

negative for complement fixing antibodies, although a reasonable number of such antibodies might still be present in the serum. With this possibility in mind, a series of complement fixation tests were carried out, using complement gradations of  $1\frac{1}{4}$  units,  $1\frac{1}{2}$  units,  $1\frac{3}{4}$  units and 2 units. The quantity of serum employed was 0.01 c.c.

It was observed that the same serum which gave negative results when 2 units of complement were employed, gave weak positive results with  $1\frac{3}{4}$  units of complement; stronger positive results with  $1\frac{1}{2}$  units; and still stronger with  $1\frac{1}{4}$  units of complement. In the last case the serum showed slight anticomplementary properties, which disappeared after about 10 minutes' incubation in the water bath.

This work is still being continued, but the results obtained thus far indicate that the employment of 2 units of complement in complement fixation tests is too great an excess of this ingredient for correct results in some cases, and that the employment of lesser quantities of complement, properly controlled, would serve as a finer measure of the complement fixing power of a given serum.

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**The thermostability of complement fixing antibodies resulting from protein immunization.**

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In a series of studies on the rate of destruction of antisyphilitic complement fixing substances by heat, recently reported by Kahn and Boyd,<sup>1</sup> it was observed that in practically all cases these substances were destroyed when subjected to temperatures ranging from 60 to 65 degrees C. These results were obtained by heating a number of fractions of syphilitic sera at different temperatures in the water bath and running regular Wassermann tests with each fraction. It was felt, however, that the results obtained with

<sup>1</sup> Kahn and Boyd, *Proceedings Society of Amer. Bacter.*, Dec., 1920. Abstr. of Bact., forthcoming Issue.

the complement-fixing substances present in the sera of syphilitic patients could not be applied to specific complement fixing substances obtained after protein injections, in view of the fact that, in the former case, the antigens employed were non-specific. This has led us to study the rate of destruction by heat of *specific* complement-fixing antibodies.

The mode of immunization as well as the complement fixation tests were conducted as indicated in the first paper of this series. The tests were carried out in each case with unheated serum and the same immune serum heated to varying temperatures, beginning with 5 minutes at 56 degrees C. and ending with 1 hour at 65 degrees C. It was soon found, however, that these temperatures did not lessen the antibody content of the rabbit serum, and that the thermal destructive point of these complement fixing antibodies existed apparently at a higher temperature level.

The sera were then diluted 1-10 with saline in order to raise the protein coagulation level (Ebersson) and placed in the water bath for 2 hours at 65 degrees C.; 1 hour at 70 degrees C.; and  $\frac{1}{2}$  hour at 75 degrees C.—without any apparent effect on the antibody content. The sera were then subjected to temperatures of 80 degrees C. and 85 degrees C. for 15 minutes. At the former temperature the antibodies were practically destroyed, while at the latter, completely destroyed.

The fact that the so-called complement fixing antibodies present in the sera of patients suffering from syphilis are destroyed when subjected to temperatures ranging between 60 and 65 degrees C. and that specific complement fixing antibodies withstand a temperature of 75 degrees C. suggests the possibility that there exists inherent biological differences between the two types of antibodies and opens a suggestive field for research.

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**The use of blood plasma in the imbedding or the dissection of small organisms.**

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Various methods have been used in order to overcome the difficulties attendant upon the carrying of a minute organism,