LVIII. THE INACTIVATION OF CATALASES FROM CERTAIN MARINE PLANTS BY OXYGEN.

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It has been shown [Marks and Fox, 1933; Marks, 1934, 1] that catalases from certain marine animals, on standing in air, slowly lose their ability to decompose hydrogen peroxide. This inactivation was found to be caused primarily by oxygen and the reaction to be pseudo-unimolecular under the given experimental conditions. The question arose as to whether or not catalases from marine plants would likewise be inactivated.

Methods.

The experimental procedure has already been described [Marks, 1934, 2]. Unless otherwise stated, the temperature at which extracts were stored and measurements were made was $20.0 \pm 0.2^\circ$. The reaction time interval was 60 minutes.

Inactivation by oxygen. Solutions were prepared by extraction with a phosphate buffer of p_H 7.0, unless otherwise specified, of a number of species of marine plants. Portions were stored in air and under nitrogen or oxygen or both. Daily measurements were made on the quantity of hydrogen peroxide decomposed by 5 ml. samples after the addition of 2 ml. of the former. Controls were run simultaneously, using portions of extract that had been heated to boiling. The hydrogen peroxide solutions were prepared in the corresponding buffer or other corresponding medium and were 0.84–0.98 *M*.

It was found possible to apply the equation for a first order reaction to the inactivation reactions, as was previously done for other catalases [Marks, 1934, 1]. Thus, in the expression, $k = \frac{2.303}{t} \log \frac{a}{a-x}$, let a = the initial number of mg. of hydrogen peroxide decomposed by 5 ml. of extract, and $(a-x)$ = the number of mg. of hydrogen peroxide decomposed by 5 ml. of extract at any time, t , in days after the start. It has been shown [Marks, 1934, 2] that the quantities of hydrogen peroxide decomposed by the catalases from *Corallina officinalis* and *Pikea californica* are directly proportional to the enzyme concentration at p_H 7.0. This has been assumed to be true for the catalases from other species of marine plants. The true concentration of enzyme would be given by multiplying a and $(a-x)$ by some unknown factor c . However, this cancels out in the logarithmic expression, and thus the procedure is justified.

Data are given in Table I and show that the rates of inactivation were always greater for the portions of extract kept under air than under nitrogen. However, since oxygen enters directly or indirectly into the inactivations, the reactions are pseudo-unimolecular [Taylor, 1930]. Thus, the true equation is $k = \frac{1}{b} \ln \frac{a}{a-x}$, in which b is the concentration of oxygen or oxygen and unknown substances. This factor remains constant for any single experiment.

Table I. *The inactivation of catalases from marine plants.*

Species	Conc. of extract %	Medium	Atmo- sphere	No. of measure- ments*	<i>k</i>	
					Range†	Mean
<i>Corallina officinalis</i>	27	Water p_H 6.2	{ Air Nitrogen	11 8	0.076-0.093 0.026-0.034	0.085 0.033
<i>Corallina officinalis</i>	20	Phosphate buffer p_H 7.0	{ Oxygen Air Nitrogen	7 7 6	0.254-0.288 0.144-0.162 0.099-0.117	0.273 0.149 0.110
<i>Corallina officinalis</i>	40	Sea water p_H 8.1	Air	6	0.145-0.167	0.157
<i>Pikea californica</i>	20	Phosphate buffer p_H 7.0	{ Air Nitrogen	13	0.041-0.063 Rate slower‡	0.044
<i>Amphiroa aspergillum</i> <i>forma nana</i>	40	Phosphate buffer p_H 7.0	{ Air Nitrogen	9 10	0.113-0.142 0.062-0.087	0.127 0.081
<i>Corallina gracilis</i> <i>forma densa</i>	20	Phosphate buffer p_H 7.0	{ Oxygen Air Nitrogen	7 9 9	0.154-0.186 0.131-0.147 0.046-0.052	0.168 0.136 0.049
<i>Plocamium coccineum</i>	35	Phosphate buffer p_H 7.0	{ Air Nitrogen	9 9	0.142-0.160 0.083-0.096	0.149 0.089
<i>Polysiphonia</i> <i>verticillata</i>	20	Phosphate buffer p_H 7.0	{ Air Nitrogen	5	0.262-0.273 Rate slower‡	0.267

* Taken at 24 hour intervals.

† Although the range of *k* seems wide in some cases, actually most of the values clustered about the mean, and only one or two observations departed greatly therefrom.

‡ Rate did not conform satisfactorily to the unimolecular equation. Compare Marks and Fox [1933].

