STUDIES ON THE INACTIVATION OF CATALASE.*

II. INACTIVATION BY ULTRA-VIOLET RADIATION AT DIFFERENT HYDROGEN ION CONCENTRATIONS.

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In a previous paper (1) the inactivation of catalase by heat at different pH was studied. It was discovered that the heat inactivation depends upon the hydrogen ion concentration of the enzyme solution which affects both the velocity and the extent of the reaction. It was also found there that 65° was the critical temperature for the enzyme preparation since at this temperature the catalase was instantly inactivated at all pH. It was further found that the degree of inactivation at different temperatures follows a rather characteristic curve, the catalase being most stable at pH 6.0 to 6.5 and losing its activity somewhat more rapidly on the acid than on the alkaline side of that pH. Catalase does not manifest a distinct optimum hydrogen ion concentration for its activity, this being covered by a range from about pH 6.5 to 8.5, so that the condition for the maximum stability of the enzyme towards heat does not exactly coincide with this range but falls only within its outside acid margin. This observation suggested that attempts to determine the chemical nature of the enzyme from its activity at different hydrogen ion concentrations must necessarily lead to erroneous conclusions and that the study of the

* The paper "Studies on the Effect of Temperature on the Catalase Reaction. VI. Heat Inactivation of Catalase at Different Hydrogen Ion Concentrations" will be considered as the first contribution on "Studies on the Inactivation of Catalase," of which the present paper is the second contribution.
resistance of the enzyme to various injurious factors at different 

pH would yield more reliable results for appraising its physico-

chemical nature. With this in view the study of the inactivation 
of catalase at different hydrogen ion concentrations by means of 
ultra-violet radiation was next undertaken.

The experiments were performed with the same catalase prepa-

ration used in the previous investigation. This was prepared 

from beef kidneys, the parenchymatous portion of which was 

finely ground and extracted several times with an equal weight 
of water saturated with chloroform. The combined extracts 

were passed through a Sharples centrifuge yielding a clear con-

centrated catalase solution which was freed of traces of hemo-
globin and of a large part of its protein content by shaking with 
one-fifth its volume of chloroform. The filtered solution, pre-

served with a little chloroform, keeps its enzymatic activity un-

altered for a very long time. A further precipitate forms on stand-
ing which may be filtered off without affecting the enzymatic 

strength of the solution.

The method for determining the catalase activity following 

various degrees of radiation with ultra-violet rays was essentially 

the same as employed in previous experiments. The activity 

was measured gasometrically by determining the volume of 

oxygen set free from 0.357 N hydrogen peroxide (equivalent to 

100 cc. of O₂) in a reaction mixture of a total volume of 50 cc. at 

21°. In order that the results should be strictly comparable in a 

quantitative way, the enzymatic concentration required to de-

compose 70 per cent of the hydrogen peroxide (yield 70 cc. of O₂ 
at 0° and 760 mm. of Hg) is taken as a basis. The degree of 
inactivation of the catalase resulting from radiation is then 
determined quantitatively from the amount of the weakened 

enzyme required to produce the same decomposition of hydrogen 
peroxide, the activity being expressed as the reciprocal of the 

enzyme concentration needed. In these experiments it was 

found more expedient to standardize the activity of the enzyme 

separately for each pH investigated. The desired pH was se-
cured by buffering the solution with an appropriate mixture. In 

all but the experiments with the highest pH the Kolthoff phos-
phate buffers were used. The exact enzyme concentration at 

each pH required to give a 70 per cent decomposition of the
hydrogen peroxide is readily obtained when the results of a series of determinations with varying enzyme concentrations are plotted. This invariably gives a curve which is a straight line and the exact enzyme concentration effecting the required decomposition is easily got from the graph. An enzyme solution, a definite volume of which will set free 70 cc. of oxygen, is then prepared from the stock preparation. This solution, spread out in a thin layer in a wide dish, was exposed to the action of ultra-violet radiation. The solution was stirred during the entire time of exposure, and samples were withdrawn at regular intervals of

radiation. The source of the radiation was a Hanovia quartz mercury vapor lamp operated at 110 volts and 5 amperes at a distance of either 25 or 50 cm.1 Three sets of experiments were performed at each pH to determine the degree of inactivation. First a set of experiments was made to find out the concentration of the enzyme preparation necessary to yield the 70 per cent decomposition of the hydrogen peroxide at the desired pH (stand-

1 To Dr. Carlton Pierce, Professor of Roentgenology, I am greatly indebted for placing at my disposal the quartz mercury lamp and for his cordial cooperation in standardizing the instrument.
Fig. 2. Inactivation of catalase solution exposed to ultra-violet radiation at 50 cm.

