with its corresponding anti-serum, there is no guarantee that the specific agglutinin is active. Similarly, unless the controls with normal serum are negative, the results with specific sera are of no account. It should be remembered that certain examples of *M. flavus* agglutinate with normal serum.

The titre of 1 : 400 is chosen for this experiment because it is a good average one. Some sera go higher, in which case the primary dilutions would, of course, be correspondingly increased.

GROUP AGGLUTININS.

It has been found that certain gram-negative cocci of the nasopharynx other than meningococci may agglutinate with these anti-meningococcus sera in their low dilutions. Such of these cocci, however, as have been met with do not agglutinate beyond the first, or the two lowest, dilutions; they have been found by absorption tests not to absorb the specific agglutinin from these univalent sera.

In order to avoid confusion from the action of group agglutinins, this differentiation of meningococci occurring in the cerebro-spinal fluid of cases in the present outbreak into three main types has been effected by controlling the result of the agglutination test by an after-test to determine whether the specific agglutinin has been absorbed or not. Strictly speaking, the agglutination test furnishes presumptive evidence only: complete identification is not established until it has been proved that the specific agglutinin of the type meningococcus has been absorbed by the coccus of test. For practical purposes, however, the agglutination test appears to be sufficient; provided that it is conducted quantitatively, and the titre of the coccus of test compares with that of a control of the homologous coccus with the same serum at the same time. It should be mentioned that Types I and III appear to be closely related, meningococci of Type I frequently, but not always, showing some agglutination with Type III anti-serum.

GENERAL REMARKS.

It will be clear from the above that when cerebro-spinal fever breaks out in a district, it is most important to test the meningococcus occurring in the cerebro-spinal fluid of the case or cases in order to determine its type. The information obtained in this way is a guide both to the correct specific serum for therapeutic use, and also to the type of meningococcus to be specially looked for in the naso-pharynx of contacts. It would seem desirable to keep a watch in the same way on meningococci occurring in the cerebro-spinal fluid of any further cases in order to see if the predominant type should alter; in which case a corresponding modification would be necessary in the defensive measures. Again, it will be of great interest to know whether more than one type of meningococcus is to be found at the same time in the cerebro-spinal fluid of a case of cerebro-spinal fever; and also whether the same type occurs in the nasopharynx as is present in the cerebro-spinal fluid of the patient.

The Medical Research Committee have arranged to supply, free of cost to medical officers specially appointed for the study and treatment of military cases of cerebro-spinal fever, sets of the necessary outfit, with standard agglutinating serum and standardized homologous agglutinable suspensions of each of the three types respectively, as part of the assistance they have given to the War Office in the scientific study of measures for the treatment and control of the disease.

All applications for the outfit, with standard sera and suspensions, should be addressed to the

CENTRAL C.S.F. LABORATORY,
ROYAL ARMY MEDICAL COLLEGE,
GROSVENOR ROAD, LONDON, S.W.

It will be observed that owing to its rarity up to that time, Type 4 was at first not included in the outfit. A few cultures of meningococcus were received about this period from sporadic cases of meningitis among children and could not then be identified. In view of later experience, and especially of Captain Tulloch's research, it is highly probable that at any rate some of these were really specimens of Type 2. To provide for the possible appearance of new types, or of types not previously identified, officers in charge of District Laboratories were asked to forward to the Central Laboratory any meningococci from cerebro-spinal fluid that refused to agglutinate with the sera sent out.

During the following months the disease recrudesced and the agglutination test was found to furnish much valuable information. The cocci received as not agglutinating with the sera were comparatively few, and the majority of them proved to be specimens of Type 4. It also was found that in the case of Type 2 some sera were better than others owing to the tendency of certain specimens of this type to become sub-typical in culture. In order to exclude error from this cause freshly isolated specimens of Type 2 were used to make the antiserum of this

type. In all cases only a single meningococcus has been used at a time, and throughout only meningococci actually isolated from the cerebro-spinal fluid of cases have been employed in the preparation of agglutinating serum.

Owing to the appearance of Type 4 in a proportion of the cases during an outbreak in a large garrison in the early part of 1916, this coccus and its antiserum were added to the outfit from that date. With one exception, however, no further outbreaks have come to notice in which this particular type of meningococcus has been at all frequent.

Now that an accurate method of identifying the meningococcus in the nasopharynx was available, the following memorandum was sent round to District Laboratories with the object of obtaining further information concerning the general distribution among the military population of meningococci of known epidemiological significance.

