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The effect of temperature on the rate of complement fixation.

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Suitable temperature is one of the most important prerequisites to the proper progress of biological reactions. Since the complement fixation reaction is used so widely at present and the relation between the temperature and rate of fixation has not been studied systematically thus far, we thought it advisable to study this question. This was especially necessary, since many authors have varied the technique of the routine complement fixation test in this respect. The practical points brought out in this study are as follows:

1. If a rapid fixation of complement is desired we found that a temperature of 37° C. is the best. For diagnosis one-half-hour incubation at this temperature is the most efficient. We found, however, that, if sufficient antibody is present in the serum (3–5 units or more), fixation of two units of complement already takes place within the first five minutes, provided the amount of antigen used contains several antigenic units. We find it possible to use this procedure for presumptive elimination of strongly positive sera from a large series of cases. One places in a tube 0.05 c.c. of the patient's serum, adds the proper amount of antigen and salt solution and incubates at 37° C. in the water bath for five minutes and then adds sensitized cells to test for free complement.[1]

2. If the time element is not so important, but complete fixation of the complement is desired, then we find that incubation in the ice box for 8–10 hours is best. These fixations on ice, however, may not be specific, for the reaction of fixation is so complete under these conditions that even traces of secondary circulating antigens and their corresponding antibodies may cause

[1] One must, of course, test 0.025 c.c. of the serum for complement at the same time, to ascertain that there are at least two units of complement originally present in the amount of serum used in the test.

fixation of complement.[1] The ice-box fixation can therefore be used only as a presumptive test to eliminate the negative cases.[2]

3. As for fixation of complement at temperatures below the freezing point we find that such a procedure produces undesirable changes in the reagents, especially in the antigen and is therefore unsuitable for the test.

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A study of the acid-base equilibrium of the blood in acute bichloride intoxications.[3]

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Thirty-two dogs have been used in the initial series of experiments. The animals were kept in metabolism cages, given 500 c.c. of water by stomach tube and fed on bread with a small amount of meat. The urine was examined qualitatively for albumin, acetone and glucose. The hydrogen ion (p. H.) concentration of the blood was determined by the method of Levy, Rowntree and Marriott, and the alkali reserve of the blood (R. p. H.) and the tension of alveolar-air carbon dioxide by the methods of Marriott. The phenolsulphonephthalein test was conducted according to the method of Rowntree and Geraghty.

The urine during two days of observation prior to the administration of the bichloride was normal. The hydrogen-ion content of the blood varied between 7.4 to 7.5, the reserve alkali between 8.05 to 8.1, while the tension of carbon dioxide in alveolar air has shown a variation between 40 to 45 mm. The total output of phthalein in a two-hour period has varied from 74 to 91 per cent.

The animals were starved for twenty-four hours prior to giving the bichloride. On the days of the experiments the animals were given 0.25 c.c. of a 4 per cent. solution of morphine sulphate

[1] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1916, XVI, p. 37.

[2] Bronfenbrenner and Schlesinger, *Am. Jour. of Spyhilis*, April, 1917.

[3] The expense of the investigation has been aided by a grant from the Rockefeller Institute for Medical Research.