# A STUDY OF THE SEROLOGICAL REACTIONS OF MENINGOCOCCI AND AN ACCOUNT OF THE METHOD OF PREPARATION OF ANTI-MENINGOCOCCUS SERUM.

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(*From the Field Laboratories, University of Cambridge.*)

(With 2 Charts.)

## CONTENTS.

## INTRODUCTION.

THE work on the meningococcus described in this report was begun in 1916 in connection with the measures instituted by Lieut.-Colonel M. H. Gordon, C.M.G., for the preventive control of cerebro-spinal fever in the Army.

These measures required large quantities of monovalent agglutinating sera for the identification of the four types of meningococci defined by Colonel Gordon. The preparation of the sera from rabbits was carried out at the Central C.-S. F. Laboratory and on account of the great demand entailed much labour. With a view to helping to meet this demand it was arranged by the Medical Research Committee, at Colonel Gordon's request, that I should prepare agglutinating sera from horses against the four types of meningococci. Unfortunately the horse sera did not prove sufficiently specific for differential purposes and the work was discontinued. Later on, the immunisation of horses was resumed with the object of testing different methods of preparing therapeutic sera.

While the above work on horses was in progress I took the opportunity to study the serological reactions of the various strains of meningococci

[1] Report to the Medical Research Committee.

which had come into my possession, and to compare the relative values of agglutinating sera from different species of animals (horse, goat and rabbit) for the differentiation of meningococci. The results of these serological tests are detailed in Part I of this report.

Part II of my report deals with the methods of preparation of anti-meningococcus serum for therapeutic purposes.

The work was done at the Field Laboratories, University of Cambridge.

## PART I.

### SEROLOGICAL REACTIONS OF MENINGOCOCCI.

*Source of strains.* The strains of meningococci used in this investigation were obtained from three different sources and each group of strains is considered in a separate section.

Colonel Gordon supplied me with 44 strains of meningococci, derived from military cases of cerebro-spinal fever, which had previously been identified either by the agglutination test alone, or by the agglutination and absorption tests with one or other of his four types.

The second series was sent to me by Dr F. Griffith from the Local Government Board's laboratory and comprised 31 strains. These were to some extent selected, many being strains which had been found to have a limited capacity for absorbing agglutinins. All were derived from the cerebro-spinal fluid of cases of meningitis which had occurred during the epidemic prevalence of cerebro-spinal fever.

The third series of 18 unselected strains from adult cases of cerebro-spinal fever were obtained from Mr H. W. C. Vines and Dr W. M. Scott.

*Preparation of Agglutinating Sera.* All the sera used in the investigation of the serological characters of the different groups of strains were prepared (with the exception of one from a goat) from rabbits with representative strains from each of Gordon's four types. The rabbits were immunised by the intravenous injection of cultures grown on glucose agar. The inoculations were made with stock suspensions and only occasionally were living cultures used. The initial dose was 5 or 10 mgrm., and this was gradually increased, at five to ten days' intervals, to a maximum of 80 mgrm. The majority of the rabbits were found to give a good agglutinating serum after from three to five weeks, when the dose had reached 40 or 50 mgrm. When the titre of a serum was satisfactory the rabbit was bled and the serum preserved with 0.5 per cent. carbolic acid.

The methods of immunising horses and goats are described in the sections dealing with the results of serological tests with the sera of these animals.

*Technique of agglutination and absorption tests.* Cultures for these tests were grown on glucose agar at 37° C. for 24 hours. The growth was scraped off, put into a previously tared sterile test tube and weighed. Sufficient 0.5 per cent. carbolic acid in 0.85 per cent. salt solution was added to form a suspension, 1 c.c. of which contained 20 mgrm. of culture. The tubes were then

heated in a water bath at 65° C. for half-an-hour. This constituted the stock suspension and was used in that strength for absorption tests. For agglutination tests the stock suspension was diluted so that 1 c.c. contained 2 mgrm. of culture. The agglutination tests were made in small tubes measuring $3'' \times \frac{1}{2}''$. Into each of these 0·5 c.c. of the serum dilution and 0·5 c.c. of the suspension of cocci were pipetted and the tubes, after being corked, were put into a 55° C. incubator for 24 hours; at the end of 24 hours readings were taken.

The greatest concentration of serum in a mixture was 1 in 100. The end point chosen was the highest dilution at which the cocci were completely clumped and sedimented, the supernatant fluid being quite clear or showing only the faintest trace of cloudiness. Each figure in the tables represents this end point. When there was well-marked clumping at 1 in 100, but the fluid remained cloudy, this result is represented by the + sign. The symbol (+) represents a slighter degree of agglutination. When there was no clumping, or only a trace at 1 in 100, the − sign is used.

For absorption tests equal quantities of the stock suspension of the coccus (20 mgrm. per c.c.) and the diluted serum were incubated at 55° C. over-night in centrifuge tubes. Usually the serum was diluted 1 in 25, but when the titre of the serum was very high a 1 in 50 dilution of the serum was employed. Thus, each cubic centimetre of a mixture contained 10 mgrm. of cocci in a 1 in 50, or a 1 in 100 dilution of the serum. After centrifuging, the supernatant fluid was used for agglutination tests on the homologous coccus, and each absorbed serum was also tested on the absorbing coccus.

## SECTION 1.

### Colonel Gordon's strains.

This series comprises 44 strains which had been classified as follows: 18 Type I, 17 Type II, 4 Type III and 5 Type IV. One of the strains in the Type I sub-group was found by me on investigation by absorption tests to be not a Type I but a Type III coccus. Gordon (1917) has stated that some of his early strains provisionally classified as Type I would probably be found on application of the absorption test to be Type III strains, and this statement has been confirmed by Tulloch (1917). As this was no doubt one of those strains it has been transferred to the Type III sub-group. The amended classification of the strains is therefore as follows: 17 Type I, 17 Type II, 5 Type III and 5 Type IV.

The object of the investigation was to ascertain whether Gordon's classification of the strains would hold good with sera prepared from some of them in a different laboratory.

Monovalent agglutinating sera were prepared by me from rabbits with strains from each of the four types of cocci, and altogether 15 of the 44 strains were used for this purpose.

The results of agglutination and absorption tests with the sera from each type of coccus will now be considered in detail. The agglutination results are set out in Tables I (a) and I (b).

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Table I (a). *Simple agglutination tests upon Gordon's strains of meningococci with sera prepared from members of each of the four types.*

| Designation of strains | Type I sera | | | Type III sera | | | | Type II sera | | | | Type IV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | "Cou" serum titre 1–800 | "All" serum titre 1–1600 | "Litt" serum titre 1–800 | "Bunt" serum titre 1–3200 | "Bunt" serum titre 1–800 | "Mac" serum titre 1–1600 | "Mer" serum titre 1–1600 | "D" serum titre 1–1600 | "Off" serum titre 1–3200 | "Fos" serum titre 1–3200 | "Harr" serum titre 1–100 | "Hic" serum titre 1–400 |
| **Type I—** | | | | | | | | | | | | |
| Cou | 800 | 3200 | 800 | 200 | 400 | 100 | 200 | 200 | 800 | 800 | 400 | 400 |
| All | 200 | 1600 | 800 | ... | 800 | 200 | 100 | 200 | 400 | 400 | 400 | 100 |
| Litt | 200 | 800 | 800 | 400 | 1600 | 800 | 800 | 200 | 1600 | 400 | 400 | 800 |
| Win | 800 | 1600 | 800 | 200 | 200 | 100 | 200 | 200 | 400 | 100 | 100 | 100 |
| Sher | 800 | 3200 | 800 | 200 | 400 | 200 | 200 | 200 | 800 | 400 | 200 | 400 |
| Kn | 800 | 3200 | 800 | 200 | 200 | 400 | 200 | 200 | 400 | 200 | 200 | 100 |
| Lis | 800 | 1600 | 800 | 400 | ... | 200 | ... | ... | ... | ... | ... | ... |
| Mit | 800 | 1600 | 800 | 200 | + | 200 | 100 | 100 | 200 | 400 | 100 | 200 |
| How | 200 | 1600 | 800 | + | + | 200 | (+) | 100 | 200 | 100 | (+) | (+) |
| Bal | 400 | 1600 | 1600 | ... | 800 | 200 | 200 | 200 | 800 | 200 | 100 | 200 |
| Fox | 400 | 1600 | 800 | 200 | 800 | 200 | 200 | 200 | 400 | 200 | 200 | 200 |
| Sco | ... | 1600 | 400 | 100 | 400 | 200 | 100 | 100 | 200 | 200 | 100 | 100 |
| Char | 400 | 3200 | 800 | 200 | 800 | 400 | 200 | 400 | 400 | 400 | 200 | 200 |
| Far | 200 | 1600 | 800 | 800 | 400 | 800 | 800 | 400 | 800 | 400 | 400 | 200 |
| Mif | 400 | 1600 | 800 | − | 200 | 100 | 200 | 100 | (+) | 100 | 100 | (+) |
| Pay | ... | 3200 | 800 | ... | 800 | 200 | 400 | 100 | ... | 400 | ... | ... |
| Jor | 1600 | 3200 | 1600 | 400 | 800 | 200 | 100 | ... | ... | ... | ... | ... |
| **Type III—** | | | | | | | | | | | | |
| Mac | 200* | 200 | 200 | 1600 | 1600 | 1600 | 1600 | 100 | 1600 | 200 | 400 | 100 |
| Bunt | + | 200 | 400 | 3200 | 800 | 1600 | ... | 100 | 800 | 200 | 400 | 200 |
| Cha | ... | 100 | 100 | ... | 1600 | 800 | 800 | 200 | 800 | 400 | 400 | 400 |
| Mer | ... | 200 | 100 | 1600 | 1600 | 1600 | 1600 | 200 | 800 | 200 | 800 | 200 |
| Jac | − | (+) | 100 | ... | 800 | 800 | 1600 | 100 | ... | 100 | ... | ... |
| **Type II—** | | | | | | | | | | | | |
| McP | − | 100 | 200 | 100 | 400 | 400 | 200 | 200 | 800 | 800 | 400 | 400 |
| Wat | − | (+) | 100 | (+) | 200 | − | − | 1600 | 800 | 3200 | 800 | 400 |
| Mi | (+) | 200 | 200 | 400 | 1600 | 400 | 800 | 1600 | 3200 | 3200 | 800 | 200 |
| Wy | − | 100 | 200 | 400 | 800 | 400 | 400 | 800 | 1600 | 3200 | 1600 | 200 |
| Glid | − | (+) | 400* | (+) | 400 | (+) | 100 | 1600 | 3200 | 3200 | 800 | 400 |
| Mat | ... | (+) | + | − | (+) | − | (+) | 800 | 800 | 800 | 200 | (+) |
| Off | − | 200 | (+) | ... | 800 | ... | 100 | 800 | 3200 | 6400 | 800 | 400 |
| Cu | − | + | 100 | 100 | 800 | ... | 400 | 1600 | 1600 | 3200 | 800 | 400 |
| New | − | 200 | 200 | ... | 800 | ... | 100 | 800 | 3200 | 3200 | 1600 | 200 |
| Mor | ... | (+) | 100 | (+) | + | ... | (+) | 1600 | 800 | 1600 | 400 | 400 |
| Harr | − | (+) | (+) | ... | + | ... | + | 400 | 800 | 800 | 100 | 400 |
| Fos | − | 200 | − | ... | 400 | ... | + | 800 | 800 | 3200 | 800 | 400 |
| Wal | ... | + | (+) | ... | 400 | 800 | 200 | 1600 | 1600 | 6400 | 800 | 100 |
| Wet | ... | + | (+) | ... | 400 | ... | 100 | 1600 | 800 | 6400 | 400 | 200 |
| Ber | ... | + | (+) | + | 400 | ... | 100 | 1600 | 1600 | 1600 | 800 | 200 |
| Lag | ... | (+) | + | ... | 100 | + | + | 400 | 400 | 1600 | 400 | 200 |
| Lin | ... | (+) | (+) | + | 200 | 200 | 400 | 1600 | 400 | 800 | 200 | 100 |
| **Type IV—** | | | | | | | | | | | | |
| Hic | − | (+) | 100 | − | (+) | − | − | 100 | (+) | 400 | 100 | 400 |
| My | − | 100 | 100 | 200 | 800 | 200 | 400 | 200 | 800 | 800 | 800 | 1600 |
| Bow | − | + | 200 | 200 | 800 | 400 | 400 | 200 | 800 | 400 | 800 | 1600 |
| Gar | − | 200 | 100 | − | + | − | − | − | 400 | 200 | + | 400 |
| P 5 | − | (+) | (+) | ... | 400 | ... | 400 | 100 | 800 | 200 | 400 | 1600 |

+ = well-marked clumping at 1–100 but fluid still cloudy.
(+) = a slighter degree of agglutination.
− = no agglutination, or only a trace.
... = not tested.