"MEMORANDUM.

(1) MODE OF SPREAD OF CEREBRO-SPINAL FEVER. EXAMINATION OF CONTROLS.

As part of the investigation of factors governing the spread of cerebro-spinal fever, it is very desirable that, as far as possible, opportunity should be taken of making control observations with a view to ascertaining what proportion of non-contacts harbour the meningococcus in their nasopharynx.

It is suggested that, other things being equal, it would be more useful to examine for this purpose small groups of men from a large number of units, rather than a large number of men from the same unit.

Before such investigations are undertaken, a particularly careful enquiry should be made to ascertain whether or no there has been any possibility of recent contact with a case of cerebro-spinal fever, or with a carrier from such a case.

No gram-negative coccus should be accepted as the meningococcus for the present purpose unless it fails to agglutinate with normal serum, and at the same time agglutinates to approximately the same titre as the homologous meningococcus with one or other of the anti-meningococcus sera supplied.

In positive cases it will also be valuable to know (1) the relative abundance of the meningococcus in the nasopharyngeal mucus of the person carrying it, and (2) the duration of such carrying.

(2) DEFINITION OF THE RELATION BETWEEN OUTBREAKS OF INFLUENZAL CATARRH AND CEREBRO-SPINAL FEVER.

In some camps severe outbreaks of coughs and colds have occurred. It is desirable to make observations on the bacteriology of these cases both from the point of view of the presence or absence of the meningococcus, and also for the purpose of determining what are the prevalent bacteria in these cases of catarrh."

CENTRAL C.S.F. LABORATORY,
ROYAL ARMY MEDICAL COLLEGE,
GROSVENOR ROAD, LONDON, S.W.
February 16th, 1916.

VI. RESULT OF PRACTICAL APPLICATION OF THE AGGLUTINATION TEST FOR IDENTIFICATION OF THE MENINGOCOCCUS.

The report of Captain Martin Flack on cerebro-spinal fever in the London District during 1916 demonstrates in a convincing manner the value of the agglutination test in actual practice, both for detecting cases of the disease, and also for identifying carriers. With regard to cases he found (1) that not more than a single type of meningococcus could be obtained from the cerebro-spinal fluid of a patient, (2) on examination of the nasopharynx of the patient he confirmed the later observation of von Lingelsheim as to the constant presence of the meningococcus there at the onset of the disease, and he showed further that this meningococcus in the nasopharynx was always of the same type as that present in the cerebro-spinal fluid of the patient when this was positive. A similar observation as to the identity of type between the meningococcus in the nasopharynx and cerebro-spinal fluid of the same case has also been reported by Major F. W. Andrewes. This constant presence of the meningococcus was found by Captain Flack to have a direct clinical application in facilitating a correct diagnosis of cerebro-spinal fever in the very cases where help of this kind was most needed, namely in cases where the symptoms of the patient were atypical, or where the cerebro-spinal fluid failed to yield a growth of the meningococcus. The importance of early diagnosis cannot be overemphasised from the point of view of successful serum treatment. Captain Flack also found that the same type of meningococcus was most prevalent both in cases and in carriers during the period that he was in charge of the London District C.S.F. Laboratory.

Another observation made by him was that, like the cases, chronic carriers are remarkably monotypical in the sense that the great majority of them carry one and the same type of meningococcus throughout the whole period of their carrying. This was confirmed by Captain Tulloch.

A good opportunity for comparing the types of meningococcus prevalent in cases and in carriers respectively occurred during an outbreak of cerebro-spinal fever in a large garrison during 1916.

Meningococci from the cerebro-spinal fluid of thirteen cases of the disease were examined at the Central Laboratory with the following results:

Type	1	2	4
Specimens	3	5	5

A very large number of men in this garrison had been swabbed by Captain R. R. Armstrong and amongst them he found a proportion who harboured suspicious gram-negative cocci in their nasopharynx. From 193 of these men Captain Tulloch obtained cultures of gram-negative cocci that agglutinated with one or other of the univalent agglutinating sera. The distribution of types among these men was as follows:

Type	1	2	4
Specimens	30	72	71

The correspondence of types in cases and carriers is remarkable. It may be added that the identification of the types in the cases and carriers was made independently, and that it was not until Captain Tulloch came to write up his report that this closeness of the grouping in the two series was noticed.

