

PRELIMINARY NOTE ON THE RAPID PREPARATION
OF HIGH-TITRE AGGLUTINATING SERUM FOR
THE MENINGOCOCCUS.

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IN the course of some recent experiments made in the Central Laboratory for Cerebrospinal Fever, for the purpose of obtaining, with the smallest possible delay, serum with a reasonably high content of agglutinin for the meningococcus, it was found that there was no sufficient certainty as to the results. The statement of Elser and Huntoon that young rabbits are preferable to full-grown ones for this purpose was confirmed. The intensive method of Fornet and Müller, by which increasing doses are given at intervals of twenty-four hours for three days in succession, was tried, with success in some cases but not in others. A method was next tried in which the rabbit was given a small initial dose, followed seventy-two hours later by three larger doses with an hour's interval between them. This method, like the preceding one, gave excellent results in some cases, but less satisfactory ones in others. It became necessary, therefore, to study the question of the production of agglutinin by the rabbit for the meningococcus more carefully than yet appears to have been done.

The following experiments were the first of a set undertaken for this purpose. All of the rabbits used were young ones. They were injected intravenously with a standardized suspension of the meningococcus (*See* "Identification of the Meningococcus," by Major M. H. Gordon). In making this suspension the twenty-four hours' growth of the coccus on legumin-agar plates is suspended in normal saline, heated to 65° C. for thirty minutes, standardized by its turbidity, and finally receives the addition of half per cent. of phenol as preservative.

The agglutinating titre of the serum was determined by the macroscopic method. Small test-tubes were used, three inches by a quarter of an inch. These are arranged in specially designed racks. The dilutions of serum are made in quarter cubic centimetre bulk in the tubes, and then an equal amount of suspension is added. The present results were all obtained at 55° C. and read off after twenty-four hours.

SERIES A.

Seven young rabbits, all weighing within sixty grammes of a kilogramme, each received one hundred and fifty millions of killed meningococcus, type I, of the present outbreak.

Rabbit I received the 150 million cocci in one dose.

Rabbit II received two doses each of 75 millions with an hour's interval between them.

Rabbit III received three doses each of 50 millions with an hour's interval between them.

Rabbit IV received the same three doses as rabbit III, but at twenty-four hours' interval.

Rabbit V received 25 millions and then, twenty-four hours later, 50 millions, and after a further interval of twenty-four hours 75 millions.

Rabbit VI received the same amounts as Rabbit V, but at forty-eight hours' interval instead of twenty-four hours.

Rabbit VII received 30 millions the first day and, seventy-two hours later, three doses of 40 millions at hourly intervals.

About half a cubic centimetre of blood was drawn from each rabbit's ear before the first injection and daily until fourteen samples had been taken from each animal; the blood on any day on which a dose was given being taken before the injection. The serum was separated from these and titrated against the standard suspension in the manner previously described.

The result is best seen in the accompanying curves (Chart I), where the limit of well-marked macroscopic agglutination on the various days is plotted.

In none of these rabbits did the serum show any degree of agglutination beyond a trace in the 1 in 10 dilution until the fourth day. On that day, however, there was definite agglutination with four of the sera up to the 1 in 100 dilution. On the fifth day the titre rose in all, except in the case of rabbits IV and VII. On the sixth or seventh day the titre reached 1 in 500 in all except rabbits VI and VII, the last of which did not reach this point until the ninth day. The titre of all the other sera began to fall about the eighth day. It is noteworthy that rabbit VI, in which the interval between the doses was longest (forty-eight hours), did not show a titre of over 1 in 200.

With one exception, the weights of these rabbits steadily rose by some two hundred or three hundred grammes during the period of this experiment. The exception was rabbit III, which died of peritonitis on the fifteenth day (apparently brought about by the bursting of a parasitic cyst in the liver).

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CHART I.

