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Thermostability of Heat-Stabile Components of Complement.*

E. E. Ecker, L. Pillemer and A. O. Kuehn.

From the Institute of Pathology and the University Hospitals of Western Reserve University, Cleveland, Ohio.

Complement is generally inactivated at 56° C for a period of 30 minutes although a much higher temperature is required to destroy its thermostabile components.

During the course of some experiments it was found that NaCl protected the third component and mid-piece (euglobulin) against the action of alkalis, zymin, etc. This observation led to the study of the thermostability of the component parts of complement with and without the addition of NaCl.

Methods. All titrations were done by the method of initial hemolysis described by Ecker, Pillemer, Wertheimer and Gradis.[1]

The inactivation of complement by the action of yeast was effected by a zymin preparation as described by Whitehead, Gordon and Wormall,[2] and of the fourth factor by the method of Gordon, Whitehead and Wormall.[3]

The general procedure for each experiment was as follows: One cc each of fresh guinea pig serum and of the same serum containing 10% NaCl by weight was pipetted into serological (10x1 cm) tubes and heated at temperatures ranging from 54° C to 66° C for a period of 30 minutes. After heating, the salt-containing serums were diluted 10 times with distilled water, and the salt-free serums with 0.9% saline. Equal parts of each of the 1:10 serums were then added to both the zymin-treated and the ammonia-treated serums. The mixtures were allowed to stand at room temperature for 15 minutes, and were titrated by the method of initial hemolysis. The heated serums, by themselves, were completely inactive. The heating was done in an especially constructed water-bath (Dewar flask) with an electrical control unit so that the variations at temperatures in the range of 64° C to 66° C were ±0.1°. At lower temperatures the variations were about ±0.05°.

Summary and Conclusions. From the accompanying graph it is

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1 Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.

2 Whitehead, H. B., Gordon, J., and Wormall, A., *Biochem. J.*, 1925, **19**, 618.

3 Gordon, J., Whitehead, H. B., and Wormall, A., *Biochem. J.*, 1926, **20**, 1028.

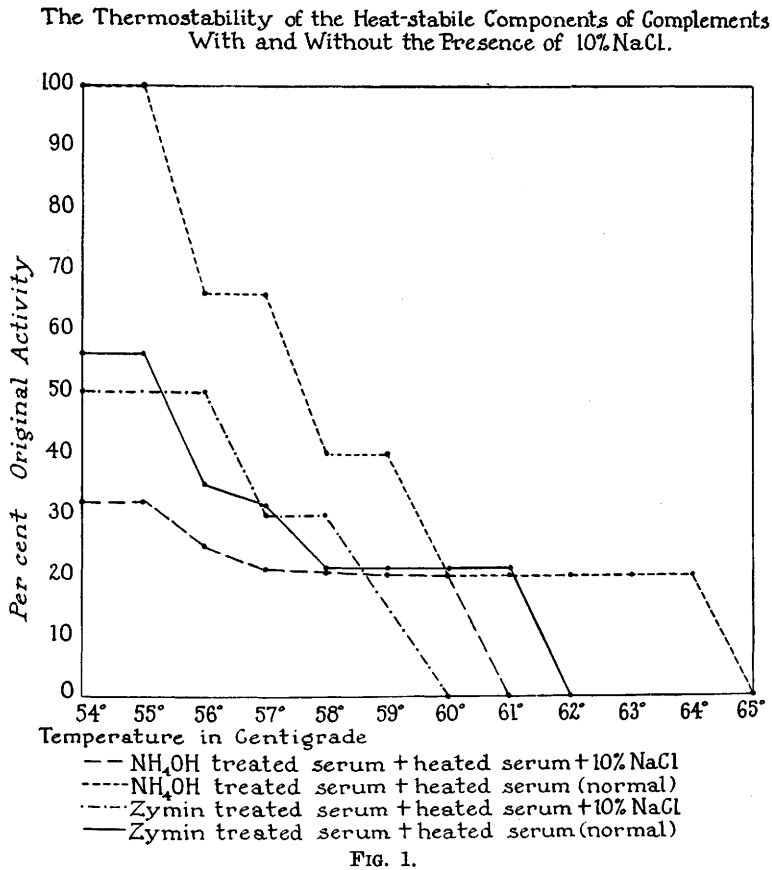

FIG. 1.

seen that the fourth component is relatively more heat-stabile than the third component. The addition of 10% NaCl to the complement prior to heating markedly diminishes the thermostability of the fourth component, while this effect is not noted in the case of the third component, except that in the presence of 10% NaCl the third component is completely inactivated at 60° C while in the absence of the salt a temperature of 62° C is required. It is also observed that the fourth component is inactivated at 65° C while the third component is inactivated at 62° C. From these findings it is evident that the third component is more intimately associated with the serum proteins than is the fourth component. It is therefore suggested that in the routine inactivation of complement for various serological purposes, inactivation be accomplished at 54° C instead of the customary 56° C, provided the temperature control is adequate.