For the last two years monotypical agglutinating sera have been in routine use at the Central Laboratory and in District Laboratories for detecting the meningococcus both in cases and in carriers with satisfactory results, and it is hoped that some of the reports of District Laboratories bearing upon this matter will be published. Meningococci isolated from troops coming from England, Scotland, Wales, Ireland, France, Gallipoli, Australia, New Zealand, Canada, and South Africa have been submitted to examination and in the vast majority of instances relegated with ease to one or other of the four types. When preparing a new circular for issue with the agglutinating outfit during August of the present year it was found on inspecting the records that during the past two years meningococci from the cerebro-spinal fluid of over 300 cases of the disease had been tested in the Central Laboratory alone, with the result that approximately 98 per cent. of them had been

identified by the agglutination test with one or other of the four types. The relative abundance of individual meningococci has varied from time to time, but on the whole their relative frequency may be put roughly as follows:

Type	1	2	3	4
Frequency	40 %	45 %	10 %	5 %

Thus while 85 % of the cases have been due either to Type 1 or to Type 2, the experience of 1915 has been confirmed throughout each of the two years that have elapsed since the types of meningococcus at work in the present outbreak were defined.

VIII. THE RESULT OF INVESTIGATION OF FURTHER SPECIMENS OF THE MENINGOCOCCUS BY THE ABSORPTION TEST.

With a view to checking previous results, and also to obtaining further information concerning the types of meningococcus occurring in cases of cerebro-spinal fever, Captain W. J. Tulloch continued the investigation of these organisms by means of the absorption test. In this way he examined with scrupulous care 100 more specimens of meningococci from the cerebro-spinal fluid of cases. Captain Tulloch's paper on this subject has been published in the R.A.M.C. *Journal* for July 1917. His investigation not only confirmed previous results, but also brought out a new and very important point, namely that in the case of meningococci belonging to Type 2—previously the most difficult of all to classify by the absorption test—no less than three distinct sub-groups are distinguishable. This sub-grouping in the case of Type 2, however, does not interfere with the practical utility of the agglutination test when identifying Type 2, for Captain Tulloch has shown that by preparing a rabbit with a sufficiently typical meningococcus of this type an agglutinating serum can be prepared that includes cocci of all its three sub-groups by the agglutination test. Captain Tulloch's research has thus been of the greatest value not only in consolidating information upon this most important matter, but also in improving the accuracy of the method in its practical application.

INTER-RELATION OF THE TYPES.

Are the four serological types of the meningococcus merely temporary variants of one and the same micro-organism, or are they pathogenically distinct members of the same group, somewhat after the manner of *B. typhosus* and *B. paratyphosus a* and *b*?

The answer to this question given by the absorption test is undoubtedly to the effect that the types are not temporary variants, but are distinct and stable entities. In order to throw further light upon the matter the following experiments have been carried out.

Superimposition Tests.

On considering this problem of the relationship of the types of meningococcus to one another it appeared that in addition to the information afforded by the absorption test, further information might be gained by comparing the effect of injecting the meningococcus of another type into a rabbit already elaborating agglutinin in response to previous stimulation with a given type of meningococcus. If the second type of meningococcus were only a temporary variant of one and the same micro-organism as the first, then the effect of superimposing it in this way should be merely to stimulate the production of the agglutinin which the animal was already in course of elaborating. If, on the other hand, the type secondarily injected were a specifically different micro-organism then the first agglutinin so far from being increased, would exhibit its normal decline; and a new agglutinin specific for the new antigen would make its appearance in the rabbit's blood.

These experiments have been described elsewhere. In the first place five young rabbits were all injected with Type 1 and when on the sixth day their blood showed a titre of from 1: 300—1: 600 for this coccus four of the rabbits received a second injection. Thus rabbit A was not interfered with: rabbit B received a second dose of Type 1, rabbit C received a dose of Type 2, rabbit D of Type 3 and rabbit E of Type 4. The result was that in the case of the rabbit that received the second dose of Type 1, the original agglutinin was increased, whereas in the case of each of the other three rabbits a new agglutinin specific for the new type, and previously absent from the rabbit's blood made its appearance. Further experiments in which Type 2 was first injected and Type 3 and 4 superimposed showed that in such cases also while the agglutinin for the primary coccus declined, new agglutinins appeared in the rabbits' blood specific for the cocci of Type 3 and 4 respectively in a precisely similar fashion as had been observed in the first experiment. Finally the superimposition of Type 4 in a rabbit already elaborating agglutinin to Type 3 resulted in the same way in the birth of a new agglutinin specific for Type 4 in the rabbit's blood.

