TEMPERATURE COEFFICIENTS OF ENZYMIC ACTIVITY
AND THE HEAT DESTRUCTION OF PANCREATIC
AND MALT AMYLASES.*

BY DONALD H. COOK.

(From the Department of Chemistry, Columbia University, New York.)

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There are in the literature many references to the action of heat on enzymic processes. In general these are not specific, and where they are they deal, as a rule, with one isolated point such as the temperature of optimum activity or that of destruction. Not much reliance can be placed on the data given in some of the earlier work (1, 2), as at that time very little was known as to the conditions for the optimum activity of enzymes. The effect of heat on enzymic activity through a wide range of temperature has been little studied. As enzymes are, in general, unstable substances, the effect of heat upon any reaction catalyzed by them is made up of a twofold effect. Euler (3) points out that a rise in temperature first accelerates the chemical velocity and second, inactivates the enzyme. Thus in plotting curves of the activity of an enzyme with increase in temperature the curve always tends to rise to a maximum. Sorensen (4) says: "Therefore, the curve of the temperature should be considered as if it were two entirely different curves, namely, a curve of the real temperature effect which increases in proportion to the temperature elevation, not only below but probably above the optimum temperature, and on the other side a curve of the destruction of the enzyme." The maximum of this temperature curve varies according to the enzyme employed, the acidity, concentration of the substrate, presence of salts, type of solvent, etc. Much of the confusion arising from apparently conflicting data in the literature is due to the lack of fulfilling the conditions of optimum activity of the enzyme.

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especially in regard to hydrogen ion concentration and activating salts. Much of the work of various investigators cannot be compared quantitatively because of failure to insure the optimum hydrogen ion concentration. At any other hydrogen ion concentration the enzyme is less active and also, according to Lüers and Lorinser (5), less stable. Sherman, Thomas, and Baldwin (6) determined the hydrogen ion concentrations at room temperature which enable pancreatic and malt amylases and taka-diastase to exert their optimum activities when acting upon soluble starch at 40°C. for 30 minutes under definitely specified conditions.

It has been stated as a general rule that for every 10°C. rise in temperature, the velocity of a chemical reaction increases two- to threefold. Harcourt and Esson (7) found for the liberation of iodine from HI by H₂O₂ that between 0° and 50° the velocity was about doubled for every 10° rise. Hudson and Paine (8), working with invertase, found the coefficient of destruction by acids and alkalies was between 2 and 3 for every 10° for the interval 0°-45°. Auld (9) working with amygdalin emulsion showed a temperature coefficient of 2 for every 10° between 15°-35°C. Vernon (10), using starch and amylase, found that the velocity of hydrolysis was twice as great at 30° as at 20°. Kendall and Sherman (11) give 2 as the approximate temperature coefficient of pancreatic amylase for an interval of 10° between 20° and 40°.

In the light of this previous work it appeared that it might be of interest to study the effect of temperature not only on the speed of hydrolysis but also on the rate of inactivation of two typical starch-splitting enzymes. Pancreatic and malt amylases were chosen for investigation.

Materials Employed.

The starch used in this work was Merck’s "soluble starch according to Lintner." It was washed nine times with distilled water, six times with triply distilled water, and air-dried.

Ordinary distilled water was twice redistilled, first over alkaline permanganate solution, then over very dilute solution of orthophosphoric acid; the distillate was condensed in a block tin condenser. This product was always used for making up starch and stock solutions and for the final rinsing of all glassware.
The primary and secondary phosphates used as buffers were purified according to the unpublished methods of Beans and Kiehl. The sodium chloride used was twice recrystallized from triply distilled water and dried at 120°C.

The two enzyme preparations were commercial products: Parke, Davis and Company pancreatin, laboratory sample No. 9, and a barley malt obtained from the Froedert Malting Co. The malt extract was prepared by grinding 100 gm. of malt and extracting with 250 cc. of triply distilled water at ice box temperature for 2 hours. The extract was then filtered and kept in the refrigerator in a steamed Nonsol bottle. The total solids and reducing substances present in the extract were determined for each new preparation. This stock solution of malt extract was tested from day to day and did not change in activity during the time of the experiments with it. The pancreatin was prepared fresh for each day's experiment and was made up by weighing out 50 mg. of the dry substance and making up to a definite volume with a cold solution of phosphate of the required concentration. The activities of these preparations were determined according to the method of Sherman, Kendall, and Clark (12). The pancreatin had an average activity of 300 on their scale and the malt extract an activity of 48.

EXPERIMENTAL.

1 per cent starch solutions were used as the substrates in these experiments. They were adjusted to pH 7 for the work with the pancreatin and contained 0.05 molar sodium chloride and 0.0005 molar disodium phosphate. In the experiments with malt amylase the starch solutions contained 0.03 molar dihydrogen phosphate and were adjusted to pH 4.5.