\* Denotes that agglutination was not quite complete at that dilution and the lower dilutions.

Table I (b).  *Agglutination tests on 19 Series I strains with sera prepared from Type II and Type IV strains.*

| | Type II sera | | Type IV sera | | | | |
|---|---|---|---|---|---|---|---|
| Designation of strains | "Wat" titre 1–800 | "Glid" titre 1–800 | "Hic" titre 1–1600 | "Bow" titre 1–1600 | "Gar" titre 1–800 | "My" titre 1–800 | "My" titre 1–800 |
| Type I— | | | | | | | |
| Litt | 100 | 100 | + | 200 | 200 | 400 | 200 |
| Cou | − | + | + | 100 | 100 | 400 | − |
| Win | − | − | − | + | 100 | 200 | − |
| Kn | − | (+) | (+) | + | 100 | 200 | − |
| Sher | − | + | + | 100 | 200 | 400 | + |
| Lis | − | + | + | 100 | 100 | 200 | − |
| Mit | − | + | + | 100 | 200 | 400 | (+) |
| Jor | − | − | − | + | + | (+) | − |
| Type III— | | | | | | | |
| Bunt | − | − | + | 100 | + | 800 | 100 |
| Mac | − | (+) | (+) | 100 | (+) | 800 | + |
| Type II— | | | | | | | |
| McP | 100 | 200 | 100 | 400 | 100 | 800 | 100 |
| Wat | 800 | 400 | 100 | 100 | 100 | 400 | − |
| Mi | 800 | 800 | 200 | 400 | 200 | 800 | 400 |
| Wy | 800 | 800 | 100 | 400 | + | 800 | 200 |
| Glid | 800 | 800 | 400 | 400 | 200 | 800 | 100 |
| Type IV— | | | | | | | |
| Hic | 200 | + | 1600 | 800 | 400 | 400 | 800 |
| Bow | 400 | 400 | 1600 | 1600 | 400 | 1600 | 800 |
| Gar | − | − | 800 | 800 | 800 | 400 | 800 |
| My | 400 | 200 | 1600 | 800 | 400 | 800 | 800 |

**Type I sera.** Monovalent agglutinating sera were prepared from three of the 17 Type I strains, namely: "Cou," "All" and "Litt."

*Agglutination tests.* "Cou" serum (titre 1–800), the first prepared, was tested against the limited number of cocci then available and was found to agglutinate Type I strains only. The second serum was a high titre serum (1–1600) and agglutinated all Type I cocci (with one exception) in dilutions up to and beyond the titre of the serum for the homologous coccus. Most of the Type III and a few of the Type II and Type IV strains were also agglutinated, but only in dilutions of 1–100 and 1–200. The third serum agglutinated all Type I cocci (with one exception) up to the full titre of the serum for the homologous coccus, but this serum had a wider range of action on cocci of other types, particularly Type III, than the first two sera.

*Absorption tests.* Each of the sera was absorbed by some or all of the Type I strains and representative strains of other types. The homologous agglutinin was readily absorbed from each of two sera ("Cou" and "All") by all the Type I cocci and was not diminished, or only to a trifling extent, by cocci of the other types.

On the other hand the homologous agglutinin of the third serum was not

readily absorbed by Type I cocci. While the coccus producing the serum removed the whole of the agglutinin, other Type I strains were able to remove it only partially, *i.e.*, reduced the agglutinative power of the serum for the homologous coccus from 1–800 to at most 1–200, and after a second application of the cocci there was very little further reduction. Type III strains removed homologous agglutinin from this serum slightly, while a strain from each of the other types (II and IV) did not remove any; after a second absorption however there was slight reduction with a Type IV strain.

SUMMARY. With two of the three Type I sera the results were clear and definite. All the 17 Type I strains were agglutinated in higher dilutions of the sera than strains of other types, and all absorbed the specific agglutinin, which was not absorbed by other type strains.

The results with the third serum ("Litt") were less well defined and showed that the coccus producing the serum was not antigenically identical with the other two strains used to prepare sera.

**Type II sera.** Altogether six monovalent sera were prepared from Type II cocci.

*Agglutination tests.* The first two sera, made in the early part of the work with "Wat" and "Glid" strains, were tested against rather less than half the strains. The results with these sera are given in Table I (*b*). The two sera agglutinated the Type II strains at 1–400 and 1–800, except "McP" which was completely agglutinated only in 1–100 and 1–200 dilutions of the sera. Three Type IV cocci were agglutinated at 1–200 and 1–400, and one Type I coccus was agglutinated by both sera at 1–100. These sera, therefore, did not differentiate Type IV strains from Type II strains.

Subsequently monovalent sera were prepared from three more of Gordon's Type II strains and from one Type II strain[1] isolated in the early part of 1919 by Captain E. H. Shaw.

With these four sera there was a considerable amount of cross-agglutination, and no line of demarcation appeared between Type II and the other three types. Three of the sera each had a high titre for the homologous coccus, while the fourth failed to agglutinate the homologous coccus completely in dilutions higher than 1–100, though agglutinating other Type II strains well.

The Type II cocci exhibited a wide range of variation in the extent to which were agglutinated by these four sera, and many strains of the other types were agglutinated in as high dilutions of the sera as· Type II strains.

The most satisfactory serum for the grouping of the cocci by simple agglutination tests was that prepared from the "D" strain. On reference to Table I (*a*) it will be seen that this serum differentiated all excepting three

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[1] This strain ("D") was subsequently identified at the Central Cerebro-spinal Fever Laboratory as a typical Type II strain; the results with the serum prepared from it are therefore included in Table I (*a*).

of the Type II cocci from other types by agglutinating them at the full or half titre of the serum.

*Absorption tests.* Absorption tests were carried out with five of the rabbit sera. Three of the sera ("Wat," "Glid" and "Off") were each absorbed with a small number of Type II strains, and only the coccus used to produce the serum was able to remove the homologous agglutinin completely. One strain ("McP") removed the homologous agglutinin from one serum ("Off") partially but failed to remove any from the other two sera. Other Type II cocci reduced the titre of each serum for the homologous coccus only slightly (to half titre at most). After a second addition of the cocci to two of the sera ("Wat" and "Glid") there was little or no further reduction with one serum ("Wat"), but with the other ("Glid") there was partial absorption in some cases. Strains of other types failed to absorb more than a trace of the homologous agglutinin.

With sera "Fos" and "D" a larger number of absorption tests were done. From one or other or both of these sera all the Type II cocci, with the exception of "McP" which absorbed only a trace from one serum, absorbed some of the agglutinin for the strain producing the serum, whereas representative strains from the other types did not remove any of it.

Even with these two sera all Type II strains did not exhibit an equal capacity for combining with the homologous agglutinin. While some strains absorbed as much as the coccus producing the serum, others were able to remove only half or quarter that amount; for example, the titre of the serum "D" was reduced from 1–1600 to 1–100 by the "D" coccus and some other strains, whereas other Type II strains reduced it only to 1–200 or 1–400.

Absorption tests were also carried out with a Type II goat serum. From this serum all but one (*i.e.* "McP") of the Type II strains tested absorbed the homologous agglutinin completely, strains from other types not removing any.

The strain "McP" mentioned above as having feeble capacity for absorbing Type II agglutinin from rabbit sera was also found to absorb only small amounts of agglutinin from this goat serum. Moreover, when a serum (goat) was prepared from this strain, it was found that Type II strains did not absorb its agglutinin. This strain, therefore, which perhaps at one time absorbed Type II agglutinin well, since it was one of the 32 cocci first investigated by Gordon, had acquired during artificial cultivation a high degree of individuality.

Summary. Agglutination tests with monovalent sera prepared from six Type II strains did not clearly differentiate Type II from the other types. Not only was there pronounced cross-agglutination with these sera but also wide variation in the extent to which Type II strains were agglutinated by them.

Absorption tests on the other hand with two rabbit sera showed that Type II strains formed a distinct group, the members of which, however, differed among themselves in absorptive capacity. With a goat serum differentiation by absorption was clearer than with the two rabbit sera.

**Type III.** Agglutinating sera were prepared from three of the five Type III strains, namely "Mac," "Bunt" and "Mer": "Bunt" was Gordon's type strain[1] of the Type III sub-group; "Mac" was the strain which had been wrongly classified in the Type I sub-group.

*Simple agglutination tests.* "Bunt" serum agglutinated all the Type III strains tested in high dilutions of the serum; it also agglutinated the majority of the Type I strains and a few Type II and Type IV strains, but not so strongly as the Type III strains.

"Mac" serum agglutinated all the Type III strains at the full or half titre of the serum; it also agglutinated all Type I strains and several Type II and IV strains, but except in two instances at less than half the titre of the serum. The third serum ("Mer") gave very similar results.

*Absorption tests.* Each of the three sera was absorbed with all the Type III strains and the majority of the other strains. All the five Type III strains absorbed completely or nearly completely the homologous agglutinin from "Bunt" serum, while strains of other types absorbed little or none.

The same five strains which had exhausted "Bunt" serum also removed the homologous agglutinin completely or nearly completely from the other Type III sera, but a good many strains of the other types were found capable of removing agglutinin from these two sera.

From "Mac" serum four Type II, one Type I and two Type IV strains effected a partial removal of the agglutinin. The experiment with two of the Type II strains ("Mi" and "Wy") was repeated several times and on each occasion there was the same partial removal.

From "Mer" serum the two Type II strains ("Mi" and "Wy") and one Type IV strain ("My") also removed the homologous agglutinin partially, while two Type I strains and one Type II strain absorbed it slightly.

SUMMARY. With one Type III serum there was clear differentiation of Type III strains from other types both by agglutination and absorption tests.

With the other two sera there was not the same clear differentiation by agglutination and, although the Type III strains absorbed the homologous agglutinin from these sera as completely as from the first serum, strains of other types, particularly Type II, were found capable of removing appreciable amounts of their agglutinins. Thus agglutination and absorption tests with these sera showed that serological relationship exists between Type III and the other types.

**Type IV.** Four of the five Type IV strains were used to prepare agglutinating sera. The results of the agglutination tests are given in Table I (*b*). The results with the two Type II sera included in this Table have already been referred to.

