THE DECOMPOSITION OF HYDROGEN PEROXIDE BY LIVER CATALASE.

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The decomposition of hydrogen peroxide by catalase has been the subject of many investigations, notably those of Senter (1), Sörensen (2), Michaelis and Pechstein (3), Yamasaki (4) and Morgulis and his coworkers (5–7). Different results have been found by these and other authors, the difference lying in the various conditions of temperature, peroxide concentration, etc., employed. The two most comprehensive studies of this reaction are those of Yamasaki and Morgulis. Yamasaki (4) studied the reaction in dilute solutions of hydrogen peroxide (0.02 to 0.1 N) using catalase extracted from vegetable sources, and found that the enzyme was inactivated during the reaction, the rate of decomposition of hydrogen peroxide and the rate of inactivation of catalase bearing a constant ratio to one another. He embodied this relationship in an integrated expression which fits his results well. This equation will be mentioned later. His work is limited, however, since he worked with unbuffered hydrogen peroxide solutions, no knowledge of the effect of pH on the decomposition, therefore, being available from his data. Sörensen (2) has shown that pH has a marked effect on the decomposition of hydrogen peroxide by blood catalase. The work of Morgulis and his coworkers (5, 6) which was performed in more concentrated solutions of hydrogen peroxide (above 0.2 N) although covering a wide range of conditions of temperature, pH and peroxide concentration loses much of its value as a physicochemical study owing to the faulty temperature control. This criticism does not apply to their last paper, Morgulis and Beber (7). In spite of the amount of work already done upon this enzyme it was thought of interest to reinvestigate certain aspects of the com-

plex problem which it presents with the object of confirming and of extending (in certain directions) the results and conclusions drawn by previous workers.

Theoretical.

It will be seen from the results quoted later that the following conclusions, in general agreement with those of previous authors, can be drawn.

A. The velocity of decomposition of hydrogen peroxide is proportional to the concentration of catalase.

B. In dilute solutions of peroxide (up to 0.1 N) the velocity of reaction is proportional to the concentration of hydrogen peroxide, but as the peroxide concentration is increased the velocity becomes independent of the hydrogen peroxide concentration. In still more concentrated solutions of peroxide a depression of the activity of the enzyme occurs.

C. It has been generally found by previous authors and confirmed by the writer, that the reaction gives falling unimolecular constants, ascribed by Yamasaki to the fact that the enzyme is also inactivated during the decomposition of hydrogen peroxide. For dilute solutions of hydrogen peroxide, the velocity of reaction is proportional to both the concentrations of hydrogen peroxide and catalase, hence

$$\frac{-dS}{dt} = k_1 \cdot E \cdot S \tag{1}$$

where S is the concentration of H_2O_2, determined in the present case by titration with standard $KMnO_4$ solution, E is the concentration of catalase and k_1 is the velocity constant.

The value of E, the concentration of catalase, cannot be measured directly. From equation (1) we see that

$$k_1 \cdot E = \frac{1}{S} \cdot \frac{dS}{dt} \tag{2}$$

The results of a typical experiment are given in Table I. The values of dS/dt and dE/dt were obtained by drawing tangents to the curves obtained by plotting the values of S and $k_1.E$ respectively against time. As will be seen from Table I, the ratio of the two velocities is

sensibly constant. Although in all the experiments the constancy is not so good as the above, yet over the greater part of the reaction a fair constancy is obtained. It is seen therefore that in the decomposition of hydrogen peroxide by catalase two simultaneous reactions are going on, namely the catalytic decomposition of the peroxide and the induced inactivation of catalase. This process is called the "induced inactivation" to distinguish it from the so called spontaneous inactivation of the enzyme which only proceeds when the temperature is raised. The velocities of the two processes, namely induced

TABLE I.

1 cc. of a catalase solution (strength 0.09) was mixed with 50 cc. of hydrogen peroxide solution of a concentration of 0.056 N. pH = 6.8, temperature = 15°C.

