Temperature and Enzyme Activity.

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With 4 Figures in the Text.

I HAVE recently carried out an experiment upon the amylolytic enzyme of the crystalline style of the scallop (*Pecten opercularis*), which has given some unexpected results. The experiment was suggested to me by Mr. C. F. A. Pantin, and I wish to take this opportunity of thanking him very much for supervising the work, which was carried out at the Marine Biological Laboratory at Plymouth.

Crystalline styles were removed from sixty-four scallops, weighed, ground up with sand, and dissolved in distilled water to form a 1% solution, which was then filtered. Three experiments were started with this solution directly it had been prepared :—

The solutions were at about pH 6.

(1) *Optimum Temperature Experiment.*

10 c.c. of style solution and 10 c.c. of 1% starch solution were placed in each of six test tubes, which were kept for three hours at constant temperatures, varying from 0° C. to 65·5° C. At the end of this period enzyme action was checked by boiling, and the solutions titrated into Benedicts solution to find how much sugar had been formed. An ordinary optimum curve was thus obtained, which is shown in Fig. 1 (Yonge, 1923).

(2) $1\frac{1}{2}$ *Hours' Incubation Experiment.*

Test tubes containing 10 c.c. of the crystalline style solution were incubated for $1\frac{1}{2}$ hours at constant temperatures varying from 0° C. to 66° C. All the test tubes were then placed at room temperature (16°), and 10 c.c. of starch solution was added to each. The tubes were left at room temperature for three hours and the amount of sugar in each estimated as before. The results of this experiment are shown by the graph in broken lines in Fig. 2.

(3) 3 *Hours' Incubation Experiment.*

Precisely the same experiment was performed, except that the test tubes were incubated at the various temperatures for 3 hours instead of $1\frac{1}{2}$. The unbroken line in Fig. 2 gives the result of this experiment.

A glance at these graphs shows at once that there is progressively increasing destruction of enzyme above a certain temperature (Bayliss, 1919). But another very remarkable and quite unexpected fact also

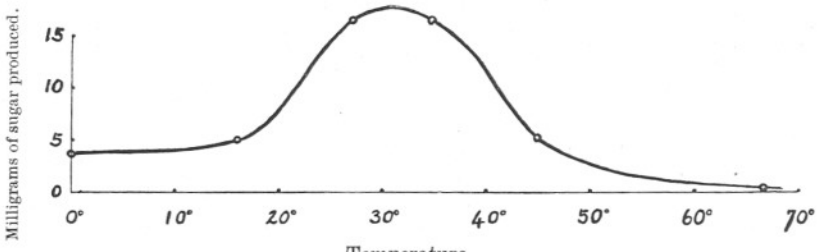

FIG. 1.

appears. This is that if one incubates the crystalline style solution filtrate at 0° C. or at room temperature before adding the starch solution, the amount of sugar subsequently produced is very much greater than if no such incubation had taken place. To put it in concrete terms, 10 c.c. of style solution produce 5·0 milligrams of sugar in 3 hours at room temperature; but if the style solution has been kept for 3 hours previously at room temperature, no less than 13·3 milligrams are produced! It was suggested to me that the reason for this may be that the protein base upon which the enzyme is absorbed is at first in

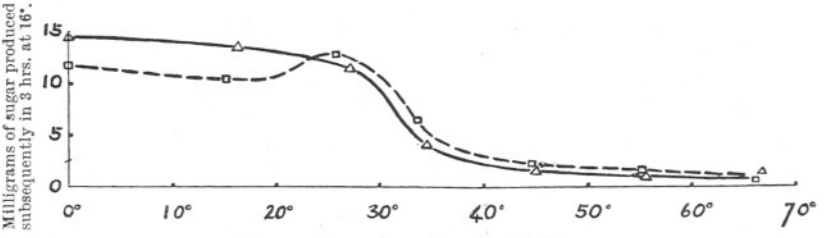

FIG. 2.

fairly large particles, which gradually become more dispersed. As they become more dispersed their surface area becomes greater and the efficacy of the enzyme is thus increased.

The action cannot be due to a co-enzyme; for this would have ample time to effect the activation of the style while in the body of the animal. It should be borne in mind that the style is a pure secre-

tion unmixed with digesting matter or tissue extracts and contains no proteases, lipases, etc.

These effects account for the form of the two curves in Fig. 2. The curves show the activity in producing sugar at room temperature (16° C.). If the pure enzyme solution has previously been kept at a low temperature (0°—10° C.) the activity is greater after three hours of such treatment than after 1½ hours. This is because there is but little destruction of the enzyme at this temperature, but there is a considerable progressive increase in activity on standing. After previous treatment at high temperatures the destructive effect is overwhelming, and the greater the incubation period at the higher temperature the greater the destruction of the enzyme. Hence the activity after 1½ hours' incubation is greater than that after three hours at high temperatures.