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[1] This strain when it had been three years in artificial cultivation was used again to produce a serum; this serum acted upon the great majority of the cocci tested, irrespective of type (see Table I (*a*)).

*Agglutination tests.* Each of the four strains produced a serum which agglutinated the Type IV strains to a higher degree than strains of any of the other types. There was some variation in the agglutinative power of the different sera on strains of Types I, II and III, two of the sera selecting mainly Type II strains, the other two agglutinating Type I and Type II strains indifferently. It will be noted also in Table I (*b*) that a particular strain was not agglutinated with equal readiness by the different sera.

The serum from one rabbit ("My") was tested at intervals during the course of immunisation. In Table I (*b*) the results with the first and last samples only, taken seven days after the third (30 mgrm.) and eighth (80 mgrm.) doses respectively, are given. The results with the different samples varied. The first sample had a wide range of action and agglutinated the majority of the strains of other types as well as, or even better than, the Type IV cocci. In subsequent samples the agglutinin for these other types diminished, and the final sample acted upon only a small number of strains, the titre of the serum for these being much lower than it had been previously.

One Type IV strain ("Hic") was used a second time to produce an agglutinating serum after it had been about four years in cultivation. The serum which was withdrawn three weeks after immunisation began had a wider range of action than the first serum produced with this coccus, the results, given in Table I (*a*), resembling closely those obtained with the first sample from the "My" rabbit.

*Absorption tests.* Each of the four sera was absorbed with the four Type IV strains as well as with representative strains of other types.

From "Hic" serum all the Type IV strains absorbed the homologous agglutinin completely or nearly completely.

Three of the four strains absorbed completely or nearly completely the agglutinins from two of the other sera, while the fourth ("Gar") removed it partially in both instances.

From the serum produced by this partial absorber ("Gar") none of the other Type IV strains was able to remove more than a slight amount of homologous agglutinin, and there was no further reduction even when a whole agar culture was used. Thirty-four other strains (Types I, II and III) were tested against this "Gar" serum with the result that some were found to remove slight amounts of the agglutinin while others failed to remove any or more than a trace. Similar results were obtained with thirty strains of Types I, II and III in absorption tests with the other Type IV sera.

The fifth Type IV strain (P 5) was tested with one serum only, *i.e.*, the second produced from "Hic," and was agglutinated by it in high dilution (see Table I *a*). This coccus also absorbed the homologous agglutinin of "Hic" serum.

SUMMARY. The above agglutination and absorption tests show that the Type IV strains form a group distinct from the other three types. All the strains absorbed the homologous agglutinin from a serum produced by one of them, and this agglutinin was not absorbed by strains of the other types. The members composing the group are, however, not completely identical, differing among themselves in agglutinogenic capacities.

SUMMARY OF RESULTS WITH THE FIRST SERIES OF MENINGOCOCCI.

The serological reactions of forty-four strains of cerebro-spinal meningo-cocci have been studied. These strains had been classified into four types at the Central Cerebro-spinal Fever Laboratory. The object of the investigation was to ascertain whether or not the classification would hold good if mono-valent sera prepared in a different laboratory from members of each type were used.

*Simple agglutination tests.* Three Type I sera were prepared and two of these clearly differentiated the Type I strains from strains of other types. The third Type I serum agglutinated all the Type I cocci to full or half titre but this serum had more influence on strains of other types than the first two sera; *e.g.*, one Type III strain was agglutinated at half titre.

The sera prepared from Types II, III and IV were less specific and there was considerable cross-agglutination. One at least, however, out of each set differentiated fairly well; that is to say, the strains (or the majority of them in the case of Type II) of the same type as the homologous coccus were agglutinated in higher dilutions of the serum than strains of other types.

Type II strains showed less uniformity in agglutination with Type II sera than did Types III and IV strains in the presence of their respective type sera, several Type II strains agglutinating less well with the Type II sera than some strains of the other types.

*Absorption tests.* All the seventeen Type I strains absorbed the specific agglutinin completely from two Type I sera; strains of other types removed little or none of the agglutinin. From the third Type I serum Type I strains were able to remove the homologous agglutinin only partially and there was in addition slight absorption with Type III strains.

The five Type III strains absorbed the homologous agglutinin completely from three Type III sera. From one of these sera strains of other types absorbed little or none of the agglutinin, but with the two others there was definite cross-absorption, particularly with Type II strains.

All the Type II strains with one exception absorbed homologous agglutinin from two Type II rabbit sera. The amount absorbed however varied widely with the different strains, some absorbing as much as the coccus producing the serum, others only half or quarter that amount. From other Type II sera the coccus producing the serum was the only one found capable of removing the homologous agglutinin completely. Strains of Types I, III and IV did not absorb, except occasionally and in very small amount, homologous agglutinin from any of the Type II sera.

All the Type IV strains removed the homologous agglutinin completely or nearly completely from one Type IV serum while representative strains of other types failed to absorb any. One of the Type IV strains absorbed the homologous agglutinin only partially from two other Type IV sera and this

coccus produced a serum from which other Type IV strains were able to absorb only slight amounts of the homologous agglutinin.

With certain sera, therefore, prepared from members of each of the four types, a division of the meningococci into four distinct groups was effected by means of the absorption of agglutinin test. These groups correspond exactly to Gordon's four types.

With other sera from each type the differentiation into similar groups could not be made or was not so well defined, thus demonstrating that all the strains included in each type are not absolutely identical.

The following conclusions are to be drawn from my tests upon this series of meningococci:

1. Gordon's classification of the strains holds good if care is taken in the selection of strains for the production of type sera.

2. All the strains included in each type are not identical in their serological characters.

3. There is inter-relationship between the types, as shown by the results of cross-agglutination and absorption experiments.

## SECTION 2.
### Local Government Board strains.

This series contains thirty-one strains the serological characters of which have been reported on by F. Griffith in this *Journal* (1918).

Before proceeding to discuss my results with these strains it will be useful to summarise briefly the view expressed in the above report on the grouping of meningococci. F. Griffith has shown that the majority of spinal meningococci can be divided into two main groups by simple agglutination tests if selected sera are used, a few being relatively inagglutinable or agglutinated equally by sera of both groups. By the application of absorption tests it was found that the strains in each of the two main groups differed among themselves in absorptive capacity and could be divided into sub-groups. This division depends upon variations in the structure of their antigens. In Group I three different antigenic components were demonstrated which were designated "A," "B," and "C," "A" being the most complex, "C" the least. Group II was much more diverse and at least four different antigens were defined.

As mentioned on page 34 the strains in this series were to some extent selected. For example, having ascertained that strains with "C" antigen had no identical representatives among Gordon's types, I obtained the majority of the members of this sub-group of Group I. Many of the Group II strains were chosen on account of their poor absorptive capacity. A few strains could not be allotted definitely to either of the two groups.

The purpose of my investigation was to ascertain whether these spinal meningococci could be classified by means of sera prepared from Gordon's four types.

The great majority of the thirty-one strains were tested against eight sera, two from each of the four types; ten strains which were received late in the investigation were tested against five sera only. In Table II the results

Table II. *Agglutination and absorption tests upon the Local Government Board strains of cerebro-spinal meningococci with sera prepared from Gordon's four types.*

| Designation of strains | Type I sera | | | Type III sera | | | | Type II sera | | | Type IV sera | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aggl. with "Litt" serum 1 800 | Aggl. with "Cou" serum 1-800 | Absorp. of "Cou" serum | Aggl. with "Bunt" serum 1-3200 | Absorp. of "Bunt" serum | Aggl. with "Mac" serum 1-1600 | Absorp. of "Mac" serum | Aggl. with "Wat" serum 1-800 | Aggl. with "Glid" serum 1-800 | Absorp. of "Glid" serum (Goat) | Aggl. with "Hic" serum 1-1600 | Aggl. with "Gar" serum 1-800 | Absorp. of "Hic" serum | Absorp. of M 15 serum |
| M 4 | 800 | 800 | N.C. | 200 | ... | 200 to 800 | ... | − | − | ... | − | 100 | ... | ... |
| M 11 | 200 | + | Nil | 200 | Nil | 200 | Nil | − | − | Nil | + | 200 | ... | C. |
| M 12 | 400 | 100 | Nil | 1600 | N.C. | 1600 | C. | − | − | ... | 100 | + | ... | ... |
| M 14 | 400 | + | Nil | 400 | Nil | 100 to 800 | Nil | + | − | Nil | + | 200 | ... | C. |
| M 15 | 400 | 100 | Nil | 200 | S. | 200 | S. | − | − | Nil | − | 200 | Nil | C. |
| M 16 | 400 | − | Nil | 100 | Nil | 100 | Nil | + | + | ... | 100 | 200 | Nil | ... |
| M 17 | 400 | + | Nil | 1600 | C. | 1600 | C. | − | − | ... | − | − | Nil | N.C. |
| M 36 | 400 | − | Nil | 200 | Nil | 200 | Nil | − | − | Nil | + | 200 | Nil | C. |
| M 40 | 400 | + | P. (twice) | 400 | P. | 400 | S. & P. | − | − | ... | − | − | ... | S. |
| M 41 | 400 | + | Nil | 200 | Nil | 200 | ... | − | − | ... | − | 200 | Nil | C. |
| M 43 | 400 | 200 | Nil | 1600 | C. | 1600 | C. | − | − | ... | + | − | ... | C. |
| M 46 | 200 | − | Nil | − | Nil | − | Nil | − | − | ... | − | − | Nil | ... |
| M 48 | 800 | 400 | C. | 200 | Nil | 200 | Nil | − | − | ... | − | 100 | ... | ... |
| M 67 | ... | 400 | C. | 400 | C. | 400 | S. | − | ... | Nil* | − | ... | ... | S. & P. |
| M 68 | ... | 400 | N.C. | 400 | Nil | 200 | Nil | − | ... | ... | − | ... | ... | ... |
| M 72 | 800 | 400 | N.C. | 200 | V.S. | 200 | Nil | − | ... | ... | − | ... | ... | ... |
| M 73 | 800 | 400 | ... | − | ... | 200 | ... | 800 | ... | N.C. | − | ... | ... | ... |
| M 77 | 100 | + | Nil | 400 | Nil (twice) | 100 | ... | 100 | ... | Nil | − | ... | Nil | V.S. |
| M 80 | ... | − | Nil | 400 | Nil | 200 | ... | − | ... | Nil* | − | ... | V.S. | P. |
| M 18 | + | ... | ... | − | ... | ... | ... | 400 | 200 | C. | ... | ... | ... | ... |
| M 19 | 100 | − | ... | 400 | ... | 800 | P. | 1600 | 800 | C. | 100 | + | Nil | ... |
| M 20 | 100 | − | ... | 200 | ... | 800 | S. | 800 | 1600 | C. | 100 | − | Nil | ... |
| M 24 | (+) | − | ... | − | ... | − | ... | 400 | 200 | C. | − | − | Nil | ... |
| M 31 | + | − | ... | − | ... | − | Nil | 100 | 200 | Nil | 100 | + | Nil | Nil |
| M 32 | − | − | ... | − | ... | − | ... | − | + | Nil | − | − | Nil | Nil |
| M 33 | + | − | ... | + | ... | 200 | ... | 200 | + | ... | 800 | 400 | N.C. | ... |
| M 35 | (+) | − | ... | − | ... | − | Nil | 400 | 200 | C. | − | − | Nil | ... |
| M 82 | ... | − | ... | − | ... | ... | ... | 400 | ... | N.C. | − | − | ... | ... |
| M 83 | ... | − | Nil | − | Nil | ... | ... | 800 | ... | S. | + | ... | Nil | Nil |
| M 86 | ... | − | Nil | − | Nil | ... | ... | + | ... | Nil | − | ... | Nil | Nil |
| M 89 | ... | − | Nil | − | Nil | ... | ... | − | ... | Nil | 200 | ... | N.C. | ... |

C. = absorption as complete as with the homologous coccus.  N.C. = nearly complete.
P. = partial.  S. or V.S. = slight, or very slight.  Nil = no absorption.
* "Wat" rabbit serum.

of these tests are given. The designation of the strains is the same as in F. Griffith's report, M 4 down to M 80 belonging to his Group I, the remainder to his Group II.