Time	C H_2O_2	dS/dt	$\frac{1}{S} \cdot \frac{dS}{dt} = k_1 \cdot E$	dE/dt	$\frac{dE}{dt} / \frac{dS}{dt}$
min.	*mols/liter*				
0	0.05680	.00227	0.04	.0038	1.7
2	0.05304	.0018	0.034	.00298	1.66
5	0.04733	.00113	0.024	.00187	1.65
11	0.04242	.00067	0.0158	.00106	1.6
15	0.03975	.00054	0.0135	.00064	1.2
20	0.03781	.00042	0.0112		
25	0.03569	.00030	0.0085		
30	0.03432				

inactivation of the enzyme and decomposition of hydrogen peroxide, are connected by the relation

$$dE/dt : dS/dt = \text{a constant} \qquad (3)$$

The value of this proportionality constant varies with catalases from different sources. It also varies with temperature, pH and the previous history and treatment of the enzyme solution. The value for a solution of catalase did not alter sensibly while it was in use, and as the experiments in each section of the present paper were performed with the same solution of catalase, the dependence of the constant upon the above mentioned factors will not affect the value of the results.

Equation (3) is the relation of Yamasaki, and embodied by him in an integrated equation. We have yet to enquire into the significance of equation (3). It states that the velocity of induced inactivation of catalase bears a constant relationship to the velocity of decomposition of hydrogen peroxide. This may mean that the enzyme is inactivated during the decomposition of the peroxide molecule, or it may be inactivated as a sequel to the reaction, the destruction of the enzyme being caused by some factor the magnitude of which is proportional to the velocity of decomposition of hydrogen peroxide. Various possibilities arise for consideration.

1. The inactivation of the enzyme may be caused by the *substrate*, hydrogen peroxide. This is implicitly assumed by Yamasaki and Morgulis. Although in his conclusions he states that either the substrate or the products may be responsible for the inactivation, Yamaski implicitly assumes that the substrate is responsible by writing the kinetic equations in the form:

$$-dS/dt = k \cdot E \cdot S \text{ and}$$
$$-dE/dt = k' E \cdot S$$

where k and k' are the respective velocity constants of the two reactions. Morgulis also assumes that the destruction of catalase in the more concentrated solutions of hydrogen peroxide which he employs consists in the oxidation of catalase by the excess peroxide. It is generally found, as instanced later, that in concentrated solutions the substrate exerts a depressor effect on the activity of enzymes. The process of induced inactivation however is going on in all concentrations, though sometimes masked when the enzyme is in great excess.

2. One factor which is proportional to the velocity of decomposition of hydrogen peroxide is the heat effect. We have called the inactivation of catalase in presence of its substrate "induced inactivation" to distinguish it from the so called spontaneous inactivation which takes place when solutions of catalase (or any other enzyme) are heated alone. Since the decomposition of hydrogen peroxide by catalase takes place at the enzyme surface a possibility of localized heating of the enzyme presents itself, in which case the induced inactivation and the spontaneous inactivation would be identical. Mellor (8) states "The decomposition of hydrogen peroxide into water and molecular

oxygen gives out sufficient heat, if confined to the components of the reaction to raise their temperature 1000°C." If the induced inactivation of catalase was the same as the spontaneous inactivation, in this case brought about by the localisation of the heat of reaction, we should expect them to possess the same critical increment. As will be seen later, the induced inactivation of catalase in dilute hydrogen peroxide solutions possesses a very small critical increment, whereas the critical increment of the spontaneous inactivation of enzymes is usually of the order of 50,000 to 100,000 calories; so that we may conclude that in dilute solutions of hydrogen peroxide, at least, the induced inactivation of the enzyme is not due to local heating.

3. Another possibility which would lead to equation (3) is the inactivation of the enzyme by the products of the reaction. The experiments of Waentig and Steche (9) show that this mode of inactivation is not produced by the molecular oxygen in solution. The other factor, and in the writer's opinion the most probable explanation, is that the induced inactivation is caused by the oxygen, while still in the activated state following its production from the peroxide at the enzyme surface. In this case, we should have

$$- dE/dt = - k_2 \frac{dS}{dt}$$

where k_2 is the velocity constant of induced inactivation.

On this latter basis we have

$$- dS/dt = k_1 \cdot E \cdot S \tag{1}$$

$$- dE/dt = - k_2 \cdot \frac{dS}{dt} \tag{3a}$$

whence $E = k_2 \cdot (S + A)$ where $A = (E_0/k_2 - S_0)$. E_0 is the initial concentration of enzyme and S_0 the initial concentration of substrate.