The measurements of pH were made either electrometrically or colorimetrically at room temperature (about 22°C.). Although the above conditions, which were chosen because they had been shown by Sherman and coworkers to give the best results with these enzymes in tests of activity carried out at 40° for experimental periods of 30 minutes, may not represent the optimum conditions for testing the activities of these enzymes at other temperatures and for different periods of time, they give a basis for quantitative comparisons of the influence of temperature when starting with the same standard initial conditions.
The direct tests of activity at the various temperatures were carried out as follows: The starch solutions prepared as described above were thoroughly mixed and poured into 100 cc. measuring cylinders which were then immersed in the thermostat at the temperature at which the digestion was to be carried out. 50 mg. of the enzyme preparation, in the case of pancreatin, were then weighed out and made into a smooth paste with a drop of cold water containing the same concentration of sodium chloride and disodium phosphate as the starch solution. This was then washed into a 100 cc. volumetric flask with the cold water-salt solution, made up to the mark, and kept at 10° or colder until used. In the case of malt extract, 2.5 cc. of the extract were made up to 100 cc. with cold triply distilled water without salts. Varying amounts of these enzyme solutions, depending on the activity, were pipetted with an accurately calibrated 1 cc. pipette into dry 200 cc. Erlenmeyer flasks. The starch mixtures in the cylinders were then poured into the flasks at 15 second intervals and the flasks immersed in the bath for 1 hour. At the expiration of this period 50 cc. of Fehling's solution were added to each flask at 15 second intervals and in the same order in which the starch was poured on the enzyme. The flasks were then immersed in a boiling water bath and held at the boiling point for 15 minutes. The cuprous oxide formed was rapidly filtered through Gooch crucibles, washed with hot water, alcohol, and ether, dried at 100°C., and weighed. Maltose was calculated from Defren's table.

The temperatures chosen were 20°, 30°, 40°, 50°, 60°, and 70°C. As a rule five determinations were made simultaneously, four at a lower temperature and one at a temperature 10° higher. Thus, each new series of experiments overlapped the previous work and was a constant check on the technique and the activity of the enzyme. Blank experiments were made not only with the enzyme preparations, but also with the starch and reagents in each series of experiments.

As the presence of starch appears to exert a protective action upon these enzymes it was also of interest to study the influence of changes in temperature upon the enzymic activities when the solutions were held at the various temperatures in the absence of the substrate before being allowed to act upon it.

For these inactivation experiments the above procedure was modified as follows: 300 cc. of a 2 per cent starch solution containing the molar concentrations of sodium chloride and sodium phosphate given above were prepared and placed in 50 cc. portions in the thermostat. 300 cc. of a water-salt solution containing the same concentrations of these salts were also prepared. As both the water-salt solution and the starch solution contained the same concentration of activating salts they would give the standard 1 per cent substrate upon being combined. The water-
salt solution was divided into 50 cc. portions which were immersed in the thermostats in test-tubes and after reaching the desired temperatures were poured on the enzyme solutions at 15 second intervals. The enzyme solutions were then placed in the thermostats and allowed to remain for 30 minutes. At the end of this time the starch solutions previously brought to the same temperatures were added to the enzyme-water mixtures at 15 second intervals and in the same order as the water-salt solutions had been added. The enzyme was allowed to act upon the starch for 30 minutes. Fehling's solution was then added and the rest of the procedure as outlined above was carried out. The method of overlapping experiments from day to day was followed here in order to guard against any deterioration of the enzyme or other possible source of error. The 30 minute period of heating without substrate was, at the higher temperatures, shortened to 15, 10, and 5 minutes in order to follow the rapid rate of destruction of the enzyme which was found to occur.

RESULTS.

As this investigation covered a wide range of temperatures it was necessary to increase the quantity of enzyme used, at 20° and 30°, in order to obtain enough cuprous oxide to weigh, while at the higher temperatures the activity of the enzymes was so increased that much smaller amounts were sufficient. In order to have a basis for the comparison of results and the plotting of curves it was decided to calculate the amounts of maltose formed by a fixed quantity of enzyme. The amount of enzyme chosen, which appeared to yield the most satisfactory values, was taken as 0.1 mg. of the dry pancreatin or of the dry matter of the malt extract. The data shown in Tables I and II were obtained in this way and give the average amount of maltose per 0.1 mg. of pancreatin or of the solids of the malt extract for each temperature. The values given under the headings "Without previous heating" in Tables I and II show the influence of temperature upon the activities of pancreatic and malt amylases in the hydrolysis of starch when acting upon the standard 1 per cent starch mixtures described above for 30 minutes at temperatures ranging from 20° to 70°C. The other data given in Tables I and II show the influence upon the enzymic activities of heating portions of the enzyme solutions in the absence of substrate in 50 cc. of the water-salt solutions described above for 30 minutes at each of the temperatures before allowing them to act upon the substrates at those temperatures. As in the latter experiments the enzyme is rapidly inactivated at.
the higher temperatures it was necessary to decrease the time of preliminary heating of the enzyme in the absence of substrate.