*Agglutination tests.* One Type I serum ("Litt"), which had been shown in the tests on Series I to have a wider range of action than the other Type I sera, agglutinated all the Group I strains tested; this serum had little or no

action on Group II strains. The second Type I serum had a more limited range of action, agglutinating only nine of the nineteen Group I strains and leaving Group II entirely unaffected.

The two Type III sera agglutinated all the Group I strains (with one exception), three at the full titre of each serum. Three strains from Group II were also agglutinated by these sera, one serum agglutinating two strains in quite high dilution.

The two Type II sera agglutinated nine of the twelve Group II strains and two of the Group I strains, one of the two latter at the full titre of the serum. Other Group I strains were not acted upon.

Of the two Type IV sera one agglutinated five strains in Group II, one, M 33, at half titre of the serum, and two in Group I. The other agglutinated only one strain (*i.e.* M 33) in Group II and eight in Group I. It should be noted that M 33 was agglutinated well by both sera.

There is therefore a rough grouping by simple agglutination tests, Types I and III sera acting mainly on Group I strains, Type II sera mainly on Group II strains. There is evidence also of sub-grouping, the Type III and Type IV sera respectively picking out certain strains and agglutinating them in higher dilutions of the serum than other strains.

*Absorption tests.* One Type I serum ("Cou") was absorbed with all the Group I strains (except M 73) and three Group II strains.

Five Group I strains removed the homologous agglutinin completely or nearly completely, one removed it partially, while none of the others was able to absorb any of it.

The other Type I strain ("Litt") was absorbed with a few strains. M 48 absorbed the agglutinin partially, while M 11, M 16 and M 46 removed it very slightly; after a second addition of these cocci there was little or no further reduction.

The two Type III sera were absorbed with the majority of the Group I strains and a few Group II strains. Three strains from Group I, *i.e.* the three which had been strongly agglutinated by the sera, absorbed completely the specific Type III agglutinin from both sera; one Group I strain (M 40), which had removed Type I agglutinin partially, removed the homologous agglutinin partially from both Type III sera; a fifth strain (M 67), which had also absorbed Type I agglutinin, removed the homologous agglutinin completely from one Type III serum and slightly from the other. Of the Group II strains, one absorbed the agglutinin from one serum ("Mac") partially, and another Group II strain absorbed the same agglutinin slightly. A parallel result was obtained in the first series, several Type II strains absorbing the homologous agglutinin partially from this serum.

The Type II serum used systematically for absorption tests in this series was from a goat; this was the only Type II serum then available from which Type II strains were able to absorb homologous agglutinin completely. From this serum five Group II strains removed the homologous agglutinin com-

pletely and one (M 83) removed it partially. Of eight Group I strains used to absorb this goat serum, one, M 73 (see below), removed the homologous agglutinin nearly completely, while the others failed to remove any.

A few absorption tests were also done with the Type II rabbit sera used for agglutination tests. "Wat" serum was absorbed with three Group II strains, namely M 18, M 20 and M 24; these removed a small amount only of homologous agglutinin, and there was little further reduction on a second application of the cocci. "Glid" serum was absorbed with five Group II strains; M 19 and M 20 removed the agglutinin partially, whereas with M 18, M 24 and M 35 the absorption was very slight, even after a second addition of the cocci. Very similar results were obtained in the first series with these sera and Type II cocci.

One Type IV serum was absorbed with all the Group II strains and all those in Group I which had not yet been identified with any of Gordon's types. Two of the strains in Group II (M 33 and M 89) removed the homologous agglutinin from the serum nearly as completely as the coccus producing the serum; none of the other strains removed any of it even after a second absorption in some cases. The result with M 33 was confirmed with two other Type IV sera ("Bow" and "My").

After the completion of the above tests I noted in F. Griffith's report that the Group I strain, M 73, which had absorbed nearly completely the agglutinin from the Type II goat serum, had failed to absorb the homologous agglutinin of M 18 (a Group II coccus), but had removed partially the agglutinin of M 15, a Group I strain. I therefore re-tested this strain on a Type I serum, two Type II sera and one Type III serum. The strain removed homologous agglutinin nearly completely from the Type I and one Type II serum, and partially from the other Type II serum. From the Type III serum there was only a trace of absorption. Here then is an example of a strain which is capable of removing both Type I and Type II agglutinins.

The results of the agglutinin absorption test on this series of strains against sera made from Gordon's four types of meningococci are summarised as follows.

Table III. *Classification of thirty-one spinal strains of meningococci by means of the absorption test with sera prepared from Gordon's four types.*

| Type I | Type III | Type II | Type IV | Indeterminate, absorbing 2 antigens | Unplaced | |
|--------|----------|---------|---------|-------------------------------------|----------|------|
| M 4    | M 12     | M 18    | M 33    | M 40 (Types I and III)              | M 11     | M 16 |
| M 48   | M 17     | M 19    | M 89    | M 67            ,,                  | M 14     | M 31 |
| M 68   | M 43     | M 20    |         | M 73 (Types I and II)               | M 15     | M 32 |
| M 72   |          | M 24    |         |                                     | M 36     | M 46 |
|        |          | M 35    |         |                                     | M 41     | M 77 |
|        |          | M 82    |         |                                     | M 80     | M 86 |
|        |          | M 83    |         |                                     |          |      |

The strains classified as Type I and Type III are Group I strains containing respectively F. Griffith's "A" and "B" agglutinin. Types II and IV correspond to sub-groups of Group II.

Table III shows that only sixteen of the thirty-one strains of cerebro-spinal meningococci were completely identified with one or other of Gordon's four types; three strains were indeterminate, two having absorbed agglutinin from Types I and III sera and one from Types I and II sera, and twelve were not identified with any type by the absorption test. By the agglutination test of the latter twelve strains, nine (all Group I strains) were evidently related to Types I or III, two to Type II, and one was not agglutinated by any of the sera used.

The results of additional tests with the unplaced strains will now be considered.

Of the twelve strains which did not absorb agglutinin from any serum made with Gordon's strains F. Griffith had shown that six, all from Group I, were able to remove homologous agglutinin completely from a serum made with one of the strains (M 15) and therefore formed a sub-group. This agglutinin he designated "C" agglutinin of Group I. A little of this serum was kindly supplied to me and I tested it against all the unclassified strains and a good many other strains which had already been typed, with the result that five of the twelve unplaced strains were found to remove the agglutinin completely from this serum and one (M 80) to remove it partially, the other six having no effect. Several Type I and some Type III strains (including M 17 and M 43 in this series) absorbed the agglutinin from M 15 serum, in the majority of cases completely or nearly completely.

Except as regards M 80 my results with M 15 serum are identical with those obtained by F. Griffith.

With the other unplaced strains my results and his are also in close agreement.

The Group I strains M 16 and M 46 did not remove agglutinin from any of the four type sera; F. Griffith found that each of these strains was able to remove agglutinin only from its own serum.

With regard to the unplaced strains of Group II: M 31 was not agglutinated very well with the two selected sera but with other Type II sera it was agglutinated in high dilution. The strain absorbed a trace of agglutinin from one Type II serum but none from any other type serum. F. Griffith records that this strain absorbed agglutinin partially from sera of Group II, which appears to include Gordon's Types II and IV.

M 32 agglutinated with none of the sera in the table but agglutinated at 1–400 with another Type II serum. This strain did not absorb agglutinin from any of the type sera. F. Griffith found that it absorbed partially one of his Group II sera.

*Tests with Local Government Board sera on Gordon's four types.*

Orientation tests were also carried out with two sera supplied to me by Dr F. Griffith, one prepared from the Group I strain M 43, the other from the Group II strain M 18.

M 43 serum agglutinated Type I and Type III strains without distinction to the full or nearly to the full titre of the serum; there was no or only feeble action upon Type II and Type IV strains. The homologous agglutinin of this

serum was absorbed only by Type III strains. M 43 strain from which the above serum was prepared was originally a complex strain from which F. Griffith separated two daughter strains, one containing "A," the other "B" antigen. The strain of M 43 used in these absorption tests evidently contained only "B" antigen.

M 18 serum agglutinated all Type II and two Type IV strains in dilutions of 1–400 to 1–1600; two other Type IV strains and the Type I and Type III strains were but feebly acted upon or not at all.

Two Type II strains ("Wat" and "Keay") and M 18 itself absorbed completely the homologous agglutinin of this serum and two (a Type II and a Type IV strain) absorbed it partially while other Type II strains removed none or only traces. It is interesting to mention here that, although the above mentioned three strains showed equal capacity for removing the agglutinins from M 18 serum, two of them (M 18 and "Keay") were unable to remove the agglutinin produced by the third, *i.e.*, "Wat."

## SUMMARY OF RESULTS WITH THE SECOND SERIES OF MENINGOCOCCI.

The general results of the investigation of this series of meningococci can be summarised under three heads.

(1) Sera prepared from Gordon's four types of meningococci do not suffice for the classification of all spinal meningococci. Out of thirty-one cerebro-spinal strains only sixteen could be completely identified with one or other of the four types; three were indeterminate and twelve were unplaced.

(2) F. Griffith's "A" and "B" agglutinins of his Group I are identical with Gordon's Type I and Type III respectively, and two of F. Griffith's four Group II agglutinins can be identified with Types II and IV.

(3) The twelve unplaced strains, not represented among Gordon's types, include a small group of strains related to Types I and III but not identical with either, and a residuum of highly individual strains.

As regards (1), the explanation why so many of the cerebro-spinal strains in this series could not be classified by absorption tests with Gordon's four type sera may be found in the fact that the source of material was not the same in the two series.

Gordon's strains were all obtained from military cases (*i.e.* adult cases) of cerebro-spinal fever, whereas the Local Government Board series was obtained from cases of meningitis occurring among the general population and included a large proportion from children.

## SECTION 3.

## Unselected strains.

This section deals with tests on eighteen strains, the serological characters of which had not been determined when they were sent to me. All the strains were derived from adult cases of cerebro-spinal fever.

I am indebted to Mr H. W. C. Vines for sixteen of the strains and to Dr W. M. Scott for two. These strains were investigated with the object of ascertaining what proportion could be identified with Gordon's four types.

Table IV gives the results of agglutination and absorption tests on these eighteen strains with six monovalent sera prepared from the four types, one each of Types I and IV and two each of Types II and III.