These superimposition experiments therefore confirm the result of the absorption tests in a very definite manner, and afford striking evidence of the serological specificity of the different types.

IMMUNITY TESTS.

In order to define the protective values of prophylactic inoculation of individual types of meningococcus, a group of rabbits was prepared against Type 1, another against Type 2 and other groups against Types 3 and 4 respectively. After each animal had received a number of doses at weekly intervals and showed a good yield of homologous agglutinin in its blood, one rabbit of each group and a control normal rabbit were saturated with Type 1, another set with Type 2, and the same repeated with Types 3 and 4. As a result it was found that in the case of Types 2, 3, and 4, the homologous rabbit survived, while the other rabbits succumbed. The protection afforded by prophylactic inoculation, therefore, with these types was univalent. In the case of Type 1, on the other hand, the homologous rabbit in spite of its previous treatment was no more protected against Type 1 coccus than a control normal rabbit. The whole experiment was repeated with new specimens of the type cocci and a similar result obtained.

So far as they go, therefore, these immunity tests indicate that in the case of Types 2, 3 and 4, the protection is mainly univalent. In the case of Type 1, it is clearly far more difficult to protect a rabbit than in the case of the others, a difficulty probably due in great part to the particularly potent endotoxin of this type.

It may be here mentioned that Kennedy and Worster Drought have drawn attention to a point observed by them with regard to the relative intensity of the illness in cases of cerebro-spinal fever, according as they are infected by one or another type of meningococcus. In their experience cases due to Type 1 are far more severe and fatal than most of the cases due to Type 2. This clinical experience appears to be in accord with what has been observed elsewhere, and is on a par with the result of the protective tests described above. Some severe outbreaks due to Type 2, however, have occurred. Fulminating cases may be produced by any of the types.

It may be of interest to mention here that a particular therapeutic serum of which much use was made during the early stages of the outbreak in 1915, with disappointing results, was tested at the time against meningococci from the cerebro-spinal fluid of the cases and found not

to agglutinate them. The coccus then most prevalent was Type 1. A sample of this serum was recently submitted for examination and tested against the four types with the result that while agglutinins for 1 and 2 were practically nil, agglutinins for 3 and 4 were found to be abundant. The inference that the horse furnishing the serum had been prepared against the wrong types is difficult to avoid. The interest of this observation, however, is not limited to the negative virtues of the serum. The only medical officer who has obtained good results with this serum (and whose request for more led to my examining the sample) is one in whose district Type 4 cases have certainly been identified, and also some cases in which the infecting coccus could not be identified with Type 1 by absorption tests and almost certainly was an example of Type 3.

The following instance of the value of monotypical serum is only a single case, but it is worthy of attention. A young woman of good physique was seized with cerebro-spinal fever, and as the serum which she received did her no good, the civilian Medical Officer in charge sent an urgent request for another brand. Having some Rockefeller serum kindly sent to me by Dr Flexner for trial, I forwarded some with the request that a sample of the patient's cerebro-spinal fluid should be submitted so that the type of meningococcus present could be determined. This material shortly arrived and the coccus was found to be a specimen of Type 3. The sample of Rockefeller serum, though strong in agglutinin for Types 1 and 2, showed very little for 3 and 4. I was not surprised therefore to hear that the Rockefeller serum had been no more successful than the other. Now we had a few bottles of Type 3 serum made by Dr Stanley Griffith for us a year previously when we were trying to get agglutinating serum from horses for identification of types of meningococcus. The horse serum proved useless for the purpose then in view, although it had a high titre, for Type 3 co-agglutinins were numerous in the lower dilutions. This serum was the patient's only hope, and it was therefore sent for trial. The effect, it is pleasing to report, was extremely satisfactory; the patient making a rapid recovery forthwith. This monotypical serum was also used on some other Type 3 cases about the same time with satisfactory results, although the routine serum had failed to benefit them.

Measures are being taken at the present time to prepare monotypical sera on a larger scale for more extensive trial in the treatment of cases of cerebro-spinal fever.

CONCLUSION.

