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Effect of Temperature on Activity of Choline-Esterase.*

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Kahane and Lévy[1] observed a temperature optimum for the combined enzymatic and non-enzymatic hydrolysis of acetylcholine in the presence of horse serum extending from 38° to 60°. In the present investigation the purely enzymatic activity was found to have a very narrow optimum at 40°. Apparently the broad maximum found by Kahane and Lévy was the resultant of increased non-enzymatic hydrolysis occurring as the temperature was elevated, and coexistant decreased enzymatic scission. Early studies on the heat-inactivation of choline-esterase were carried out by Abderhalden and Paffrath,[2] who found that the choline-esterase in the press-juice of the small intestine of the pig was unaffected by heating at 55-58° for 5 minutes, whereas after 2 hours a certain loss of activity resulted. Heating for 5 minutes at 70-75° caused total destruction of the enzyme. Loewi and Navratil[3] found that frog heart extracts had a less destructive action upon acetylcholine when they were heated at 56° for 20 minutes. Plattner and Hintner[4] later confirmed the gradual inactivation of the enzyme in organ extracts when the latter were held at 56°.

Balls and Matlack[5] observed that the splitting of benzyl butyrate or stearate by pancreas extracts changes from a zero order reaction at 40° to more of a monomolecular reaction as the temperature is lowered. Experiments presented in this paper show that the course of the enzymatic scission of acetylcholine is unaffected by a temperature change from 40° to 25°.

Because of the ease with which the course of the hydrolysis could be followed, the manometric method employing the Warburg apparatus was used for these experiments in a manner essentially the same as that previously employed.[6] Correction for non-enzymatic hydrolysis was made in every case. One-half ml of 2% horse serum was used in the side arm of the reaction flask and 1.5 ml of acetyl-

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1 Kahane, E., and Lévy, J., *Compt. rend. Acad.*, 1936, **202**, 781.

2 Abderhalden, E., and Paffrath, H., *Fermentforsch.*, 1925, **8**, 299.

3 Loewi, O., and Navratil, E., *Arch. ges. Physiol.*, 1926, **214**, 678.

4 Plattner, F., and Hinter, H., *Arch. ges. Physiol.*, 1930, **225**, 19.

5 Balls, A. K., and Matlack, M. B., *J. Biol. Chem.*, 1938, **125**, 539.

6 Glick, D., *J. Biol. Chem.*, 1938, **125**, 729.

choline chloride solution (5 mg per ml) in the main chamber. Readings were taken every 10 minutes for 60 minutes to obtain the slope of the initial linear reaction curve, and the points in Fig. 1 represent the hydrolyses after 60 minutes. The course of the reaction was linear for the 60-minutes period at all temperatures except 50°. In this case an extrapolation from the linear portion to the 60-minutes point was made. The temperature coefficients in Table I were calculated from data taken from Fig. 1. In order to observe the effect upon the course of the reaction of a change from 25° to 40°, an experiment was performed using only 3 mg of substrate in each vessel. The quantity of substrate was reduced in order that a shorter time be required for following the reaction to about 50% of the total hydrolysis. The temperature constants in Table II were calculated from these data.

The curve for the effect of temperature upon the activity of choline-esterase (Fig. 1) drops sharply on both sides of the optimum. The temperature coefficient, which is the ratio of the enzymatic activity at a given temperature to that at a temperature 10° lower. may be seen to fall to a small extent as the temperature increases toward the optimum; this behavior is common for hydrolytic enzymes. Clark, Raventós, Stedman and Stedman[7] found the rate of

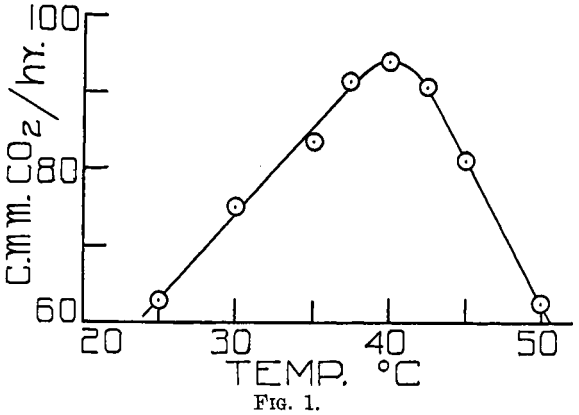

FIG. 1.
Effect of Temperature upon the Activity of Choline Esterase.

TABLE I.
Temperature Coefficients of Choline Esterase.

Temp. range, C°	$K_{t+10} : K_t$
25-35	1.36
30-40	1.27
40-50	0.66

[7] Clark, A. J., Raventós, J., Stedman, E., and Stedman, E., *Quart. J. Exp. Physiol.*, 1938, **28**, 77.