The results were clear and definite with fourteen strains which could be classified as follows:

| Type I | Type II | Type III |
|:---:|:---:|:---:|
| 1 | 9 | 4 |

Table IV. *Agglutination and absorption tests upon a series of unselected strains of cerebro-spinal meningococci.*

| | Type I serum ("Cou" 1 in 800) | | Type III sera ("Bunt" 1 in 3200) | | ("Mac" 1 in 1600) | | Type II sera ("Wat" 1 in 800) "Glid" (Goat) | | Type IV serum ("Hic" 1 in 1600) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Aggl. | Absorp. | Aggl. | Absorp. | Aggl. | Absorp. | Aggl. | Absorp. | Aggl. | Absorp. |
| Smi | 400 | N.C. | 400 | V.S. | 100 | Nil | − | ... | ... | ... |
| Cha | 200 | N.C. | 400 | P. | 800 | P. | − | ... | − | ... |
| Eld | 400 | N.C. | 800 | N.C. | 800 | N.C. | − | ... | − | ... |
| Bay | + | P. | 200 | V.S. | 200 | Nil | + | ... | + | ... |
| Sim | 100 | Nil | 800 | N.C. | 800 | N.C. | − | ... | − | ... |
| Bat | 100 | Nil | 800 | N.C. | 800 | N.C. | − | ... | − | ... |
| Am | + | Nil | 400 | P. | 400 | C. | − | ... | + | ... |
| Rou | − | Nil | 400 | N.C. | 800 | C. | − | ... | + | Nil |
| Chid | − | ... | − | ... | ... | ... | 1600 | C. | + | ... |
| Mitch | − | ... | − | ... | ... | ... | 800 | C. | − | ... |
| Pow | − | ... | − | ... | ... | ... | 800 | C. | + | ... |
| Mill | − | ... | − | ... | ... | ... | 1600 | C. | − | ... |
| And | 800 | ... | 200 | Nil | 200 | ... | 800 | C. | 200 | Nil |
| Newb | − | ... | − | ... | ... | ... | 1600 | C. | 100 | ... |
| Bay | − | ... | − | ... | ... | ... | 800 | C. | − | ... |
| Mor | − | ... | + | ... | − | Nil | 800 | C. | + | Nil |
| Keay | − | Nil | − | ... | − | Nil | 400 | C. | + | ... |
| Ri | + | Nil | 400 | Nil | 200 | Nil | + | Nil | − | V.S. |

The Type II serum used for absorption tests in this series was the goat serum from which, as has been recorded above, Type II strains readily removed the homologous agglutinin.

A few absorption tests were also carried out with two Type II rabbit sera ("Wat" and "Gl"); from these sera none of the strains (except the homologous coccus in each instance) was able to remove more than a slight amount of the homologous agglutinin at one absorption but after a second addition of the cocci one strain ("Mor") removed the whole of the agglutinin from both sera. It is interesting that this strain ("Mor") was able to combine with the agglutinins produced by two different strains ("Wat" and "Gl"), neither of which was able to exhaust the serum produced by the other ("Gl" or "Wat").

One of the Type III strains ("Rou") was atypical in agglutinogenic capacity. A rabbit was under treatment with this coccus a total period of two and a half months, the final dose being 80 mgrm., and during this period its serum was frequently tested. The last sample of serum failed to agglutinate the homologous coccus at 1–100 and only on one previous occasion did the titre rise to 1–100. The final sample of serum agglutinated chiefly Types I and III strains and these were equally acted upon but only in dilutions of 1–100 and 1–200.

The remaining four of the eighteen strains gave the following results: Two of the strains ("Cha" and "Eld") absorbed the homologous agglutinin from two type sera (I and III)[1]. A third strain ("Bay") although it was not agglutinated by the Type I serum appeared to absorb its agglutinin partially, an observation twice made. The fourth strain ("Ri") agglutinated best with the two Type III sera but failed to absorb agglutinin from either serum; there was also no absorption with this strain from five other sera, two Type I, two Type II and one Type IV.

With a view to studying the agglutinogenic capacity of this latter strain a serum was prepared from it. This serum, with a titre of 1–800, acted best on Type I and Type III strains, agglutinating the majority of them to the full titre of the serum; a good many Type II strains were also agglutinated but agglutination with one exception ("Glid" 1–800), was complete only at 1–100 or 1–200. Agglutinin absorption tests were carried out and the strain producing the serum was the only one which removed the homologous agglutinin completely. Type I and Type III, as well as cocci containing "C" agglutinin, removed it partially. There was very slight or no absorption from the serum with the Type II and Type IV cocci used.

### SUMMARY OF RESULTS WITH THE THIRD SERIES OF MENINGOCOCCI.

Out of eighteen unselected strains fourteen could be identified with one or other of three of Gordon's four types. The remaining four were not typical; two were agglutinated best with Type III sera but absorbed agglutinin from both Type I and Type III sera; one gave a doubtful result, absorbing slightly from a Type I serum which had not agglutinated it; and one could not be identified by absorption tests with any type but was apparently related to Type III according to the agglutination test.

### Tests with agglutinating sera from horses.

Altogether five horses were used for the purpose of producing agglutinating sera. Four of the animals were intravenously inoculated at five to seven days' intervals with gradually increasing doses of living meningococci. The initial dose in each case was 10 mgrm. and the largest dose given was 360 mgrm. The procedure in the fifth horse was a little different. This horse was immunised with Type IV cocci, and as an agglutinating serum for this type was then urgently required for trial a more intensive method was adopted. After three preliminary small doses during four days, the horse received the relatively

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[1] Gordon (1915) has recorded a strain with similar combining capacities which he has termed "amphoteric."

large dose of 100 mgrm. on the sixth day and 200 mgrm. on the eleventh day, rising by stages to 300 on the twenty-eighth day. These doses were well borne but the serum could not, for the reasons stated below, be used for agglutination tests.

In this series of five horses no desensitising doses were given, and none of the horses died as the direct result of the injections. One, however (Mi 4, Type II), died twelve to fourteen hours after an injection of 300 mgrm. but the immediate cause of death was judged to be due to distension of the stomach following an over-feed of green food.

Samples of the sera were sent to Col. Gordon at frequent intervals, and when these gave satisfactory agglutination with the homologous cocci the horses were bled.

None of the sera, however, were used for the purpose for which they had been prepared, as it was found by Col. Gordon, after trial on a variety of strains, that they were not specific; that is, they did not differentiate the meningococci into the same types as rabbit sera. Some of the serum was used therapeutically in a few cases (one Type II and two or three Type III cases) apparently with good result, all the cases treated recovering.

For the purpose of comparing the relative specificity of different animal sera, the sera of these horses, as well as of others immunised later on for the production of therapeutic sera, were tested by me against a large number of strains of meningococci.

In Table V are given the results of agglutination tests with five horse sera and one donkey serum on thirty strains, all of which, with one exception (M 15), have been identified by means of rabbit sera with one or other of Gordon's four types.

Horse 1 was immunised with eight strains sent to me as representative of Type I. One of these strains ("Mac"), however, was subsequently found to be not a Type I but a Type III strain. It is not therefore surprising that there was no differentiation between Type I and Type III cocci, which were the only strains agglutinated well with this serum.

The donkey serum was produced with four typical Type I cocci and like Horse 1 serum divided the series into two groups, Types II and IV strains being unaffected, while Types I and III (except Bunting) were agglutinated in dilutions from 1–200 to 1–800.

The other Type I serum was a univalent serum with a high titre for the homologous coccus. This serum was one of the latest produced sera and some of the early strains were not then available for test. Many later ones were however included and altogether fifty-two strains were tested. Sixteen Type I strains were agglutinated in dilutions 1–1600 to 1–6400; thirteen Type III were complete at 1–400 to 1–800; seventeen Type II were complete at 1–400 to 1–800 and four Type IV 1–150 to 1–400. Thus, with this serum, while there was no sharp line of demarcation between Type I strains and the other types, all Type I cocci were agglutinated in higher dilutions than any other type.

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Table V. *Agglutination tests with horse sera upon various strains of meningo-cocci from the three series.*

| Designation of strains | | Type I sera | | Type III | Type II | Type IV |
|---|---|---|---|---|---|---|
| | Horse 1 | Donkey | Horse 35* | Horse 3 | Horse 4 | Horse 5 |
| | Multivalent | | | Monovalent | Multivalent | |
| **Type I—** Litt | 1600 | 800 | 1600 | – | 800 | 200 |
| Cou | 400 | 800 | 3200 | – | 400 | + |
| Win | 400 | 800 | 6400 | + | 400 | (+) |
| Sher | 1600 | 400 | 3200 | + | 200† | 100 |
| Kna | 400 | 400 | 3200 | + and 200 | 400 | + |
| Lis | 800 | 800 | ... | – | 400 | (+) |
| Mit | 400 | 800 | 1600 | 100 | 400† | + |
| Jor | 1600 | 800 | ... | 400 | 400 | ... |
| M 4 | 800 | 800 | ... | + | 800 | (+) |
| M 48 | 800 | 200 | ... | – | + | ... |
| **Type III—** Bunt | (+) | (+) | 400 | 1600 | 1600 | 400 |
| Mac | 400 | 200 | 800 | 1600 | 800 | 400 |
| M 12 | 800 | 200 | ... | 1600 | 1600 | 400 |
| M 17 | 400 | 200 | ... | 800 | 3200 | 100 |
| M 43 | 400 | 200 | 800 | 800 | 1600 | 400 |
| **"C"—** M 15 | 100 | 200 | ... | – | + | 100 |
| **Type II—** McP | (+) | – | 400 | 200 | 3200 | 200 |
| Wat | – | – | 400 | – | 3200 | 100 |
| Mi | 100 | ... | 400 | 400 | 1600 | 400 |
| Wy | + | ... | 400 | + | 1600 | + |
| Glid | (+) | (+) | 800 | – | 800 | 800 |
| Keay | – | – | ... | – | 800 | + |
| M 19 | 100 | – | ... | 400 | 3200 | 800 |
| M 20 | – | – | ... | 400† | 1600 | 400 |
| M 24 | – | – | ... | – | 800 | + |
| **Type IV—** M 33 | – | – | ... | (+) | 800 | 800 |
| Hic | + | – | 200 | + | 800 | 400 |
| Gar | 200† | + | 200 | – | 800 | 400 |
| Bow | 100 | + | 400 | 200 | 1600 | 800 |
| My | + | (+) | 100 | 200 | 1600 | 800 |

* Horse 35 serum was tested on 34 strains additional to those in the table with the following results: 10 Type I agglutinated at 1–1600 to 1–6400; 10 Type III, 1–400 to 1–800; 12 Type II, 1–200 to 1–800; "Eld," 1–1600; "Ri," 1–200.

† Denotes that agglutination was not quite complete at that dilution and all lower dilutions.

Horse 3 was immunised with a single Type III strain. The serum agglutinated five Type III strains up to 1–800 and 1–1600 and agglutinated also a few strains from each of the other three types at lower dilutions.

Horse 4 was at first immunised with a single strain but, when it was found by Col. Gordon that the serum after six weeks' immunisation did not agglutinate all Type II cocci equally well, three other strains were added. With these four strains a serum of high titre was obtained, but it did not differentiate Type II from other types. Similarly the Type IV (multivalent) serum was not specific in its action, strains from other types being picked out irregularly

and agglutinated in as high dilution as the Type IV strains. The serum from this horse became useless after continued immunisation for another reason, namely, the occurrence of the phenomenon of pro-agglutination. Agglutination of the homologous and other cocci did not occur until the serum had been diluted 1–800 or more, and then only exceptionally was there complete agglutination of the cocci. The phenomenon of pro-agglutination was frequently observed with horse sera but usually it was evident only in the low dilutions of the sera.