Fig. 3. Inactivation of catalase solution exposed to ultra-violet radiation at 25 cm.
A second set of experiments was made with the enzyme preparation radiated for varying lengths of time, the catalase activity being determined with the same amount of the radiated enzyme. The time required for the complete inactivation is thus determined. A third set was then performed exactly as before, samples being withdrawn at the same intervals of radiation, but the residual activity was determined on variable amounts of the enzyme solution. The longer the radiation progressed and the smaller, therefore, was the residual catalase activity, the larger was the sample employed in the experiment. By plotting the quantity of the enzyme radiated a definite length of time as abscissae and the catalytic activity in cc. of oxygen set free as ordinates, the concentration necessary to effect the standard enzymatic work of 70 per cent decomposition is found from the graph. The zero time sample (i.e. the enzyme solution before being radiated) was always adjusted to give this amount of decomposition and this catalase activity was given the value of 100. The residual activity of this catalase solution at various intervals during the radiation varies inversely as the quantity of the solution required compared to that found for the zero sample.

This may be demonstrated on a specific example. At pH 4.0, 9.5 × 10⁻² cc. of the stock catalase preparation were required to liberate at 21° 70 per cent of the oxygen available in the H₂O₂ used. On being exposed to ultra-violet radiation at a distance of 50 cm., progressively larger amounts of the enzyme preparation were needed to accomplish the same catalytic action. Thus:

<table>
<thead>
<tr>
<th>Time of exposure, min.</th>
<th>Enzyme for 70 per cent decomposition, cc.</th>
<th>Residual activity, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.5 × 10⁻²</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>11.0 × 10⁻²</td>
<td>86.4</td>
</tr>
<tr>
<td>15</td>
<td>15.0 × 10⁻²</td>
<td>63.3</td>
</tr>
<tr>
<td>30</td>
<td>24.0 × 10⁻²</td>
<td>39.5</td>
</tr>
<tr>
<td>40</td>
<td>52.5 × 10⁻²</td>
<td>18.2</td>
</tr>
<tr>
<td>60</td>
<td>172.5 × 10⁻²</td>
<td>5.5</td>
</tr>
</tbody>
</table>

The results of the experiments are presented in a series of curves. In Fig. 1 the course of inactivation when the properly buffered catalase solution has been exposed to ultra-violet radiation at 50 cm. is shown on the acid side and in Fig. 2 on the alkaline side of
pH 7.0. Fig. 3 shows the same thing when the exposure was made at half that distance; namely, at 25 cm. At the shorter distance the inactivation, of course, is much more rapid but the course of the inactivation is practically the same. At the 25 cm. distance there is a tendency for the temperature of the radiated solution to rise but this did not exceed 8° and we can therefore be sure that the change in temperature is not a factor in the inactivation reaction, inasmuch as it has been shown before that no appreciable heat inactivation of catalase occurs below 50°.

We observe from this series of curves that at 25 or 50 cm. the inactivation of the catalase at pH 6, 7, and 8 is a linear function of the time of exposure; in other words, it proceeds at a uniform rate. However, both on the acid and alkaline side of this pH range there is no longer this direct relationship between the inactivation and time of radiation but the rate varies, being much more rapid during the first few minutes of radiation and becoming slower subsequently. In fact, at pH above 8.0 the inactivation becomes even slower than at pH 6.0 at which pH the catalase shows a maximum resistance to the ultra-violet rays, just as previously we found a maximum resistance to heat at that pH. On the alkaline side of pH 8.0 the complete inactivation is not attained in less than an hour or even a longer length of time.