The agglutination test controlled by the absorption test, therefore, has proved practically of the greatest possible use in dealing with the outbreak of cerebro-spinal fever among the military forces. By its means a serious error has been eliminated when identifying carriers of the meningococcus; diagnosis has been facilitated—particularly in those cases where it was most needed; and the meningococci occurring in the cerebro-spinal fluid of cases have been differentiated into four separate types which breed true and while closely similar in characters of minor importance, are clearly distinguished from each other by the reaction to them of the tissues of the living animal.

The full results of this differentiation have still to be reaped, but a severe practical trial of the agglutination test during the past two years on a scale that is without precedent in this country, has demonstrated beyond all reasonable doubt that this method constitutes at the present time by far the most valuable of all known methods of identifying the meningococcus.

APPENDIX.

NOTE ON SOME RECENT OBSERVATIONS BY OTHER WORKERS WITH REGARD TO THE CLASSIFICATION OF MENINGOCOCCI.

During the early stages of the present outbreak, Arkwright and Ellis observed independently that meningococci occurring in the cases, fell into two distinct groups. Both observers relied upon simple agglutination.

Prior to the present outbreak, the work of Arkwright in this country and of Dopter in France had paved the way for the present differentiation. Arkwright made a collection of meningococci isolated from cases and tested their agglutinative characters. By this means he obtained evidence of the presence of several different kinds of them, and he noticed that diversity was more marked in case of meningococci from sporadic than in those from epidemic cases. The *Comptes Rendus* from 1909—1914 contain a set of papers by Dopter that are of special interest, because they show the stages by which, from an entirely independent standpoint, this distinguished Medical Officer of the French Army arrived at a conclusion very similar to that reached by us as the result of a systematic serological analysis by the agglutination + absorption test of meningococci from military cases during the present outbreak. In the first place his researches on the gram-negative cocci from the nasopharynx of soldiers led to the identification of a group of cocci

indistinguishable from the meningococcus in morphological, cultural, and fermentative respects, but distinct in agglutinative characters and in the absorption test. To this group he gave the name parameningococcus. On proceeding to examine meningococci from the cerebro-spinal fluid of cases of cerebro-spinal fever in the same way, he found that serologically two different kinds could be distinguished. The first he regarded as the meningococcus, the other he called parameningococcus. The investigation of meningococci from the cerebro-spinal fluid of further cases led to the definition in the same way of still further parameningococci until by May, 1914, he had distinguished no less than three parameningococci all serologically distinct from the meningococcus and from one another.

M. Nicholle of the Department of Serotherapy of the Pasteur Institute has received specimens of our four types of meningococcus, and regarded Types 1 and 3 as belonging to the meningococcus group, and 2 and 4 to the group of parameningococcus. I understand that he has not applied the absorption test: without this of course the action of co-agglutinins cannot be excluded.

In a volume recently issued by the Medical Department of the Local Government Board, certain criticisms are made of the procedure with reference to identification of meningococcus summarised in the present paper, and an attempt is made to throw doubt upon the value of the absorption test. The work thus criticised, however, is its own witness; and the demonstrated success of the quadrivalent agglutination test in practice during the last two years for identifying the meningococcus both in cases and in carriers among the forces is sufficient to dispel these doubts based to a large extent upon theoretical considerations, which, as Captain Tulloch shows, are extremely insecure from the scientific point of view.

Definition of the relationship between pharyngococci and meningococci is a matter of research rather than of philosophy: research moreover of a distinctly arduous character. The final criterion so far has been the capacity of a given coccus to combine with a specific agglutinin *in vitro*. Now in its practical application to gram-negative cocci the absorption test upon which the final decision rests is work demanding a very high degree of dexterity that can only be acquired by continuous and persevering effort. The test is a process of balancing, dependent upon a series of very accurate measurements and quantitative adjustments; and even minute errors may mar or upset the result. In our experience at the Central Laboratory it requires at least

six weeks hard work before even a trained bacteriologist with considerable serological experience can sufficiently master the technique to obtain consistently satisfactory results in absorption tests of these delicate micro organisms. Similarly when he goes for a holiday—even for a week—it requires at least another week's work before the necessary unconscious manipulative dexterity returns. After that degree of skill is reached, irregular results are far less frequent than before. Granted that this factor of technique is equal in both sets of workers, the following sources of fallacy merit attention.