Tests made with the sera of other horses confirmed as a rule those first obtained. Moderately specific sera were produced by Types I and III strains, while sera made with II and IV strains were generally, though not invariably, found to act well on strains of other types.

A few absorption tests were carried out. From Horse 4 serum (when monovalent) three Type II strains removed homologous agglutinin completely and four Type IV removed it partially.

Horse 35 (Type I) serum was absorbed with twenty-four strains. Four Type I and the "amphoteric" strain ("Eld") removed the homologous agglutinin completely, two Type III and one Type IV removed it partially, while the rest, including representatives of II, III and IV, removed only a slight amount or none at all.

From Horse 3 serum all the Type III cocci removed the homologous agglutinin completely, but some II and IV strains (*i.e.* those which combined with the homologous agglutinin in "Mac" rabbit serum) were also able to remove a good deal of it.

Agglutination and absorption tests with horse sera were in the main, therefore, in agreement with those obtained with rabbit sera.

### Tests with agglutinating sera from goats.

Seven goats in all were immunised with three of Gordon's types of meningococci, three with Type I, three with Type II and one with Type III.

Goats were found to be very susceptible to the intravenous inoculation of meningococci. An initial dose of 1 mgrm. produced severe symptoms, and although the doses were very cautiously increased the animals were often unwell and refused their food twenty-four and more hours after an injection; they also lost weight. Three of the goats died after an injection two-and-a-half and four months after treatment began. None of these sera had attained a titre for the homologous coccus higher than 1–200. One goat died from causes unconnected with the experiment and in another case the experiment was discontinued.

Only two of the goats produced a serum with a satisfactory titre for the homologous coccus.

The serum of Goat 1 ("Litt") agglutinated the homologous coccus at 1–800 and other Type I cocci in dilutions 1–200 to 1–800. With the exception of one strain ("Gar," complete 1–100) no coccus belonging to other types was

acted upon at 1–100. This serum was absorbed with a selection of strains, and all the Type I cocci tested absorbed the homologous agglutinin, while none of the other types removed any.

The second satisfactory serum was made with a Type II coccus ("Glid"), which was agglutinated at 1–1600 after the goat had been about four months under treatment; other Type II cocci were agglutinated in dilutions 1–400 to 1–1600. The Type IV cocci and one Type I coccus were agglutinated at 1–100, while other cocci were unacted upon at that dilution. The serum of this goat proved very useful for absorption tests; all Type II cocci (with one or two exceptions) absorbed the specific agglutinin, whereas no coccus of other types was able to combine with it.

The two strains ("Litt" and "Glid") used for the immunisation of Goats 1 and 6 were also used to immunise rabbits. While from each of the goat sera the specific agglutinin was readily absorbed by the cocci of the same type as that used in producing the serum, from the rabbit sera specific agglutinin was removed completely by the homologous coccus only, other allied strains removing it only partially or slightly.

My conclusion from the above experiments is that goats are not suitable for the production of meningococcic agglutinating sera on account of their susceptibility and the tardy appearance of agglutinins. In the two cases where sera of good titre were obtained the agglutinins produced appeared to be specific both in agglutinative action and in relation to the absorption test.

## Variability of serological results.

All who have worked with the meningococcus have noted irregularities in the results of serological tests.

The most common irregularity is variation in agglutinability of a strain. In the course of this investigation agglutination tests with the same serum and coccal suspension have often been repeated and the results have not always been identical, although the divergence has not been great. The widest variations between results on different occasions have been observed when different suspensions of the same strain have been used. This has been the case with Type II strains especially, but a similar observation has been made with a Type III and a Type IV strain. This particular irregularity has caused great trouble in absorption tests with Type II. On several occasions when it was necessary to use a fresh suspension of the coccus homologous with the serum, the new suspension has been found to agglutinate in very much lower dilution than the previous one. In one instance one suspension agglutinated at 1–3200 while a later, fresh, suspension was complete only at 1–100. Gordon (1917), Tulloch (1917), Scott (1917) relate similar examples.

In absorption tests there have been slight variations in the degree to which a particular coccus has removed homologous agglutinin from a serum on different occasions, but in general the results of repeated absorption tests have been remarkably constant.

It is sometimes the case that the strain used for producing the serum agglutinates at a less high titre than other strains of the same type. On two occasions, once with a Type II strain and once with a Type III strain, this feature was very marked. Elser and Huntoon (1909) express the view that inagglutinable but agglutinogenic strains are of frequent occurrence, but as Eastwood (1916) points out, their recorded laboratory data in support of this view are too scanty to justify such a generalisation.

## Varieties of meningococcus antigen.

The results of the serological tests on the first series of meningococci indicate the existence, in this particular series of strains, of four distinct meningococcal antigens. Each of the four types of meningococci, into which Gordon has divided these strains, is distinguished by the predominance of one or other of these special antigens.

The different results obtained with sera made from strains belonging to the same type are attributable to variations in the amount and kind of the other antigenic components associated with the type antigen.

In certain strains one type of antigen greatly preponderates and hence it arises that sera made from such strains are very specific, picking out and agglutinating at high titre strains with the same antigen. If strains of this character are used for the preparation of sera for the classification of meningococci the division into types appears clear and definite. In other strains there are associated with the predominant antigen one or more components identical with or allied to the other type antigens. When sera made from such strains are employed the division of meningococci into types can no longer be maintained.

Of the different antigens, Type I appears to be more often present in a relatively pure state than the others and on this account it is easy to produce a specific Type I serum.

The strains of Type II show greater diversity of antigenic structure than those of Type I. There appears to be a variety of antigen common to all the Type II strains, but this is often associated with antigens related to the other types, as shown by the agglutination tests. There is, however, no uniformity in these associated antigens, which vary in different strains in kind and quantity. This is a possible explanation why Type II sera vary so much among themselves in range and degree of capacity to agglutinate meningococci of all types, and why the agglutinins of Type II sera are absorbed irregularly by Type II strains.

Type III antigen is commonly associated with antigens which agglutinagenically are related to Type I, but one strain produced a serum which was able to agglutinate strongly some Type II as well as Type I strains, and from which Type II strains absorbed the homologous agglutinin.

Type IV strains contain in common an antigen which is different from the other type antigens. This antigen is usually present with components which

produce agglutinins with combining affinities for Type II strains, but one of the Type IV strains showed definite evidence of serological relationship to Type I.

The four antigens described above are apparently not the only antigens which are common to groups of strains. Scott (1917) has distinguished by means of the absorption test at least eight distinct sub-groups in a series of 131 strains of meningococci (60 cerebro-spinal and 71 pharyngeal). F. Griffith (1917) has demonstrated three main antigens, designated "A," "B" and "C" (two of these, "A" and "B," are identical with Gordon's Type I and Type III respectively) in his Group I, and at least four in his Group II.

I have had experience with only one of these additional antigens, *i.e.*, the one producing F. Griffith's "C" agglutinin, the existence of which I have confirmed. This antigen is related to Types I and III but appears to be less complex than either, since the two latter can absorb the "C" agglutinin but the "C" antigen is unable to remove either Type I or Type III agglutinins.

### SUMMARY AND CONCLUSIONS.

The serological reactions of three series (comprising ninety-three strains) of cerebro-spinal meningococci sent to me from three different laboratories have been studied.

The first series of forty-four strains came from the Central C.-S. F. Laboratory and were obtained from military cases of cerebro-spinal fever. They had already been classified into four types by means of Gordon's type sera. I prepared monovalent agglutinating sera from members of each type and with certain of these sera I was able by means of the absorption test to divide the strains into four classes corresponding to Gordon's four types. When, however, sera made from other members of each type were used the same differentiation of the strains could not be made, and in others was less well-defined.

The second series (thirty-one strains) came from the Local Government Board's Laboratory and were derived from cerebro-spinal fever cases occurring among the general population, including both adults and children. Simple agglutination tests with certain type sera made from Gordon's strains effected a division of the majority of the strains into two main groups, in agreement with F. Griffith's classification, a few strains being agglutinated by both sera, and a few not being placed on account of insufficient agglutination. With Type III and Type IV sera there was definite indication of sub-groups within the two main groups.

By application of the absorption of agglutinin test it was found that:

(1) Of the Group I strains four were identical with Type I and three with Type III (these strains were agglutinated to the full titre of the Type III sera); three absorbed agglutinin from more than one type serum, two absorbing the agglutinin from I and III and one from I and II; the remaining nine

could not be identified with any of Gordon's types. Six of these nine unplaced strains were shown by the use of a serum made from one of them to form a well-defined sub-group. This sub-group had already been defined by F. Griffith who designated the antigen present in these strains "C" antigen. Type I corresponded to strains of Group I containing his "A" antigen and Type III to his "B" antigen. Thus the "C" antigen of Group I which F. Griffith considers to be less complex than "A" or "B" was not represented in Gordon's military cases.

(2) Of the twelve Group II strains seven were identified with Gordon's Type II and two with Type IV. The remaining three could not be identified with any of Gordon's types.

The third series (eighteen strains) was derived from adult cases of cerebro-spinal fever. One of the strains was identified with Type I, nine with Type II and four with Type III. Of the remainder two absorbed agglutinin from Type I and Type III sera, one was indeterminate and one could not be identified by absorption tests with any of the four types.

It should be noted here that it was with an exceptional goat serum that the Type II strains in Series 2 and 3 were classified by absorption; none of the rabbit sera defined Type II strains so well as this goat serum.

My conclusions from the above results are as follows:

(1) If carefully selected type sera be taken as standards Gordon's four types can be well defined.

(2) These four types are not sufficient to include all meningococci which may be obtained from cases of cerebro-spinal fever.

(3) The proportion of strains which do not fall into any of Gordon's four types is greatest in cases of meningitis occurring among the general population.

(4) The division of the first series of meningococci into four types depends upon the existence of four chief antigenic components.

## PART II.

### THE PREPARATION OF ANTI-MENINGOCOCCUS SERUM.

The demonstration of the existence of at least four types of epidemic meningococci is of great practical importance from the point of view of the treatment of cerebro-spinal fever.

In the early years of the war cases of meningitis were treated with multivalent serum from different sources. The results were disappointing and it was realised, in view of Gordon's findings, that one reason for this lack of success was that the serum used had not been prepared with strains which corresponded to the type of meningococcus infecting the patient.

In order therefore to ensure provision of more specific sera for the treatment of cases of cerebro-spinal fever in the Army, cultures of the four types of

meningococci were supplied by the Central Cerebro-spinal Fever Laboratory to the various makers of anti-meningococcus serum.

Clinical experience with these type sera during the epidemic of 1917 indicated considerable variation in the therapeutic value of different batches of anti-meningococcus serum, and it was recognised that experimental investigations were desirable for the purpose of defining the best methods of preparing and standardising anti-meningococcus serum.

Having already had some experience in the immunisation of horses, I was instructed by the Medical Research Committee to co-operate with Colonel Gordon in an attempt to improve the therapeutic value of anti-meningococcus serum.

Facilities in the way of accommodation for horses and of extra laboratory space were generously provided by the Committee of the Field Laboratories, University of Cambridge.

It was arranged that the testing of the serum was to be done by Colonel Gordon at the Central Cerebro-spinal Fever Laboratory and that no serum was to be issued for use which had not been approved by him.

*Preliminary Experiments.* The work began in the summer of 1917 when four horses were procured. These were used to test the relative values of the intravenous and intramuscular methods of injection, one pair being immunised with a Type I coccus, the other with a Type II coccus. Later on, other horses were obtained for the purpose of comparing the antigenic value of the living with that of the dead coccus.

