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Heat from reactions between antigens and antibodies: Special reference to diphtheria toxin and antitoxin.

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Although Arrhenius[1] calculated from the effect of temperature upon the equilibrium constant that 5480 calories would be produced by the reaction between 1 gram molecule of tetanolysin with 1 gram molecule of antilysin, no one has actually measured the amount of heat liberated by this or any similar reaction between an antigen and its antibody. The substances are not available in a pure state, and hence immunological units must be used in place of exact quantitative terms. This is unsatisfactory. Nevertheless, as a basis for future work, the heat produced by two types of antigen antibody reaction was studied by me in the laboratory of the Department of Pathology of the Johns Hopkins Medical School during the period 1920-1923.

The instrument used was the differential microcalorimeter devised by A. V. Hill.[2] A delicate thermocouple, a White potentiometer, designed for thermoelectric measurements, and a suitable galvanometer were employed to obtain the calorimetric readings in terms of microvolts. This type of calorimeter is admirably suited to the study of the heat of a relatively slow reaction, as the differential arrangement of the vacuum thermos flasks balances the temperature effects due to changes in the environment. The heat effects due to dilution, and unknown constituents in the fluids containing the antigens and antibodies can also be cancelled by an appropriate mixture in the flask, serving as a control to the flask containing the reacting fluids. In Hill's paper, to which reference has been made, the laws governing the cool-

[1] Arrhenius, S., *Immunochemistry*, 1907, p. 181.

[2] Hill, A. V., *J. Physiol.*, Cambridge, 1911-12, xliii, 261-285.

ing of these flasks, the method of finding their coefficients of temperature loss, by which they can be adjusted to lose or gain temperature at the same rate, the method of correcting for loss of temperature and of computing the total heat evolved are set forth in adequate detail, and will not be repeated here.

After calibration of the apparatus, testing the heat production of known chemical mixtures and after a verification of the necessary controls, the heat produced by the union between diphtheria toxin and antitoxin, and by the agglutination of bacteria by an immune serum were measured.

Two preparations of diphtheria toxin and two preparations of antitoxic serum and pseudoglobulin were mixed in differing proportions, and the production of heat by their reaction was observed. The reaction was found to be exothermic. The average value obtained was based upon the heat liberated by the complete saturation of 1 unit of antitoxin by sufficient toxin to equal, in antigenic content, the equivalent by flocculation of 1 unit of antitoxin. In terms of the unit recently introduced by Glenny and Okell,[3] 1 unit of antitoxin combining with 1 L_f amount of toxin liberated 0.0645 gram calories.

At present, it is not possible to reduce this value to a more exact quantitative basis, as the amount of active substance, in grams, in 1 unit of antitoxin is not known, and the weight of 1 L_f amount of toxin is equally unknown. From the available data, however, it seems that the amount of heat produced, as found by these experiments, is a large amount. It is probably more of the order of that produced by many chemical reactions than of the magnitude of the heat produced by the mutual flocculation of oppositely charged colloids, as measured by Kruyt and van der Speck.[4]

Heat is evolved also when dead bacteria are agglutinated by an immune serum. Observations upon this effect were made upon a suspension of *B. typhosus* mixed with the serum of an immunized horse. Heat production during this reaction took place in two periods; the first corresponded to the period of the union of the bacterial antigen with the antibody, the second to the

[3] Glenny, A. T., and Okell, C. C., *J. Pathol. and Bacteriol.*, 1924, xxvii, 187-200.

[4] Kruyt, H. R., and J. van der Speck, *Kolloid-Zeitschrift*, 1919, xxiv, 145-155.

period of mechanical flocculation of the bacteria. The heat liberated by the combination between agglutinin and the antigen in this bacterial suspension, which consisted of 19 x 10^9 bacteria in saline, was 29 gram calories. The heat liberated by the clumping and flocculation of these bacteria was 10.8 gram calories. The immunological units employed in this instance have no quantitative significance.

Summary

The differential microcalorimeter was found to be suitable for the study of the heat produced by reactions between antigens and antibodies. Approximate values, which cannot be given definite quantitative meaning because of the nature of the crude solutions which must be employed, were obtained for the first time as follows:

The heat produced by the combination between 1 unit of diphtheria antitoxin and its equivalent L_f amount of diphtheria toxin was found to be 0.0645 gram-calories.

The reaction between dead typhoid bacilli and their specific agglutinin was also exothermic. Heat is produced during this reaction in two periods; the first corresponding to the phase of the union of the antigen with the antibody, the second to the clumping and flocculation of the bacteria.

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The weight curves of castrated kids.

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Twin kids, born February 5, 1923, were used for the experiments. One, without horns, was used as the control; the other with horns was castrated at the age of 74 days. Later another kid with horns born April 1, 1923, was castrated at the age of 83 days and was added to the experiment. The age at which the operation was performed, it is believed, caught the young goats at about half way to the age of puberty. Daily records (except Sundays) were kept of the weights since the operations.