IDENTIFICATION OF THE MENINGOCOCCUS.

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The following preliminary observations were made for the purpose of determining whether the capacity of the meningococcus for absorbing its own agglutinin could be used practically for the purpose of identifying the micro-organism of the present outbreak.

**Method.**—The emulsion used in these experiments was a suspension in normal saline of a twenty-four hour growth of the coccus, and this suspension was heated for thirty minutes to 65° C. in order to kill the micro-organism and to inactivate the autolysin. Raymond Koch has shown that a suspension of meningococci heated in this way keeps for months; and gives as reliable results at the end of that time as in the first place. The macroscopic method of agglutination was used; and the results were read off after twenty-four to forty-eight hours at 55° C., as recommended by Kutscher. A control with normal horse serum was put up in each experiment and proved negative in all.

(1) *Does the serum prepared against meningococci of previous epidemics agglutinate strains of the present outbreak?*

The anti-meningococcus sera tested were as follows:

- Flexner's serum
- Mulford serum
- Burroughs and Wellcome serum
- Lister Institute serum (M. 10).

These four sera were tested in 1 in 50 dilution against three meningococci isolated from the cerebro-spinal fluid of recent cases, and against fourteen meningococcus-like organisms isolated from the nasopharynx of recent contacts.

**Result.**—None of the three meningococci were agglutinated by any of the four sera. Six of the fourteen meningococcus-like organisms from the nasopharynx showed well-marked agglutination, the rest were negative.

(2) *Does serum prepared against a strain of meningococcus isolated from the cerebro-spinal fluid of a case during the present outbreak agglutinate strains from the cerebro-spinal fluid of other cases in the same outbreak?*

In order to prepare a specific serum quickly, the intensive method of Fornet and Müller was used. A rabbit was injected
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with increasing doses of meningococcus (Strain L.) on three successive days. A twenty-four hours' culture of the meningococcus on legumin agar was emulsified in 5 c.c. of saline, and 0.2, 0.3, and 0.4 c.c. of this per kilo, injected intravenously into a young rabbit at intervals of twenty-four hours. On the twelfth day the rabbit was killed, its blood collected, and the serum separated off and tested on four meningococci isolated from the cerebro-spinal fluid of four recent cases.

The agglutination results produced by it were as follows:

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<thead>
<tr>
<th>Meningococcus strain</th>
<th>Dilution of serum</th>
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<tbody>
<tr>
<td></td>
<td>1 in 30</td>
</tr>
<tr>
<td>L.</td>
<td>+</td>
</tr>
<tr>
<td>D.</td>
<td>+</td>
</tr>
<tr>
<td>B.</td>
<td>+</td>
</tr>
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<td>J.</td>
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</tr>
</tbody>
</table>

It is clear that the serum prepared against Strain L. agglutinated the other three meningococci almost as well as the homologous strain.

(3) Do these four meningococci absorb completely the specific agglutinin from the serum of the rabbit prepared against one?

Experiment.—Six loopfuls of a twenty-four hour culture were added to 1.5 c.c. of a 1 in 10 dilution of the specific serum in normal saline. This suspension was kept for three hours at 37° C., followed by one hour at 55° C. The cocci were then centrifuged out, and the titre of the serum determined for the coccus with which it had been saturated, and also for the particular strain of meningococcus with which the rabbit had been injected.

Result.—Three of the four meningococci completely absorbed the specific agglutinin. The fourth coccus failed to entirely absorb the whole of the agglutinin for itself, so in that case the result is not yet complete, and the test is being repeated.

The above results, so far as they go, appear to indicate that the four strains of meningococcus examined are alike in their agglutinating behaviour towards a serum prepared against one of them, and that three of the four absorb this specific agglutinin.

(4) Application of the results of the experiments described under 1, 2, and 3.

Application was now made of this test for the purpose of
determining whether certain meningococcus-like organisms isolated from the nasopharynx of recent contacts, and of cases of illness of doubtful character suspected of being cerebro-spinal fever, were or were not identical with this meningococcus from three cases of the present outbreak.

The cocci have been tested against the same serum as the foregoing and examined with regard to:

1. Agglutinating titre.
2. Capacity of absorbing the specific meningococcus agglutinin.

Case 1.—Lieutenant B. This case was an alleged carrier who had been in isolation for some time. His coccus was practically indistinguishable from the meningococcus in cultural and fermentative characters.

When examined with the rabbit’s serum, it was found that the agglutinating titre was as follows:

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<thead>
<tr>
<th></th>
<th>1 in 20</th>
<th>1 in 40</th>
<th>1 in 80</th>
<th>1 in 160</th>
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</thead>
<tbody>
<tr>
<td><strong>Meningococcus</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lieutenant B.’s coccus</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
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</tbody>
</table>

The serum was saturated with Lieutenant B.’s coccus, and the agglutinin for that organism thus removed.

The serum was then examined once more against both cocci with the following result:

<table>
<thead>
<tr>
<th></th>
<th>1 in 20</th>
<th>1 in 40</th>
<th>1 in 80</th>
<th>1 in 160</th>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

It is clear that while the coccus in question removed its own agglutinin, it failed to bind the agglutinin of the meningococcus of the present outbreak, and therefore could not be identified with that micro-organism.

Case 2.—Another coccus closely resembling the meningococcus was isolated from the nasopharynx of a case suspected clinically of being cerebro-spinal fever without meningitis. Culturally, and in regard to fermentative characters, this coccus could not be distinguished from the meningococcus; but as it failed to show agglutination with the specific serum, even when that was only
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diluted 1 in 20, this coccus could not be identified with the meningococcus.

Case 3.—A peculiar form of pharyngitis affected a certain regiment in camp. Although there were no cases of meningitis, the regimental medical officer suspected that the pharyngitis might possibly be due to the meningococcus. A swab taken from two of the cases yielded a coccus closely resembling the meningococcus in morphological, cultural and fermentative characters.

The coccus was examined in the same way as the previous two, and was found to agglutinate with the specific serum in the 1 in 20 and 1 in 40 dilution, although it did not agglutinate in the 1 in 80 dilution as the meningococcus did at the same time. Absorption tests showed that while it absorbed its own agglutinin from the serum, this coccus failed to diminish the true meningococcus agglutinin. It was, therefore, not identified with the meningococcus.

The pharyngitis has since abated, and there have not been any cases of meningitis in this camp up to date.

Conclusion.—These observations appear to suggest that the capacity of the meningococcus for absorbing its own agglutinin can usefully be employed in certain cases for the purpose of identifying that micro-organism. It must be borne in mind, however, that possibly and even probably there are several different strains of the meningococcus capable of producing meningitis, and some of these meningococci may not absorb the specific agglutinin of others. This would appear likely from the fact, amongst others, that all three meningococci from cases of the present outbreak failed to agglutinate with the samples of anti-meningococcus sera on the market, against which they were tried.

How many different strains there are at work in the present outbreak is obviously a matter of prime importance in the present connexion, and further observations are needed before the practical application and limits of the absorption test for the purpose of identifying the meningococcus can be regarded as defined. For the present, it is particularly desirable to collect meningococci from the cerebro-spinal fluid of as many cases as possible and to examine them by the absorption method. Where it is impossible to keep cultures of these micro-organisms going, a suitable emulsion of one, or better of several twenty-four hour cultures might be heated to 65° C. for thirty minutes and put aside for examination at a later date.

I wish to thank Mr. E. G. Murray for the great assistance which he has rendered in carrying out these experiments.
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