It was found that the catalases in 10-20 % extracts prepared from *Pelvetia fastigiata*, *Colpomenia sinnora*, *Dilophus flabellatus*, *Scytosiphon lomentarius* and *Sargassum agardianum* in a phosphate buffer at p_H 7.0 almost completely lost their activity on standing in air for 24 hours at 20°.

Inactivation at different p_H values. That the inactivation occurs irrespective of hydrogen ion concentration is shown in Fig. 1 for catalase from *Corallina officinalis*. It must not be inferred that because higher magnitudes of *k* are obtained at lower p_H values the reactions proceeded more rapidly. Reference to curves 1, 2 and 6 of Fig. 2 in the paper of Marks [1934, 2] shows that such results can be ascribed to the greater slope of portions of the proportionality curves. These portions do not pass through the origin. It is not now possible to say just what effect hydrogen ion and salt concentrations have on the reaction rates.

Previously, sharp changes in the slopes of the curves were noted when the data for the inactivation of catalases from certain marine animals were plotted logarithmically [Marks, 1934, 1]. Similar discontinuities (Fig. 1) have been found for the inactivation of catalase from *Corallina officinalis* at p_H 8.0 and 9.0. Again these changes are not to be ascribed to a difference in reaction rate, but to discontinuities in the proportionality curves. Thus, referring again to Fig. 2 [Marks, 1934, 2] for p_H 10.0, the change occurs at about 22 mg. on the ordinate, and in Fig. 1 (p_H 10.0) of the same paper, an apparent change in reaction rate is found at about 20 mg. ($\log_{10}=1.30$), i.e., both changes occur at approximately the same catalase concentration.

Inactivation of catalases at different temperatures. It is to be expected that the rate of inactivation will increase with rise in temperature. Extracts were prepared in phosphate buffer p_H 7.0, divided into several portions and stored at given temperatures which varied from their mean values by about $\pm 0.2^\circ$.

Daily measurements were made at $20.0 \pm 0.2^\circ$, the sample first being kept for 20–30 minutes in a room at this temperature before the introduction of hydrogen peroxide.

Fig. 1. Inactivation of catalase from *Corallina officinalis* at different p_H values. Respective concentrations of extracts and p_H of buffers used are in the order of the numbers of the curves: 40 %, 40 %, others 20 %; p_H 4.5, 6.0, 8.0, 9.0 and 10.0. All buffers phosphate except 9.0 and 10.0 which were H_3BO_3 -KCl-NaOH mixtures.

Fig. 2. Inactivation of catalases from marine plants. Curves showing the application of Arrhenius's equation for change in reaction rate with change in absolute temperature. Curves A and B, *Corallina officinalis* (20 % extracts); curve C, *Pikea californica* (40 % extract).

Initial values of a were determined by the extrapolation method [Marks and Fox, 1933]. These extrapolated values were not precisely the same for the different temperatures for a given extract, but only approximately so.

Application of Arrhenius's equation. Arrhenius's equation was previously found applicable to the change in inactivation rate with change in temperature for mussel catalase [Marks and Fox, 1933]. Since again it is assumed that unknown

factors, including oxygen, remained constant under the given conditions, the integrated form of Arrhenius's equation [Taylor, 1930] may be written as follows:

$$2.303 \log \frac{k_1}{k_2} = \frac{Q}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right),$$

where $k_1 = k'b$, and $k_2 = k''b$.

In Fig. 2 are plotted data obtained for catalases from *Corallina officinalis* and *Pikea californica*. Since the curves are straight lines, the Arrhenius equation is applicable. It is to be noticed that the slopes of curves *A* and *B* for two different extracts of *C. officinalis* having the same percentage concentration are quite different. Thus the values of Q are not the same in the two cases. This effect can be ascribed to an unknown factor or factors which remain constant for a given extract. It is hardly to be ascribed to oxygen alone as there is no reason for believing that the concentrations of this gas were significantly different in the two preparations at the different temperatures.

SUMMARY.

1. Catalases from certain species of marine plants are inactivated directly or indirectly by oxygen and the reactions have been found to be pseudo-unimolecular under the given experimental conditions.
2. Catalase from *Corallina officinalis* slowly loses its activity over the p_H range 4.5–10.0 at $20.0 \pm 0.2^\circ$.
3. The change in the rate of inactivation of catalases from *Corallina officinalis* and *Pikea californica* with change in temperature is expressed by the Arrhenius equation over a range of 4.0 – 37.5° .

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