### TABLE I.

**Experiments with Pancreatin.**

(Average of all results calculated to 0.1 mg. of enzyme.)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>20°</th>
<th>30°</th>
<th>40°</th>
<th>50°</th>
<th>60°</th>
<th>70°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without previous heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td>22.9</td>
<td>48.7</td>
<td>87.1</td>
<td>123.7</td>
<td>95.8</td>
<td>5.7</td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td>18.3</td>
<td>39.1</td>
<td>70.5</td>
<td>100.2</td>
<td>77.5</td>
<td>4.5</td>
</tr>
<tr>
<td>After 30 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td>23.3</td>
<td>48.9</td>
<td>68.8</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td>18.7</td>
<td>39.4</td>
<td>55.7</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 15 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td></td>
<td>72.8</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td></td>
<td>58.8</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 5 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td>2.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td>1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

### TABLE II.

**Experiments with Malt Amylase.**

(Average of all results calculated to 0.1 mg. of enzyme.)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>20°</th>
<th>30°</th>
<th>40°</th>
<th>50°</th>
<th>60°</th>
<th>70°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without previous heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td>4.5</td>
<td>8.4</td>
<td>13.6</td>
<td>17.9</td>
<td>13.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td>3.6</td>
<td>6.7</td>
<td>10.9</td>
<td>14.4</td>
<td>11.0</td>
<td>0.6</td>
</tr>
<tr>
<td>After 30 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td>4.4</td>
<td>7.5</td>
<td>10.6</td>
<td>13.5</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td>3.5</td>
<td>6.0</td>
<td>8.5</td>
<td>10.8</td>
<td>0.4</td>
<td>0.0</td>
</tr>
<tr>
<td>After 15 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cu₂O, mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Maltose, mg.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>After 10 min. heating.</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After 5 min. heating.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cu₂O, mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltose, mg.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This time was progressively decreased from 30 minutes to 15, 10, and 5 minutes. With these progressively smaller times
the rates of destruction at the lower temperatures became unmeasurable.

From the data in these tables the rate of destruction of pancreatic and malt amylases under the conditions of these experi-

Fig. 1. The upper curve shows the rate of destruction (inactivation) of pancreatic amylase when heated in water-salt solution at 50° previous to acting on starch. The lower curve shows the rate of inactivation under the same conditions at 40°.
ments as shown in Figs. 1 and 2 were calculated as follows: The amount of sugar produced in the hydrolysis of the starch by the enzyme solutions which had not had previous heating at a given temperature was called 100 per cent. That produced after the

![Graph showing the rate of destruction (inactivation) of malt amylase.](http://www.jbc.org/)

**Fig. 2.** The upper curve shows the rate of destruction (inactivation) of malt amylase when heated in water-salt solution at 50° previous to acting on starch. The lower curve shows the rate of inactivation under the same conditions at 40°.
different times of preliminary heating was calculated to per cent and subtracted from 100. The curves obtained from these values therefore represent the relative velocity of inactivation of these enzymes under these experimental conditions. At 50° the pancreatic amylase is about 97 per cent inactivated in 5 minutes while the malt amylase has lost only 25 per cent of its original activity after \( \frac{1}{2} \) hour. At 60°, which is much above the point of complete inactivation of pancreatic amylase, the malt amylase retains a slight activity even after 30 minutes.

**DISCUSSION.**

It is of interest to find that increasing temperature influences the hydrolytic activities of both these enzymes in a similar manner. The values showing the activity of each obtained in the direct tests of hydrolysis at each temperature rise to a maximum at 50° with both enzymes. Evidently the increase of chemical velocity is greater up to 50°, than the inactivation of the enzymes. Beyond 50°, however, the rate of inactivation under the conditions of these experiments increases to such an extent that it becomes the predominating factor and thus the values drop very rapidly.

The effect of increasing temperature upon the activities of the enzyme solutions when they have been held without substrate at the various temperatures previous to their action upon starch at those temperatures is also of interest.

With pancreatic amylase the activity is entirely lost upon 15 minutes heating at 50°, and at 40° the values obtained upon 15 and 30 minutes heating are much below the values obtained in the direct tests of hydrolysis of starch at that temperature. This would indicate that the rate of inactivation of this enzyme increases rapidly with time and temperature. The results with malt amylase while similar to those with pancreatic amylase are less marked and indicate that malt amylase is much more stable than pancreatic amylase. This is confirmed by unpublished data from this laboratory and work carried on by Ernström (13).

