FURTHER NOTES ON THE RAPID PREPARATION OF HIGH TITRE AGGLUTINATING SERUM FOR MENINGOCOCCUS.

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In the October number of this Journal a preliminary note was published on the method of rapidly obtaining a serum agglutinating the meningococcus, of a titre sufficiently high for test purposes; the same investigation is here carried a step further.

Although the titre of 1 in 800 obtained in eight days in the preceding Series B was sufficiently high and speedy to be of use for agglutination reactions, it was thought that the method might be improved, and a better serum obtained by a further injection without the loss of much more time. Accordingly, after some further tests with the rabbits of Series B, in which some of the sera attained a titre of 1 in 1,200 on the twenty-fifth day, the following experiments were carried out.

Four rabbits, of about 1,300 grammes each, were injected, as in Series B, with a standard suspension of the same coccus type II, freshly made; the total dose being again 3,000 millions. It was intended to give them a further single dose of 4,000 millions on the ninth day. Three of the rabbits survived; rabbit III, however was found dead about one hour after its second injection. A second rabbit was therefore procured and the first and second doses of 1,000 millions repeated; about half an hour after its second dose, however, this rabbit showed uneasiness with spasmodic kicks of its hind quarters, while its breathing became prolonged and laboured. It quickly developed an increasing paralysis of its fore-quarters and respiratory muscles, until, in about five minutes from the onset, the animal turned on its side unconscious, with no respiratory or other movements, and died, its heart continuing to beat for some three minutes after respiration had ceased.

It was obvious that the suspension used, though of the same coccus, was more toxic than that injected in Series B, and so on the third day a third rabbit was given three doses of 500 million cocci, half the former dose, at hourly intervals. This rabbit survived, though it appeared somewhat disturbed after the third dose. None of the other rabbits showed any untoward symptoms.

A sample of blood was collected every day from each rabbit as
Chart III.

RABBIT C I

RABBIT C III

C V

C VII

DAY

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300
before, and on the ninth morning (the eighth for rabbit III), they each received 4,000 million cocci in a single dose.

Chart III shows the agglutination curves of this experiment, and it will be seen that after a first rise similar to that in Chart II (see October number) a fall occurred—after the injection of the second dose of 4,000 millions—from a titre of 1 in 1,000 or thereabouts to about half this amount; in the case of rabbits III and VII a rise quickly followed to 1 in 1,200 on the twelfth day, while in rabbits I and V it only attained 1 in 1,000. The weights of the rabbits increased normally, though the second injection as well as the first temporarily lowered their weight by a few grammes. It thus appeared that the second dose increased the titre by fifty per cent above that in Series B within four days.

The methods of injection used with rabbit III and rabbit VII here again gave the best results. As the "rabbit III" method is a much simpler one, provided too ambitious a dose is not attempted at first, it would seem the better of these two; moreover, it should be noted that in this case rabbit III had only half the initial dose of the other rabbits, i.e., 1,500 millions as against 3,000 millions, though all received the second dose of 4,000 million cocci on the eighth or ninth day.

To determine if the period between the first and second dose could be lessened and a high titre agglutinin content attained in a still shorter time, it was decided to treat three rabbits with 1,500 millions of the sr-me type II coccus divided into three equal doses at hourly intervals, and then to give each rabbit a further single dose of 3,000 millions; one rabbit on the fifth day, a second on the sixth, and the third on the seventh day.

One of these rabbits was so ill after its second dose that the third was not given and the animal died the same night.

The other two rabbits had their 1,500 millions as arranged and seemed little the worse for them; one rabbit had its second dose (3,000 millions) on the sixth day, and the other on the seventh day.

A sample of blood from each rabbit was taken before the first injection and on each day from the sixth to the twelfth.

Chart IV shows the agglutination curves of this experiment as before.

In this case, the second injections being given on the rising curve did not seem to have the checking effect observed in former charts when the second dose was given at the highest point or on a descending curve. In both animals, D 1 and D 2, the agglutination titre rose to 1 in 1,200 or over on the eighth or ninth day after
Notes on Agglutinating Serum for Meningococcus

**Chart IV.**

<table>
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<th>DAY</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>800</td>
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<td>500</td>
</tr>
<tr>
<td>RABBIT DII</td>
<td>1500</td>
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<td>1300</td>
<td>1200</td>
<td>1100</td>
<td>1000</td>
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- 500 millions
- 500
- 500
the first injection and showed a tendency to remain nearer this titre than in the previous experiments in which the titre fell very shortly after attaining its greatest height.

CONCLUSIONS.

(1) That, as regards the rapid production of agglutinin for meningococcus by the rabbit, the injection of three suitable equal doses at hourly intervals on the first day appears to be more efficacious (as far as the variations of dosage tested are concerned) than either a single one of equal total amount, or a similar dose distributed in equal or varying fractions over a period of days.

(2) That, if after an interval of five to seven days a further single injection of double the initial amount be given, a serum is obtained in a little over a week from the commencement, having as high an agglutinin content as can be desired for practical purposes. (See Chart IV.)

(3) That Gordon and Horder's observation (Report Medical Officer, Local Government Board, 1907-8, pp. 341-358) that repeated doses of meningococcus are more toxic than a single dose of an equal or larger aggregate is confirmed.