TABLE II.
Velocity and Temperature Constants of Choline Esterase.

t min	25° mm³ CO_2 evolved	k_1	40° mm³ CO_2 evolved	k_2	Calories*
60	55	.00268	80	.00405	5100
120	103	.00272	144	.00411	5100
180	145	.00276	194	.00412	4950

*$A = \frac{\log k_2 - \log k_1}{0.4343} R \frac{T_2 T_1}{T_2 - T_1}$. k_1 and k_2 are velocity constants, R is the gas constant, and T_1 and T_2 are absolute temperatures referring to k_1 and k_2 respectively.

hydrolysis at 18° to be two-thirds that at 37°. The magnitude of the coefficients is of the same order as that found by Kastle and Loevenhart[8] for the action of pancreas and liver extracts upon ethyl butyrate, namely 1.34 (10-20°), 1.26 (20-30°), and 1.36 (10-20°), 1.10 (20-30°) respectively. Terroine[9] obtained higher values for the hydrolysis of ethyl butyrate by pancreas: 2.03 (5-15°), 1.96 (15-25°).

It was observed that the rate of enzymatic hydrolysis at 50° fell off rapidly after 45 minutes, whereas at 45° the reaction was still linear after 90 minutes. A progressive destruction of enzyme by heat was no doubt the basis of this effect. There seem to be two factors operating: a lowered activity independent of heat destruction and requiring no time interval to become manifest, shown by the decrease in the slopes of the linear portion of the reaction curves at 42.5 and 45° as compared to that at 40°, and a gradual destruction of enzyme by heat, demonstrated in addition to the first effect, at 50°.

The temperature change from 25° to 40° had little effect upon the course of the hydrolysis. During the second hour the reaction deviated from the linear to an essentially monomolecular one. Velocity constants calculated for the second and third hours according to the monomolecular formula: $k = 2.3/t \log a/a-x$ were in turn used to calculate temperature constants. (Table II). The values found fell between those reported for ester hydrolysis by Kastle, 4100, and Terroine, 11,600, quoted by Haldane.[10]

Summary. The activity-temperature relationship for choline-esterase in horse serum was investigated and a narrow optimum at

[8] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491.

[9] Terroine, E. F., *Biochem. Z.*, 1910, **23**, 404, 429.

[10] Haldane, J. B. S., *Enzymes*, New York and London, 1930, 67.

40° was observed. The decreased activity above 40° is believed to result from two factors, a slowing of the reaction independent of enzyme destruction, and destruction itself, first found to occur at 50°. The course of the reaction was not appreciably affected by a change in temperature from 25 to 40°. Temperature coefficients and constants for the action of this enzyme were calculated.

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Germicidal Action of Bromine.

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The halogens, fluorine, chlorine, bromine, and iodine are strong oxidizing agents which combine readily with protoplasm. Chlorine is widely used as a disinfectant in water-treatment while iodine is a much used skin-disinfectant. Fluorine is probably too active to use in disinfection. Less work has been done with bromine, which strongly resembles chlorine in its general properties. Review of the literature gives little information on relative resistance of pure cultures of microörganisms to bromine under known conditions. Beckwith and Moser[1] reported that chlorine, bromine, and iodine were about equally effective toward *Escherichia coli,* that is, 0.2 parts per million of each halogen showed 95 to 97% efficiency in 60 minutes. Wood and Illing[2] found that after 5 minutes, water containing *Escherichia coli* was sterile in presence of 0.15, 0.35, and 0.45 ppm. Henderson[3] patented a device for "antisepticizing" water with bromine. Hildesheim[4] used bromine for treatment of swimming-pool water. Bromine has also been used as a seed-disinfectant by DeZeeuw,[5] Wilson,[6] Matskov,[7] and LaRue.[8] Other work has been

1 Beckwith, T. D., and Moser, F. R., *J. Am. Water Works Assn.*, 1933, **25**, 267.
2 Wood, R. D., and Illing, E. T., *Analyst*, 1930, **55**, 126.
3 Henderson, C. T., U. S. Patent, 1935, 1,995,639.
4 Hildesheim, H., *Tech. Gemeindeblatt*, 1936, **39**, 56.
5 DeZeeuw, R., *Exp. Sta. Rec.*, 1912, **26**, 819.
6 Wilson, J. K., *Am. J. Bot.*, 1915, **2**, 420.
7 Matskov, F., Physiol. Untersuch. Zuckerriibe Erste Artikelserie, Ukrainisches Inst. Angew. Bot. Sect. Pflanzenphysiol. Charkiv 156-190, 1930.
8 La Rue, C. D., *Science*, 1937, **85**.