Unfortunately these experiments were not carried far enough for definite conclusions to be drawn owing to the death or defect of one of the horses in each pair.

Horses were at that time very difficult to obtain and several were purchased which on account of age or infirmity were unable to stand the strain of immunisation with the meningococcus. Experience showed that only young healthy horses are suitable for this work. The horse is very susceptible to the meningococcus toxin, and after an injection almost invariably lies down. If the animal is aged or defective in wind or limb there comes a time, generally in the later stages of the immunisation, when the animal goes down and is unable to get up again unaided. My difficulties in this regard were removed in December, 1917, when arrangements were made with the Army Veterinary Department by which I was enabled to obtain horses which, while no longer fit for military duty, were suitable in every respect for serum production.

The intravenous and intramuscular comparisons were, however, carried sufficiently far to indicate that the latter method was inferior to the former in the production of agglutinins and opsonins, and, as the intramuscular injections produced large painful swellings which sometimes broke down, this method of immunisation was abandoned for the intravenous method. Intravenously inoculated animals were given gradually increasing doses at weekly intervals, according to the method employed at the Pasteur Institute, Paris[1],

[1] I am indebted to Dr Dujardin-Beaumetz and M. Victor Frasey, Médicin Vétérinaire de l'Institut Pasteur, for demonstrating to me in December, 1915, the methods employed in the production of anti-meningococcus serum at the Pasteur Institute, Paris.

at first of killed culture, then of living culture; when the doses began to cause serious symptoms desensitising doses were administered two hours before the main dose as recommended by Dopter (1910).

The sera from these intravenously inoculated animals after nearly six months treatment on these lines were carefully tested at the Central C.-S. F. Laboratory for agglutinins, opsonins and anti-endotoxin.

Gordon's method of testing for anti-endotoxin was as follows: the growth from young cultures of virulent strains of meningococci were killed by ether, dried *in vacuo* and powdered; 0·1 grm. of this powder was carefully and thoroughly ground in an agate mortar and 5 c.c. of distilled water slowly added; the heavier particles were centrifuged out and an opaque watery extract was obtained of which 0·1 to 0·2 c.c. was lethal to mice inoculated intra-peritoneally.

One minimal lethal dose of this extract and 0·5 c.c. of the serum were mixed together and incubated for 30 mins. at 37° C. The mixture was then inoculated intraperitoneally into a mouse. Control mice were inoculated with toxin and normal horse serum. With potent toxin the survival or death of the mouse was the criterion, but with weaker toxin the presence or absence of illness was regarded as a good index of the antitoxic value of the serum.

As evidence that a good serum must contain anti-endotoxin, Gordon (1918) records the following observation: he found that multivalent serum which gave the best results in military cases was differentiated from serum of less therapeutic value by ability to neutralise in 0·5 c.c. amounts one minimal lethal dose of the endotoxin of both Types I and II of the meningococcus.

In consequence of this observation it was decided to use, as far as was practicable, for the treatment of cases of the disease only those sera which contained a definite amount of anti-endotoxin.

The samples were reported to be excellent as regards agglutinins and opsonins but deficient in anti-endotoxin.

As the doses then reached were fairly high and had caused severe symptoms even after desensitisation, and as it appeared to me that further increase of dose to the level apparently necessary to make the serum protective might be fatal for some of the animals, I therefore decided to adopt the method of Amoss and Wollstein (1916) for the rapid preparation of anti-meningitis serum.

*Method of Amoss and Wollstein.* This method, which is, in principle, the intensive method of immunisation of Fornet and Müller (1910), was recommended by Amoss and Wollstein because it enabled them to prepare anti-meningitis serum within six to eight weeks and to give large doses of meningococci with safety.

The plan was to begin with small doses of living meningococci injected daily for three days followed by a period of rest of seven days, when another series of injections was made. After the first series the dose given on the first day of each subsequent series corresponded to that given on the last day of the preceding series. The temperature was taken hourly, beginning at the fourth hour, until it had reached its maximum and begun to decline. If the rise did not equal 2·5° C. to 3° C. the conclusion was drawn that the dose was too small. It was increased, therefore, for the injection twenty-four

hours later above the usual rate of increase. If, on the other hand, the temperature did not fall to normal within eighteen to twenty-four hours the conclusion was drawn that the dose given was too large. By following this plan doses could be regulated with nicety, and a maximum of reaction obtained with a minimum of danger. After the third or fourth series of injections a desensitising dose was given before the first injection in each series. The greatest reaction, as a rule, was that produced by the first injection, whereas the succeeding injections on the second and third days tended to produce less severe reactions. Hence the increase between the second and third injections might be larger than that between the first and second. The doses were measured by suspending the growth from one agar slant in 2 c.c. of physiological salt solution, giving definite amounts of this suspension beginning with 0·1 c.c. or one-twentieth of an agar slant. The largest amount of any single injection was one-fourth of each slant from seventeen different strains. Meningococci and para-meningococci were used alternately in the series of injections. When autolysate was also given this formed one series alternating with two series of living meningococci.

My method at first differed from the above only in the way the dose was estimated and in the use in the early stages of the immunisation of killed instead of living culture. The doses were weighed and in the first series were 5, 7·5 and 15 mgrm. or 5, 10 and 20 mgrm. according to the susceptibility of the horse. Each horse was immunised with one type of meningococcus only, the number of strains used varying from 1 to 6. Autolysates and sensitised cocci were administered, but not in any regular manner.

In series subsequent to the first the doses were increased very cautiously, and it was recognised later that the increase between the second and third doses was never so large as it might have been. Nevertheless, as reports from Colonel Gordon testified, the sera of the horses quickly showed a distinct advance, both in agglutinins and opsonins, on those produced by single weekly doses, and several also proved strong in anti-endotoxin. Eventually all the horses then under treatment yielded a serum which was declared to be sufficiently anti-endotoxic for use in cases of the disease.

My results, therefore, confirmed Amoss and Wollstein's statement that good anti-endotoxic serum can be produced by the method of three successive intravenous injections of meningococci at stated intervals.

For the successful routine use of the method, however, it appeared that considerable experience was necessary in the matter of dosage. If the dose, and particularly the third dose in each series, were not properly adjusted to reactive capacity, *i.e.* if it were insufficient or excessive, the serum of the horse instead of rising in titre would appear actually to fall.

No definite scheme of dosage can be laid down, for the doses will vary with the individual horse, but according to the method as practised here the following may be regarded as a normal course, the figures representing mgrms. of culture.

| Series 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|
| 5 | 15 | 30 | 50 | 90 | 140 | 150 |
| 7·5 | 20 | 35 | 60 | 125 | 200 | 225 to 250 |
| 15 | 30 | 50 | 100 | 150 | 300 | 350 |

Desensitising injections were given before the first dose in series 5, 6 and 7.

A horse which had received such a course of injections and had reacted typically to the third dose could be bled after the sixth or seventh series. This course could be shortened in a resistant animal by giving a larger dose at the end of the fifth series and substituting the seventh for the sixth. It will be noted that the first dose of the seventh series does not correspond with the last of the sixth. When the third dose exceeded 150 mgrm. (in this instance it was 300 mgrm.) it was of course impossible to begin the next series with that dose and increase in the same proportion as before, for then the animal would have been overwhelmed. It was found that good reactions were obtained with a dose slightly in excess of the preceding first dose, subsequent doses increasing, however, at a higher rate.

When the total weight of cocci given in the three days had reached 750 to 800 mgrm. the horse was allowed to rest for fourteen to twenty-one days, when the injections were begun again. After this period of rest horses were often found to be hyper-sensitive, and in order to avoid accidents it was necessary to give relatively small doses for the first series. Another plan, and one to be recommended when the horse had been some time under treatment, was to change the type of coccus. In such horses immunisation proceeds more rapidly than in new horses.

After having used the method for a period I found myself unable in one respect to confirm Amoss and Wollstein's statement, that the method is a safe one. In the later stages of immunisation the horse may develop hyper-sensitiveness after the first or second injections of a series, and then the cocci instead of producing a reaction are strongly toxic. One horse died after the second injection in a series, the dose being 200 mgrm.; another was gravely ill after this dose, but survived. Another horse died after the third injection (dose 300 mgrm.).

The symptoms in all these cases were the same. Two or three hours after, the dose the horse begins to sweat; the temperature is only slightly raised above the normal; the respirations may be very greatly accelerated, and the animal lies prone; gradually the sweating extends over the whole body and becomes profuse; between the fourth and fifth hours the animal dies, or it may apparently recover for a time, and then die suddenly. These accidents can be avoided if the effects produced by the preceding injection are carefully studied. It is my experience that if after an injection the temperature rises only slightly and is still raised next morning it would be fatal to give another large injection. Also if the temperature rises very high, from 41° C. to 42° C., and the general reaction is intense with some sweating, another injection on the next day is contra-indicated. In such cases it is advisable to intermit the

injections and begin again five or six days later, when the series may be given without danger.

*Modification of Amoss and Wollstein's Method.* The occurrence of fatalities and other considerations, however, had led me to try modifications of the method at the stage when the horse was liable to develop hyper-sensitiveness. It had frequently been observed in the later stages of immunisation that the third dose of a series often failed to excite a satisfactory reaction, and the possibility occurred to me that repeated large doses might in some cases be having the effect of neutralising anti-endotoxin already formed in the body instead of stimulating its further production. I began therefore as immunisation progressed to drop out one of the three doses and give only two, increasing the size of the second dose beyond that which I would have given had a third dose been contemplated. Then after one or two such series single large weekly doses were given which had been preceded in the afternoon of the previous day by a preparatory dose equivalent to about one-tenth the amount of the main dose. Thus, there was gradual concentration of dosage, and instead of three moderate reactions one single maximum reaction could be secured.

The plan was found to answer well and no horses were lost. Two series of doses are presented which were given to horses whose sera were passed for therapeutic use. Horse 43 was very sensitive and reacted well to every injection.

HORSE 43.   TYPE II.

Series I.
    Nov. 25.    5 mgrm.
        26.    5   ,,
        27.    10   ,,

Series II.
    Dec.  5.    10 mgrm.
        6.    15   ,,
        7.    25   ,,

Series III.
    Dec. 16.    25 mgrm.
        17.    30   ,,
        18.    50   ,,

Series IV.
    Dec. 27.    50 mgrm.
        28.    60   ,,   Very strong reaction,
                3rd dose therefore not given

Series V.
    Jan.  6.    60 mgrm.
        7.    80   ,,
        8.    110   ,,

Series VI.
    Jan. 17.    10 mgrm. and 2 hours later
                100   ,,
        18.    150   ,,

HORSE 35.   TYPE I.
(Changed from Type II.)

Series I.
    Jan. 30.    10 mgrm.
        31.    15   ,,
    Feb.  1.    30   ,,

Series II.
    Feb. 10.    30 mgrm.
        11.    40   ,,
        12.    80   ,.

Series III.
    Feb. 20.    75 mgrm.
        21.    100   ,,
        22.    150   ,,

Series IV.
    Mar.  3.    15 mgrm. and 2 hours later
                135   ,,
        4.    200   ,,

Series V.
    Mar. 11.    35 mgrm. at 4 p.m.
        12.    350   ,,      11. a.m.

Series VI.
    Mar. 23.    50 mgrm. at 4 p.m.
        24.    550   ,,      11 a.m.
                Reaction not satisfactory

HORSE 43.    TYPE II.

Series VII.

    Jan. 27.    25 mgrm. at 4 p.m.