Substituting this value for E in (1) and integrating we obtain

$$k_3 = k_1 \cdot k_2 = \frac{1}{A \cdot t}\left(\log_e \frac{S_0}{S} - \log_e \frac{(S_0 + A)}{(S + A)}\right) \tag{4}$$

This differs from the equation of Yamasaki in that on the present writer's interpretation the final constant k_3 is the product of the two

velocity constants k_1 and k_2 (k_1 referring to the decomposition of hydrogen peroxide and k_2 to the induced inactivation of catalase) whereas the final constant obtained by Yamasaki and denoted by him, k', is the single constant of the inactivation process alone. This difference lies in the different significance ascribed to equation (3). There is no direct method of comparing the validity of these two interpretations of the relationship $dE/dt : dS/dt$ = a constant. In general, with other enzymes, it is found that the products of reaction are the potent inactivators of the enzyme, the substrate having no such action in dilute solution. In the case of catalase, it is impossible to vary the concentration of substrate and product of reaction independently. Indirect evidence in favour of the writer's point of view may be obtained by a comparison of the behaviour of k_3 with k_2, the constant of

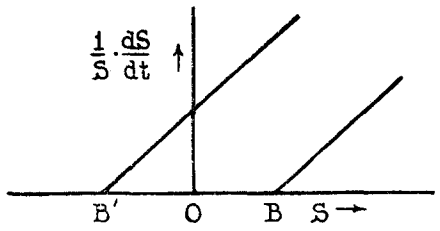

FIG. 1

inactivation alone. Thus whereas k_2 passes through a minimum at pH 7.0 (see Tables V and VI) k_3 shows no such regular behaviour.

Equation (4) has been used by the writer to calculate the results given in Section II of the present paper. The apparent order of the reaction alters with the value of the integration constant, A, as pointed out by Yamasaki: (*a*) If $A = 0$, the reaction gives a bimolecular constant. (*b*) If A is positive and E_0 is much greater than $k_2.S_0$ the reaction will give a unimolecular constant. These are the conditions chosen by the majority of workers. (*c*) If A is negative, the peroxide is in excess, and the enzyme will be inactivated before all the hydrogen peroxide has been decomposed. All these conditions have been realised in the course of this work.

The methods employed to calculate the values of A given later in the tables are the same as those of Yamasaki, namely:

A. Graphical Method.—The value of $\frac{1}{S}\cdot\frac{dS}{dt}$ obtained from the concentration time curve for the experiments is plotted against the concentration of hydrogen peroxide, when an approximately straight line is obtained. The value of A is given by the intercept OB' when A is positive, and by OB when A is negative (*cf.* Fig. 1.)

B. Calculation.—If S_0, S_1, S_2, S_4 are the concentrations of hydrogen peroxide at times 0, t, $2t$, $4t$ and $A_{1,2}$ is the value of A obtained from S_0, S_1, S_2, then

$$A_{1,2} = \frac{2 \cdot S_0 \cdot S_1 \cdot S_2 - S_1^2 (S_0 + S_2)}{S_1^2 - S_0 \cdot S_2}$$

Similar values of A_1 namely $A_{2,4}$ etc. are obtained in an analagous manner.

The mean result obtained by Methods A and B has been employed throughout.

Calculation of k_1 and k_2.—Substitution of the value of A obtained by the above methods in equation (4) gives the value of $k_3 = k_1.k_2$. Now $A = (E_0/k_2 - S_0)$, and hence $k_3 (S_0 + A) = k_1.E_0$. The value of $k_1.E_0$ is also obtained by dividing the initial velocity, obtained graphically, by the initial concentration of hydrogen peroxide. This is used as a check on the value of $k_1.E_0$ obtained *via* equation (4). The value of $k_1.E_0$ obtained by equation (4) will be referred to later (*cf.* Tables IV, IV*a*, IV*b*) as $k_1.E_0$ (calculated); the value of $k_1.E_0$ obtained by dividing the initial velocity by the initial concentration of hydrogen peroxide will be referred to as k_1,E_0 (observed).

Definition of Catalase Unit.—To find the value of k_1, we must fix some arbitrary unit for E_0, the initial enzyme concentration, since the molecular unit of an enzyme is not known. The only way of determining the enzyme concentration is to fix conditions of pH and temperature, and define the unit in terms of the velocity constant given. *In this investigation the unit of catalase is chosen as that amount which in a solution of pH 6.8 (obtained with Clark and Lubs' $K H_2 PO_4$ — $NaOH$ mixture) at 20°C. gives a velocity constant (k_1) of unity,* calculated

from equation (4), time being expressed in minutes. It is found by experiment that at 20°C. and at pH 6.8, the value of $k_1.E_0$ obtained for the standard solution of enzyme preparation used in this work is 0.4760. Hence all observed values of k_1,E_0 (obtained with this catalase) must be divided by 0.4760 to reduce them to the unit defined above. This has been done in all values quoted in the present paper.