At pH less than 6.0 the time required for the complete inactivation is not materially different from that at pH 6 or 7, but the course of inactivation is distinctly different, as is revealed at once by an examination of the series of curves both at the 25 and 50 cm. exposure. At pH 3.5 the inactivation already proceeds very rapidly and is completed in about two-thirds of the time required at pH 4 to 6. But at this low pH the enzyme becomes very unstable and even undergoes spontaneous inactivation. This factor necessarily complicates the experimental procedure, but both in this instance, and more particularly on the alkaline side at pH 11, where this spontaneous inactivation is even more serious, the standardization is carried out at the end of a specified length of time. All the experiments on the radiated enzyme are conducted after the same lapse of time so that the changes due to spontaneous inactivation are automatically corrected. Of course, where the spontaneous inactivation becomes so extensive as to overshadow the inactivation brought about by the
specific factor studied, such procedure could lose much of its significance and for this reason no attempts were made to study the inactivation by ultra-violet radiation at pH less than 3.5 or above 11.

At pH 6, 7, or 8, as was already mentioned, the inactivation is a linear function of the time of radiation and the rate increases from pH 6 to 8. With further increase of the pH (9 and 10) we note again, as was observed on the acid side at pH 3.5 to 5, that the inactivation proceeds at variable rates, being most rapid for the first few minutes and slowing down subsequently when the curves flatten out. The time required for complete inactivation becomes progressively greater as the pH increases. At pH 11 the situation is particularly striking, as may be seen from the corresponding curves in Figs. 2 and 3. At this pH the inactivation commences and proceeds for 5 minutes in a manner very similar to that observed at pH 8, and is therefore appreciably slower than at either pH 9 or 10. Then the rate of inactivation becomes very slow so that at the long exposure (50 cm.) the reaction is not completed, even after 75 minutes of radiation. Owing to the fact that spontaneous inactivation of the enzyme becomes a factor in experiments at such alkalinity, it is not practicable to prolong the experiments beyond that time. However, at the 25 cm. exposure complete inactivation was achieved in about 30 minutes, which is about twice the time required at pH 8.0.

The peculiar behavior of catalase at pH 11, manifesting itself in the extremely slow inactivation by ultra-violet radiation, is apparently not due to the nature of the buffer employed. The first experiments were made with a glycine-NaOH mixture as buffer for the pH 11. Since the buffer system employed in the other experiments was a phosphate mixture, we repeated these experiments, using a phosphate-NaOH mixture instead, with exactly the same results. We are justified, therefore, in concluding that the stabilizing effect and the increased resistance of the enzyme to radiation are the result of the high OH concentration of the medium.

The influence of the ultra-violet radiation at different hydrogen and hydroxyl ion concentrations is demonstrated most clearly in Fig. 4, where the residual catalase activities for a given time of exposure are plotted as ordinates against pH as abscissæ. We
note, in the first place, that at pH 6.0 the catalase is most resistant to the action of ultra-violet radiation; secondly, that at pH below 4 this resistance drops very abruptly. In this respect the inactivation by ultra-violet radiation is like that found previously for heat inactivation, and this is also manifested at the alkaline range of pH 8 to 9. With further increase in the OH ion concentration, however, and especially at an exposure of 50 cm., the inactivation requires considerably longer periods for its completion. If we tabulate the data on this point we get the following series of values:

<table>
<thead>
<tr>
<th>pH</th>
<th>Min. required for complete inactivation.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 25 cm.</td>
</tr>
<tr>
<td>3.5</td>
<td>12</td>
</tr>
<tr>
<td>4.0</td>
<td>17</td>
</tr>
<tr>
<td>5.0</td>
<td>20</td>
</tr>
<tr>
<td>6.0</td>
<td>21.5</td>
</tr>
<tr>
<td>7.0</td>
<td>15</td>
</tr>
<tr>
<td>8.0</td>
<td>13</td>
</tr>
<tr>
<td>9.0</td>
<td>12 (?)</td>
</tr>
<tr>
<td>10.0</td>
<td>27</td>
</tr>
<tr>
<td>11.0</td>
<td>28</td>
</tr>
</tbody>
</table>

At pH 3.5 the inactivation proceeds very rapidly and is completed in about two-thirds of the time required at pH 4 to 6, while at pH above 9 the enzyme seems to regain its resistance to the ultra-violet radiation so as even to exceed that at pH 6. In other words, in an excess of OH ions the enzyme must undergo some transformation imparting to it a protection against the inactivating effect of radiant energy. The nature of this effect is not clear.