(1) When we were working out the meningococci of the present outbreak by the absorption test, the urgency of reaching a decision in the shortest possible time saved us from a pitfall into which the Local Government Board workers may have fallen. In addition to the quality of the antigen, a factor of first-rate importance in the absorption of agglutinin test is the quality of the agglutinin used. Now it was observed at an early stage of the investigation that our sharpest cut results with the absorption test were obtained with the "First-Born" agglutinin: that is to say the agglutinin that appears first in the rabbit's blood in response to injection. While strictly specific, this agglutinin is in our experience more ready to unite with the antigen employed than older agglutinin. The agglutinating serum used by us throughout came from young rabbits (1000 grams) which had all received intensive treatment. Most of them had not been under preparation for more than ten days before their blood was collected. The serum from rabbits immunised over several months is not in our experience the best for absorption tests.

A concrete instance may be given to illustrate the importance of the quality of the agglutinin in this test. During 1915 a univalent agglutinating serum prepared against Type 1—which as a rule is the easiest to work with—had been employed on a number of occasions for absorption tests, and was promptly returned to the cold store when not in use. After several months, however, the agglutinin in this serum—while still agglutinating its type cocci practically as well as before—was found to have lost much of its power of combining with the specific antigen, and the serum had to be replaced by another one freshly prepared. This gave the same sharp-cut results that we had been accustomed to.

In this relation it may be mentioned that in our experience horses' serum is not suitable for absorption tests with gram-negative cocci. For some reason, the agglutinin in the serum of horses appears to be

less ready to combine with these organisms *in vitro* than the agglutinin made by the young rabbit. It should be added, however, that our experience in this matter has been limited to specimens of antimeningococcus serum supplied for therapeutic use, and that these specimens of horse serum were of some standing.

(2) Another conceivable reason why the results of these workers are not in harmony with our own is the possibility that the difficulties of Type 2 (perhaps the most widely diffused type of all) which Captain Tulloch succeeded in overcoming by an arduous research did not yield to their attack.

(3) A third possible reason—and, in view of Arkwright's observations on the matter, this is the most probable explanation of all, is that serological diversity amongst meningococci from cases in children of the civil population—from whom much of their case-material appears to have been derived—is greater than in that of meningococci obtained from soldiers during an epidemic. My present experience of them, though small, has certainly led me to suspect that meningococci from sporadic cases in children are serologically less uniform, and therefore more difficult to define, than those obtained from military cases during an outbreak of cerebro-spinal fever.

That the difficulties, however, of a complete classification of meningococci are by no means insuperable, is indicated by the following facts.

(1) In a letter received from him in July last Colonel C. J. Martin wrote, "I think I told you that when I was at home last winter we tested out upwards of 100 strains which had been kept going at the Lister, and found that all but one would fall comfortably into one or other of your four groups."

(2) Captain Pullon, Government Bacteriologist at Capetown, investigated a series of meningococci obtained from cases of cerebro-spinal fever in South Africa, and having differentiated them with agglutinating sera prepared by himself, forwarded suspensions of these cocci to the Central Laboratory, for examination. The suspensions arrived a few weeks ago and were tested against the four univalent sera in the routine manner with the result that of sixteen meningococci from cerebro-spinal fluid, nine were specimens of Type 1, five were specimens of Type 2, and two were specimens of Type 3. No example of Type 4 was found. On reference to the enclosure in Captain Pullon's letter giving his own results, it was seen that the classification of these cerebro-spinal fluid strains effected by our sera was identical with that made by him; the only difference being that his group A was our Type 3,

his Group B, Type 2, and his Group C, Type 1. There is good reason to believe, therefore, that a limit obtains with regard to the diversity of meningococci, and that at any rate the most important pathogenic members of the group have now been defined.

(3) Since the above was written, Staff-Surgeon P. Fildes and Surgeon S. L. Baker, R.N., have published in *The Lancet* of January 19th, 1918, a paper on "The Grouping of Meningococci into Types." Full particulars are given of the reactions shown by meningococci isolated from 46 cases of cerebro-spinal fever at Haslar to the agglutination and absorption test with four univalent sera prepared independently by themselves. The conclusion reached is as follows. "Gordon's statement that practically all meningococci capable of producing cerebro-spinal fever are found to belong to one of his four groups is confirmed by our results. It therefore follows that an unknown coccus from the throat or elsewhere which does not belong to one of these four groups is not likely to be a pathogenic meningococcus. This rule may be taken to be invariable for practical purposes."