THE TEMPERATURE COEFFICIENT OF INACTIVATION OF CRYSTALLINE PEPSIN BY ULTRA-VIOLET RADIATION

WORK BY FREDERICK L. GATES*

(From the Laboratory of General Physiology, Harvard University, Cambridge)

(Accepted for publication, May 2, 1934)

In determining the destruction spectrum of Northrop's crystalline pepsin (Northrop, 1929–30, 1932–33, 1933–34; Gates, 1933–34) and the changes in the absorption spectrum with inactivation of the enzyme by ultra-violet irradiation, it was noted that increase in the temperature to 65°C. for 5 minutes resulted in the inactivation of more than 50 per cent of the pepsin. The question remains as to how fluctuations in temperature will affect the rate of inactivation produced by the absorbed radiant energy.

From the nature of the reaction, *i.e.* the indicated single quantum relationship between the inactivation of the pepsin and the incident energy (Gates, 1934–35), it might be assumed that the process was direct or physical rather than chemical, and that a low temperature coefficient approaching unity would be obtained. To test this point and also to determine the effect of temperature fluctuations during the exposure period, experiments were performed in which crystalline pepsin (Lot 4) in sodium acetate buffer, pH 5.0, was diluted to 1 in 500 with M/100 HCl, pH 2.1, and exposed for different periods at several temperatures to radiation of wave-length 2357 Å.u.

The solutions and controls were exposed in the quartz-faced glass cells previously described (Gates, 1933–34), in a large quartz monochromator (Gates, 1929–30), with the temperature maintained at the desired level by a glass water chamber constructed on the side of the cell away from the exit slit of the quartz monochromator. Into this

* This paper is one of several in which are presented results of work completed by Dr. Frederick L. Gates before his death on June 17, 1933. The manuscripts have been prepared by Professor W. J. Crozier and Dr. R. H. Oster.

chamber a constant stream of water flowed from a storage vessel which could be heated by an electric heater or cooled by ice cubes. During the exposure the control cell was protected from direct radiation by the exit slit of the monochromator.