**DISCUSSION AND SUMMARY.**

Waentig and Steche (2), experimenting with different portions of the visible spectrum, found that the catalase activity was affected in varying degrees depending upon the purity of their enzyme preparation. The inactivation by the light, however, was such as to indicate that the ultra-violet is the most effective portion of the spectrum. These investigators found, furthermore, that the inactivation by light was greater in an alkaline than in either an acid or neutral medium. Without actual knowledge of the hydrogen ion concentration this statement is, of course,
of no particular significance since our experiments also show that at pH 8 the catalase is more affected than at pH 4 to 7, yet at greater alkalinity the enzyme shows increased resistance. Talarico (3), experimenting on catalase from horse liver, observed that red and green light had no effect, while the other parts of the spectrum inactivated the enzyme in the order blue > violet > yellow.

More recently Pincussen studying the effect of ultra-violet radiation on enzymes extended his experiments also to catalase (4).

![Fig. 4. Per cent of residual catalase activity following exposure to ultra-violet radiation at 25 and 50 cm. at different pH and for different lengths of time.](image)

Using a quartz mercury vapor lamp operated at 60 volts and 2 amperes at a distance of 10 cm., he radiated catalase solutions for a period of 10 to 40 minutes. He varied the hydrogen ion concentration of the medium from pH 6.24 to 7.35 and claims to have found the greatest inactivation of the enzyme at about pH 6.8 which, he assumes, is also the optimum pH for the catalase activity. On the basis of these assumptions Pincussen finds that his results on the inactivation of catalase fit his general idea of the inactivation of enzymes by radiant energy best expressed by a
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quotation from his own paper: At pH 6.8 the activity of the catalase is injured most seriously by light (?) which corresponds to our experience with other enzymes also. According to the results obtained by us so far, the pH at which inactivation through radiation is greatest may be regarded also as the pH at which the enzyme shows optimum activity. This fact gives us a method for determining the optimum hydrogen ion concentration for enzyme action.

Both the observation and the general conclusion which Pincussen draws from this are directly contradicted by our experimental findings on the radiation effect on catalase. In the experiments where the inactivation was brought about by ultraviolet radiation, as well as in the previous experiments on inactivation by heat, the catalase was least affected at pH 6.0, which in the case of catalase corresponds to the acid side of the optimum pH range for its catalytic activity. As was pointed out previously, catalase activity shows no definitely limited pH optimum. On the contrary, judged by the rate of its inactivation, catalase seems to have an optimum stability at pH 6.0 which is apparently its isoelectric point. We are unable to offer an explanation of the second peak of maximum resistance at pH 11.

Another point of interest in our experiments is the fact that the curves of inactivation of catalase at pH 6, 7, and 8 are straight lines, which is not the case at other pH. Baker and Nanavatty (5), studying quantitatively the effect of ultra-violet radiation upon bacteriophage, show that in the case of both bacteriophage and bacteria the curve of inactivation is of the type with a steep drop in the beginning followed by a more or less gradual descent subsequently. In other words, the curves are of the kind shown by our catalase preparation at pH 3.5, 4, 5, and also at pH 9 and 10. The curves of inactivation they obtained with complement, amboceptor, and various enzymes are of the type shown by our catalase at pH 6, 7, and 8. Baker and Nanavatty seem to imply from their evidence that the straight line curve of inactivation is peculiar for enzymes, whereas the other type is characteristic for living things, with the obvious implication that bacteriophage must be a living thing rather than an enzyme. This differentiation on the basis of the course of inactivation by ultra-violet radiation seems to lose its significance in the light of our experi-
ments which show that both types of curve are realized with an enzyme solution (catalase) and that the determining factor seems to be primarily the hydrogen ion concentration of the medium in which the inactivation process takes place.

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