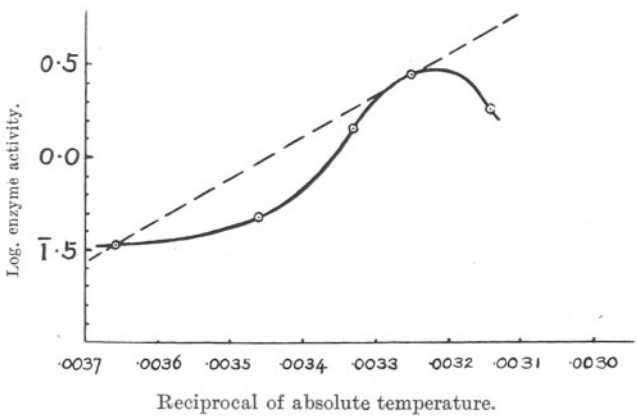

FIG. 3.

It appears, then, that two antagonistic processes are at work while the enzyme is mixed with the starch: (1) Destruction of the enzyme is taking place, particularly at high temperatures. (2) The enzyme is becoming more and more effective as time goes on, possibly as a result of dispersion of its protein base. An attempt has been made to calculate the true velocity of enzyme action, when these two processes are discounted, in a comparable way to that in which Pantin extrapolated a curve for the rate of movement of Amœba above the temperature at which destructive processes are beginning to act (see *Brit. Journ. Exp. Biol.*, Vol. I, No. 4, pp. 519–38).

In Fig 3 the relative velocities of glucose formation are given for different temperatures. The sugar produced appears to be directly glucose: the style being without action on Maltose (Yonge, 1923.)

This is peculiar, inasmuch as in the normal course of digestion Maltose is an intermediate compound from which glucose is produced.

The log. of the velocity is plotted against the reciprocal of the absolute temperature. According to the Arrhenius formula these should be related linearly, the slope of the line depending on the value of μ (Critical thermal increment, cf. Crozier, 1925).

The unbroken line in Fig. 3 gives the results of my experiments. The curve is not taken above 45° C., because the amounts of sugar produced at higher temperatures are too small to give significant results.

The obvious departure from a straight line is probably not due to experimental error. There is no necessity to suppose that Arrhenius' law holds in a heterogeneous system where the effective enzyme surface may vary with temperature.

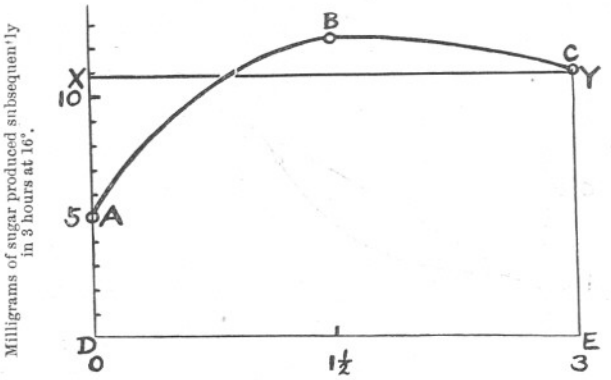

FIG. 4.

I will explain very shortly the method, suggested to me by Mr. Pantin, by which the figures were obtained on which the curve is based. I wish to thank my brother, Mr. S. J. Baker, for assistance in carrying out Mr. Pantin's suggestions.

Owing to the variation of enzyme activity with time, it is necessary to determine the *mean* enzyme activity.

Five graphs were drawn to find the *mean enzyme activity during* 3 *hours* at five different temperatures, namely, 0°, 16°, 27°, 35°, and 45°. I Fig. 4 I give the graph for 27° to serve as an example of these five graphs. The curve ABC shows the enzyme activity after various periods of incubation at 27°. The *mean* enzyme activity is found as follows: A line XY is drawn parallel to the abscissa DE at such a distance from it that the area of the rectangle XYED equals the area of the figure ABCED. The distance of the line XY from the abscissa DE gives the mean enzyme

activity for a period of 3 hours expressed in milligrams of sugar subsequently formed after another 3 hours at 16°.

The values thus obtained are given in the fifth column of the table below. They show the (combined) effect of destruction and (?) dispersion on the enzyme. As we are trying to obtain figures *discounting* destruction and (?) dispersion, we must *divide* the results obtained in experiment (1) by them. The results of this division are given in the sixth column. We now have figures proportional to enzyme activity when destruction and (?) dispersion are discounted. I have not attempted to calculate absolute values.

Centigrade Temperature.	Absolute Temperature.	Reciprocal of absolute Temperature.	Mg. of sugar produced in 3 hours with no previous incubation (Exp. 1.)	Mean Enzyme Activity.	Col. 4 divided by Col. 5.	Logarithms of numbers in Col. 6.
0	273	·00366	3·8	11·0	0·35	1·5441
16	289	·00346	5·0	10·0	0·5	1·6990
27	300	·00333	16·5	10·8	1·5	0·1761
35	308	·00325	16·6	5·8	2·9	0·4624
45	318	·00314	5·1	2·7	1·9	0·2788

SUMMARY.

The enzyme contained in the crystalline style of Pecten is destroyed more and more rapidly as temperature increases. On the other hand, it becomes more and more active if incubated at fairly low temperatures, possibly as a result of the progressive dispersion of the protein base on which it is absorbed. A graph has been calculated showing the effect of temperature on enzyme activity when both destruction and (?) dispersion are discounted.

REFERENCES.

Bayliss, W. M. 1919. The Nature of Enzyme Action. 4th edition.

Crozier, W., and Federighi, H. 1925. Journ. Gen. Physiol., VII, 565.

Yonge, C. M. 1923. Brit. Journ. Exp. Biol., I, p. 15.