        28.    250   ,,.     11 a.m.

Series VIII.

    Feb.  6.    45 mgrm. at 4 p.m.

        7.    450   ,,     11 a.m., strong
            reaction

      12.    Serum sample weakly anti-endotoxic

      14.    Bled⎫ serum strongly anti-endotoxic
      15.    Bled⎭

      17.    Serum sample weakly anti-endotoxic

Series IX.

    Feb. 24.    50 mgrm. at 4 p.m.

        25.    550   ,,     11 a.m.

    Reaction identical with that following Series VIII

    Serum on 7th and 8th days not approved.

HORSE 35.    TYPE I.

(Changed from Type II.)

Series VII.

    April  7.    50 mgrm. at 4 p.m.

        8.    600   ,,     11 a.m.

Serum approved on the 3rd, 5th and 6th days, but not on the 7th, 9th and 12th days.

Horses were bled after the 450 mgrm. and again after the 550 or 600 mgrm. dose, if the reactions had been satisfactory. A good febrile and general reaction after the dose preceding the bleeding of the horse was regarded as important and a few typical temperature reactions are reproduced (Charts I and II). The first curves are after a three-series and a two-series course of injections; the other two followed single injections.

During the course of the work many attempts were made to determine what interval of time should follow an injection before bleeding a horse. It was hoped that information on this point might be obtained by taking samples of the serum at frequent intervals after an injection and sending them to be tested by Colonel Gordon. If the reports on the early samples were favourable the horse was bled.

The results were very conflicting and appeared to indicate a good deal of individual variation. Some samples were reported as protective on the first, second or third days, and when the horse was bled five to seven days after the injection, the serum was found not to be protective. Other sera were not protective during the first few days but were reported as being so later on, fifth to eighth days. Another horse would yield a protective serum on every occasion on which it was tested during the first eight days.

The observations made, therefore, do not enable me to state what is the best day for bleeding a horse after an injection. Horses were bled as a rule on the seventh or eighth days, and these are probably the most suitable intervals.

The evidence was also conflicting in regard to the stage of immunisation when a protective serum might be expected. As the sera which were passed almost invariably came from horses which had reacted strongly it was hoped that intensity of reaction might be used as an index when to bleed. But it was found that of two horses, comparable in respect to dosage, reaction and interval after injection, the serum of one would be approved, while that of the other would be rejected. Also a horse which had yielded a protective

serum on one occasion would on the next, after a larger dose, yield a serum without protective property, although the reactions to the inoculations on both occasions had been identical.

The explanation of these irregular results may perhaps be found in individual variation in the time and duration of maximum production of
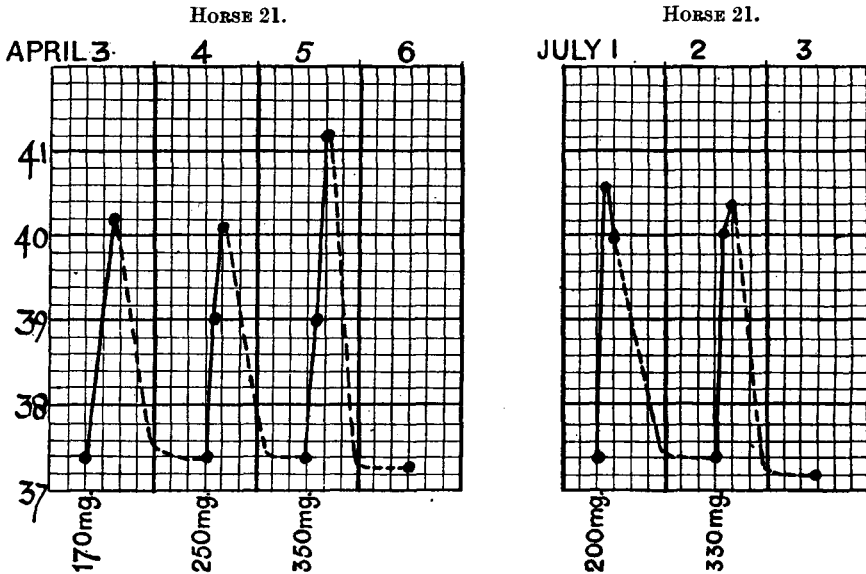


**Chart I.** Two hours before the first dose in each of these two series of injections a desensitising dose of 15 and 20 mgrm. respectively was given.
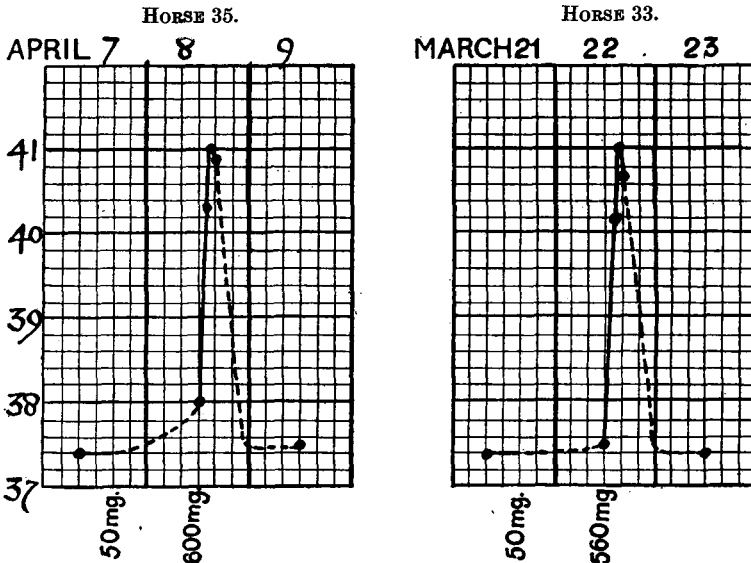


**Chart II.** Each vertical space in the charts represents an interval of six hours. The thick vertical lines mark off the days.

anti-endotoxin. In some immunised horses the curve of anti-endotoxin may be steeple-shaped, rising to its maximum on different days in different horses, and this may be the result when the immunising dose is insufficient to provoke a strong reaction. In such cases it would be a matter of chance whether the horse was bled on the right day or not. In other horses the anti-endotoxic curve is broad and protective sera may be obtained on several occasions from such animals. On the other hand the test for anti-endotoxin may not always give consistent results. Some experiments are described in a subsequent section which show that the mouse varies greatly in its susceptibility to the meningococcus toxin.

For these reasons I have not drawn any final conclusions as to what is the best method of injecting horses for the production of anti-meningococcus serum. While I have produced sera by two methods which have given satisfactory clinical results, the modification of the method of Amoss and Wollstein which I have described (p. 63) appears to me to have some advantages over the original from the point of view of safety as well as for technical reasons.

### Immunisation of horses with dried cocci.

At Colonel Gordon's request I immunised four horses with dried cocci, two with Type I and two with Type II, according to a scheme of dosage proposed by him.

The horses were under treatment for a period of about three months and were given three doses weekly. The initial dose was 0·5 mgrm. and the final 12 mgrm., each dose being repeated before the next higher dose was administered. The dried cocci had no bad effect upon the horses and the reactions were for the most part very mild.

Samples of the serum were sent to Colonel Gordon to be tested. No anti-endotoxin was demonstrated on any occasion in the Type II horses. The sera of the Type I horses were reported protective on the first and third days after the 8·5 mgrm. doses, and one was protective on the third day after the 11·0 mgrm. dose, while the serum of the other was not protective after the 12·0 mgrm. dose.

The serum of the first of the Type I horses was put up for trial and comparison with the sera produced by the injection of the living coccus.

### Tests of the potency of anti-meningococcus serum.

As stated above the routine testing of the sera of the horses was done at the Central Cerebro-spinal Fever Laboratory[1].

This plan was adopted not only because it was impossible for me, working single-handed as I was, to carry out in addition to the detailed work of producing the serum the very numerous tests which were requisite in the investigation, but also because the method of test was then in the experimental stage.

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[1] I wish here to thank Colonel Gordon for the promptness with which he reported on the large number of samples submitted to him.

Subsequently, when the test had been more or less standardised, I was able to do a few tests for which Colonel Gordon kindly supplied me with some dried cocci. The sera chosen for testing were those which had already been passed by Colonel Gordon.

The mode of preparation of the toxin was as described in the *British Medical Journal* (*loc. cit.*) except that an ordinary instead of an agate mortar was used to grind up the cocci and the toxin and serum were incubated for half-an-hour in small test tubes instead of in watch glasses.

In a preliminary experiment the toxicity of the Type II toxin was tested. Two mice each received 0·1 mgrm. and two mice each received 0·2 mgrm. of the toxin. All remained well.

In the next experiment the same toxin was used but the dose was increased to 0·25 mgrm. Of two control mice, one died in thirty-six hours, the other survived. Two mice to which toxin + anti-meningococcus serum had been administered remained well, while a third died within twenty-four hours.

In a third experiment fresh Type II toxin was used, and the dose was 0·2 mgrm. The two controls died within forty-eight hours, while of four mice receiving toxin + anti-meningococcus serum three died and one remained unaffected. In a fourth experiment with Type II toxin the dose was 0·15 mgrm. (two controls received this dose and a third received 0·2 mgrm.). The three control mice lived and of eight receiving anti-meningococcus serum + 0·15 mgrm. of toxin seven remained well and one died.

The next three experiments were with Type I toxin. In the first toxin only was injected, four mice receiving 0·1 mgrm. and five mice 0·15 mgrm. Of the four two died and two survived; of the five three died and two survived.

In the next experiment there were six controls, two of which received 0·1 mgrm., two 0·15 mgrm. and two 0·2 mgrm. The second pair and one out of each of the other pairs survived, the remaining two dying within twenty-four hours. Three mice received toxin + anti-meningococcus serum, the dose of toxin for each mouse being 0·2 mgrm.; of these two survived and one died, *i.e.*, there was exactly the same proportion of survivors among the serum treated mice as among the controls. In a third experiment with Type I toxin three samples of anti-meningococcus serum from the same horse, taken on different days after an injection, were tested; one of these sera had failed to pass Colonel Gordon's test. For each sample three mice were used; there were three controls; the dose of toxin for each mouse was 0·15 mgrm. Of the three controls two were dead on the next day and one survived. The same result was obtained with one serum sample. From each of the other two sets (including the one inoculated with the rejected serum) one mouse was dead within two days and the other two survived.

It is interesting to point out that, after eliminating Experiment 4 where the dose of toxin was insufficient, the percentage of mice which survived the inoculation of toxin and normal serum is practically identical with that for mice receiving toxin and anti-meningococcus serum.

Two experiments were carried out with living meningococci from eighteen-hour old cultures on glucose agar. In the first experiment four mice were used, and the dose was 10 mgrm. of a Type II coccus. The two controls remained well, while the two mice receiving anti-meningococcus serum died within twenty-four hours.

The second experiment was with a Type I coccus and the dose for each mouse was 15 mgrm. There were eight controls and eight inoculated with Type I serum. Of the eight controls seven died within two days and one survived. Of the eight·serum treated mice five died within three days and three survived. Here the evidence was in favour of the presence of protective bodies in the serum. But if the figures of the two experiments with living cocci are combined it is found that, as in the experiments with endotoxin, the percentage of mice which survived the injection of culture only is the same as that for the serum treated mice.

The results of these tests, therefore, are not in agreement with those obtained by Colonel Gordon with the same sera, but the experiments are too few in number from which to draw final conclusions as to the value of the test for estimating the anti-endotoxic potency of anti-meningococcus serum.

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