At temperatures sufficiently low where the inactivation of the enzyme plays a small part, the ratio of the sugar formed by equal amounts of enzyme at the lower temperature compared to that formed at the next higher temperature is a fair measure of the velocity of the action at these two temperatures. Thus for pan-
creatin in the interval 20–30° the ratio is 2.1 and between 30° and 40° it is 1.8, while for malt at the same points it is 1.9 and 1.6. From these results it would appear that the temperature coefficient of the hydrolysis of starch by these two enzymes is about that of inorganic reactions. These results are also in accord with the data of other investigators as cited above. The velocity of the inactivation of the enzyme is, however, much more rapid than this; pancreatin, for instance, under the conditions of these experiments is destroyed thirty times as rapidly at 50° as at 40° if we compare times of equal destruction.

Malt amylase shows a greater stability toward heat, as the maximum value obtained in the inactivation experiments (Tables I and II) lies at 50° instead of at 40° as is the case with pancreatin. It also seems that the presence of starch has less protective action with malt amylase than with pancreatic amylase. That this is due to the lower activity of the malt amylase and the probable presence of impurities that may act as protecting agents is hardly a valid assumption in the light of preliminary experiments carried out with an old preparation of pancreatin of about one-third the power of sample No. 9, which would presumably contain decomposition products which would act as protective agents. This gave identical points of maxima on both the velocity and destruction curves. It would seem that malt amylase is inherently more stable than pancreatic amylase.

There are two possible explanations.

1. The inactivation of the enzyme may be an hydrolysis. The concentration of the enzyme is at all times very low. Even with the commercial preparations worked with there is never more than 2 mg. of the enzyme preparation in the 50 cc. of water used in the experiments upon heat destruction. It was first thought that possibly the dilution was a factor of importance and a series of tests were made in which the enzyme concentration was decreased to one-half, and one-fourth, but no change in the rate of inactivation could be noticed. This was interpreted to mean that the dilution was already so high that hydrolysis was at a maximum. Due to the activity of the sample and the rapid rate of starch cleavage it was impossible to increase the concentration of the enzyme much beyond the amount used. As was pointed out the destruction of pancreatic amylase at 50° in these experiments
was much more rapid than the ordinary hydrolytic reactions which have been studied previously—Arrhenius (14).

2. If the amylases are protein in nature it is possible that their inactivation might be due to heat coagulation, which, with most proteins, occurs between 50° and 70°. Sherman and Schlesinger (15), working with purified preparations of pancreatic and malt amylases, found a faint opalescence in their solutions at 50°, with increasing coagulation up to 70°, at which point, after filtering, no further precipitation would occur, even at high temperatures. It is quite possible that the previous formation of this coagulum would, on the later addition of starch, prevent any action of the enzyme. On the other hand, since enzyme action is probably a surface reaction, in the presence of the starch the enzyme at coagulation temperatures would be precipitated on the starch molecule, and though its activity would probably be less, still the action would continue beyond the point where it could react with the substrate after a preliminary heating that brought about coagulation. This theory is in accord with the evidence shown.

From the data at hand neither theory is susceptible to proof. The work of Sherman, Walker, Caldwell, and Naylor (16, 17, 18) on the protective action of certain amino acids on the amylases supports the theory that hydrolysis plays an important rôle in the heat destruction of the enzymes, but this is entirely consistent with the view that there may also be destruction by heat coagulation.

SUMMARY.

The rates of starch hydrolysis by pancreatic and malt amylases used in the forms of good grades of commercial pancreatin and malt have been determined under certain specified conditions for the temperature range of 20° to 70°C. At temperatures below the point where destruction of the enzyme plays an important rôle the rate of hydrolysis is about doubled for every 10° rise in temperature.

The temperature and rate of destruction of these enzymes in water-salt solutions have been determined and malt amylase is found to be much more stable than pancreatic amylase, the latter being completely destroyed in 15 minutes heating at 50°, while malt amylase still shows a trace of activity after 30 minutes at
60°C.; pancreatic amylase is apparently inactivated approximately thirty times as fast at 50° as is malt amylase.

The results obtained for the rate of destruction of these enzymes show a wide divergence from those giving the rates of destruction of vitamins B (19) and C (20), and make it appear doubtful that any advantage is to be gained by classing vitamins as enzymes as has sometimes been suggested.

The results obtained support the view that the heat destruction of the enzyme may be a process of the nature of the coagulation of a protein, probably accompanied by partial hydrolysis also.

BIBLIOGRAPHY.
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