EXPERIMENTAL.

The catalase used in these experiments was prepared by the method employed by Batelli and Stern (10) and Dixon (11). The brown powder so obtained was stored in a dark bottle. In making solutions for experiments, a weighed amount of the enzyme was ground carefully with small quantities of CO_2-free water, which had been well boiled and cooled to below 10°C., and made up to 100 cc., any insoluble matter being removed by filtration. The strength of the enzyme solution was determined by performing an experiment under the standard conditions mentioned above.

The solutions of hydrogen peroxide were prepared by diluting Merck's perhydrol to the required dilution with distilled water and solutions of buffer salts. The buffer mixtures used were those described by Clark (12) and Kolthoff (13), the latter for the wider pH range. The pH of the solution was measured colorimetrically by means of indicators.[1]

The experiments were performed in a thermostat accurately controlled to within 0.05°C. of the required temperature. The solutions were stirred mechanically by a glass stirrer doing 50 revolutions per minute. The decomposition was followed by titration of samples withdrawn at regular intervals into ½ cc. of strong sulfuric acid (which stopped the reaction immediately), with standard $KMnO_4$ solution. Blank experiments were performed to guard against errors due to the spontaneous decomposition of hydrogen peroxide. It was found that no such decomposition took place during the time of the experiments described in the following sections.

I.

The Effect of Catalase Concentration.

Experiments were performed both in concentrated (0.2 N) and dilute (0.057 N) solutions of hydrogen peroxide, varying the catalase concentration. It will be seen that the initial velocity is proportional to the amount of catalase taken. In the dilute solution used in the

[1] The criticism brought forward by McBain, Dubois and Hay (14) is not applicable here, as the comparator solutions were made up with the same salts as those used in the preparation of the buffered solutions.

experiments summarised in the last section of Table II, the activity of the enzyme is given by $k_1.E_0 = \frac{1}{S_0}.\frac{ds}{dt_t} = 0$ (*cf.* equation (2)), while in the more concentrated solutions of the first two sections of Table II the velocity is independent of the concentration of hydrogen peroxide, as seen below. In this latter case, the true activity of

TABLE II.

Stock catalase solution, strength 1.1 (units defined above). pH = 6.8, concentration of H_2O_2 = 0.22 N, temperature = 20°C.

Relative amount of catalase	Initial velocity	Relative activity
	mols H_2O_2/min.	
4	0.06	4.02
2	0.03	2.01
1	0.0143	1.0

Stock catalase solution, strength 5.4. pH = 6.8, concentration of H_2O_2 = 0.22 N, temperature 20°C.

1.0	0.27	1.0
1.5	0.428	1.59
3.0	0.857	3.17
5.0	1.429	5.29

Stock catalase solution, strength 0.084. pH = 6.8, concentration of H_2O_2 = 0.057 N, temperature 20°C.

Relative amount of catalase	Activity given by $k_1 \cdot E_0$	Relative activity
1.0	0.04	1.0
1.5	0.063	1.57
2.0	0.078	1.95
2.5	0.10	2.5

the enzyme would be given by the initial velocity divided by some constant representing the concentration of hydrogen peroxide at which the velocity becomes independent of it. As is seen, the activity of catalase is proportional to the amount of catalase taken. This is the general fact found for all enzymes.

II.

The Effect of Hydrogen Peroxide Concentration on the Activity of Liver Catalase.