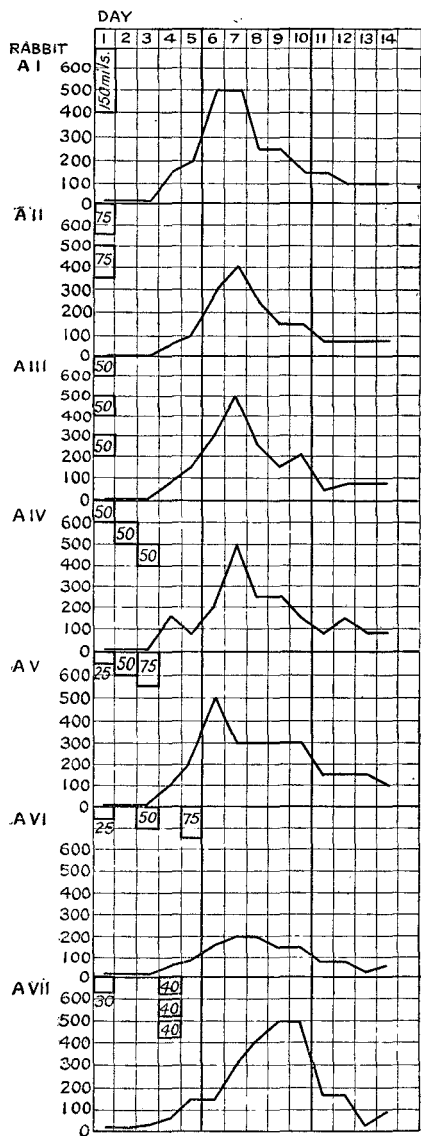
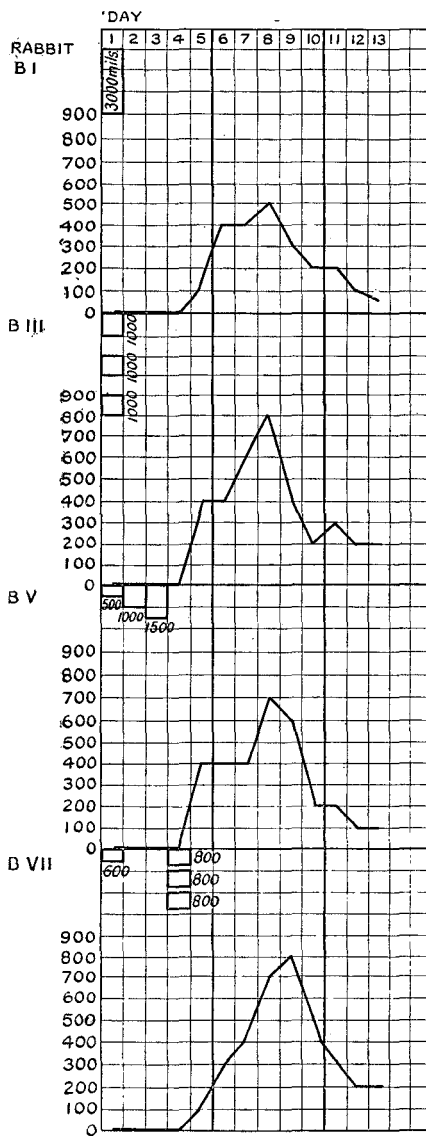


CHART II.



The figures on the left of each chart show the dilution of the serum, i.e., 700 means 1 in 700 dilution. Each dose of cocci is indicated by a rectangle, the numbers showing the number of millions per dose.

SERIES B.

In order to repeat the salient features of the preceding series, and to see at the same time if a better titre was to be obtained by increasing the size of the dose, four further rabbits were injected, each weighing just over one thousand one hundred grammes. The four rabbits in this series each received three thousand million of killed meningococci Type II. They were injected in the same way and with the same proportionate dose as rabbits I, III, V, and VII respectively of Series A had been, but in the present case each rabbit received twenty times the dose then given.

The results are shown in the curves recorded in Chart II. As in the case of the first series, the weights of the rabbits increased normally during the experiment.

The larger dose seems to have delayed the appearance of the agglutinin somewhat, but to have made it about sixty per cent higher. Rabbits III and VII gave the best results; that is to say, one large dose seems to be less efficacious than fractions of it given at short intervals, though aggregating the same.

The titre of 1 in 800 thus obtained in eight days is quite a workable one for the practical purpose of identifying the meningococcus, and, as these results may be useful to other workers, they are therefore now described. Further experiments amplifying them are in progress.