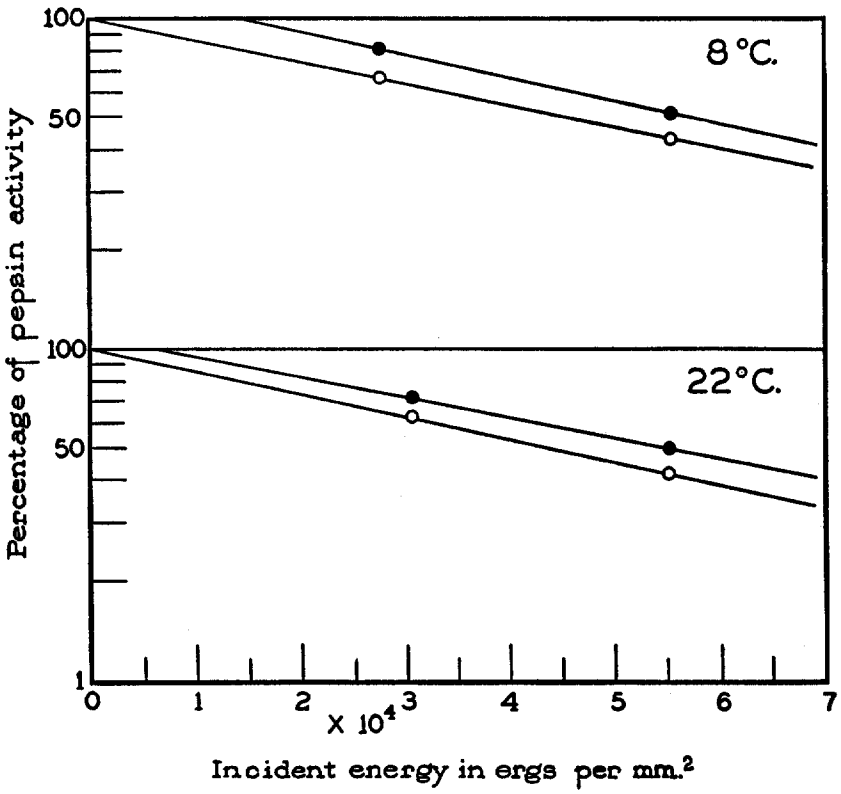

FIG. 1. The rate of inactivation of crystalline pepsin, at pH 2.1, by ultra-violet irradiation at 2537Å.u., for different periods of time, at two different temperatures. Solid circles represent calculations on the basis of 100 per cent activity for the corresponding controls. Clear circles represent points calculated on the basis of 100 per cent for the flask control.

After irradiation the exposed specimens and their controls, along with flask controls, were tested for pepsin activity by the hemoglobin method of Anson and Mirsky (1932–33).

The protocol of tests carried out at 22° and 8°C. is given in Table

I. The pepsin activity for each specimen and control has been calculated by the equation used by Anson and Mirsky (1932–33, p. 61). To determine the inactivation due to the absorbed ultra-violet energy alone, the per cent activity has been calculated on the basis of 100 per cent activity for each control.

TABLE I

Experiments on the Inactivation of Pepsin Solutions by Ultra-Violet Irradiation at 2537 Å.u., pH 2.1, at Two Temperatures. Pepsin Activity Determined by the Hemoglobin Method

Specimen	T.	Period of exposure	Energy			Colorimeter reading average	Hb (P.U.) ml. × 100	Pepsin activity		Energy to inactivate 50 per cent of pepsin. Incident	
			Incident	Transmitted	Absorbed						
	°C.		ergs/mm.²	ergs/mm.²	ergs/mm.²			per cent	per cent		
A_1	8	37′24″	27,800	7,850	19,950	14.9	1.152	80.2	66.7*	56,600	45,300*
B_1		—				12.3	1.438	100			
A_2		74′48″	55,600	15,700	39,900	22.0	0.739	51.0	42.8*		
B_2		—				12.2	1.449	100			
C_0		Flask control				10.4	1.728		100*		
D_1	22	37′24″	30,880	8,960	21,920	15.9	1.080	71.8	62.5*	54,700	44,000*
E_1		—				10.7	1.675	100			
D_2		74′48″	55,200	16,080	39.12	22.0	0.739	49.2	42.8*		
E_2		—				11.8	1.505	100			

* On the basis of flask control = 100 activity.

On plotting the per cent activity of each specimen at the given temperature as a function of the incident energy, a straight line may be drawn through the points (Fig. 1) according to the one-quantum relation found in previous tests (Gates, 1933–34; Northrop, 1933–34). For the values calculated on the basis that each corresponding control

represents 100 per cent pepsin activity (solid circles) the line does not strike the origin, due probably to stray light and other external factors. The temperature coefficient on this basis, obtained by taking the reciprocal of the energy ratios for a 10° change in temperature (Column 11, Table I), was found to be 1.024. If we use the activity of the flask control as 100 per cent activity the resultant curves strike the origin (clear circles) and the temperature coefficient is 1.020. In either case the temperature coefficient is so near to unity that a direct inactivation of the enzyme by the absorbed energy is indicated. Hussey and Thompson (1925–26) found a similar situation for the inactivation of pepsin by radiations from the radioactive products in equilibrium with radium emanation.

CONCLUSION

Determinations of the temperature coefficient of inactivation of pure crystalline pepsin solutions by ultra-violet irradiation give values very close to unity (1.02).

BIBLIOGRAPHY

Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932–33, **16,** 59.

Gates, F. L., *J. Gen. Physiol.*, 1929–30, **13,** 231; 1933–34, **17,** 797; 1934–35, **18,** 265.

Hussey, R. G., and Thompson, W. R., *J. Gen. Physiol.*, 1925–26, **9,** 315.

Northrop, J. H., *J. Gen. Physiol.*, 1929–30, **13,** 739, 767; 1932–33, **16,** 41; 1933–34, **17,** 359.