

Thus the first series were run both at water-bath and ice-box temperatures with the following fixation periods: 15 minutes, 30, 45, 60, 90 and 120 minutes. In view of the fact that complement has a tendency to be destroyed when exposed for too lengthy periods in the water-bath, the fixation tests at this temperature were not extended beyond 2 hours. Neither were the fixation periods extended beyond this time when the fixations were at room temperature. In the case of the ice-box, however, the fixation periods were continued for 3, 4, 5 and 6 hours, and occasionally longer. At the end of each fixation period, standard amounts of sheep cells and amboceptor were added to each set and placed in the water-bath to determine whether or not the complement had been "fixed."

The results indicate:

(1) That the phenomenon of fixation of complement goes on equally well at water-bath, room or ice-box temperature.

(2) That from 50 to 75 per cent. of fixation takes place during the first hour and that fixation is completed in the neighborhood of 4 hours at ice-box temperature.

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**The quantitative relation between complement and
complement fixing antibody.**

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In the course of investigation on precipitin and complement fixing antibodies produced by injections of edestin, it was observed that, while the serum of a rabbit immunized with this protein showed the presence of precipitin antibodies, it did not show any complement fixing antibodies when employing the usual 2 units of complement in the fixation tests. It appeared reasonable at first to accept this finding as evidence of the lack of relation between these two types of antibodies. It seemed, however, that possibly the employment of 2 units of complement in the tests might give a sufficient excess of this ingredient to render a serum

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.

83 (1665)

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 antisymphilitic complement fixing substances by heat, recently reported by Kahn and Boyd,¹ 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

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