It is generally found for enzyme reactions that whereas the velocity of decomposition is directly proportional to the concentration of enzyme, no such proportionality exists between the rate of reaction and the substrate concentration except in very dilute solution. Senter (1) showed that while the rate was proportional to the concentration of hydrogen peroxide in dilute solution, in solutions more concentrated than 0.1 N the velocity becomes independent of peroxide concentration. To examine this behaviour of liver catalase with varying concentrations of hydrogen peroxide more fully, experiments

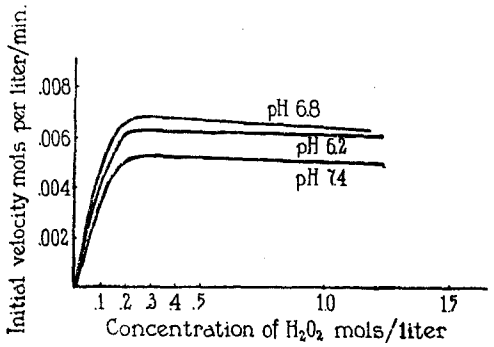

FIG. 2. Variation of initial velocity with H_2O_2 concentration; temperature 15°C.

were conducted over a large range of peroxide concentrations. The experiments were carried out at 15° and 20°C. and at pH 6.2, 6.8 and 7.4 (the optimum activity of catalase being shown by Morgulis and others and confirmed by the writer to be at pH = 6.8). The decomposition was followed for about 10 minutes, the concentration being determined by titration every minute. The values thus obtained were plotted against time and the initial tangent drawn carefully, thus giving the initial velocity. The strength of the catalase solution used in these experiments was 0.18, the strength being calcu-

lated in terms of the unit already defined. The experimental results are plotted in Figs. 2 and 3. It is seen that only for a small range of hydrogen peroxide concentrations does the velocity of decomposition vary in direct proportion to concentration. For pH = 6.8 at 20°C. the relationship has been studied in greater detail. The results for

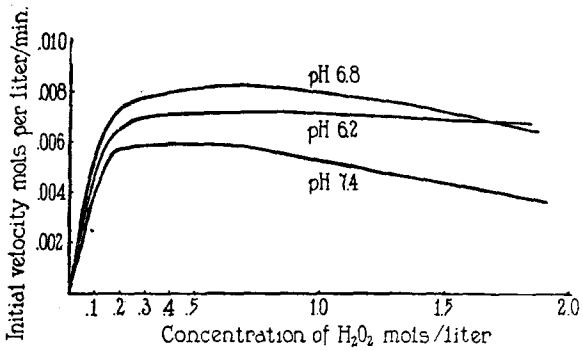

FIG. 3. Variation of initial velocity with H_2O_2 concentration; temperature 20°C.

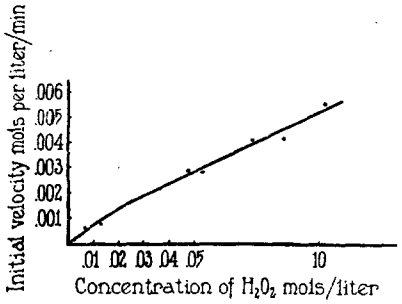

FIG. 3*a*. Variation of initial velocity with H_2O_2 concentration for low concentration values; pH 6.8, temperature 20°C.

the more dilute solutions of peroxide are given in Fig. 3*a*. It will be seen that at very low concentrations of hydrogen peroxide the velocity increases more rapidly than is the case for the next portion of the curve which is characterised by a linear relation. As shown in Fig. 3, at still higher concentrations the velocity varies relatively little with

peroxide concentration. In this higher range of concentration, the velocity passes through a flat maximum in the neighbourhood of 0.5 N solutions of hydrogen peroxide. Evans (15) found the maximum to lie between 0.5 and 1.0 N solutions. Morgulis (5) places the maximum activity of catalase at 0.56 N. Curves similar to the first portion of the curves given above (*i.e.* prior to the maximum) have been obtained with other enzymes, with lactase by Armstrong (16), with invertase by Hudson (17) and Nelson and Bloomfield (18), and with malt amylase by Edie (19). These curves are similar to adsorption isotherms in shape. The results of Fig. 3*a* obey a Freundlich isotherm $v = k.C_{H_2O_2}^{0.87}$ which is of the same order as that found by MacInnes (20) for the decomposition of hydrogen peroxide by colloidal platinum. Further support to the view that the decomposition of hydrogen peroxide by catalase involves a process of adsorption is

TABLE III.

Concentration of H_2O_2	$k_{uni.}$	Concentration of H_2O_2	$k_{uni.}$
mols/liter		*mols/liter*	
0.08575	0.0262	0.008573	0.065
0.05257	0.0413	0.006636	0.0686
0.0174	0.064	0.004458	0.0684
0.01256	0.0668		

afforded by the data of Table III. Unimolecular constants have been calculated from the experiments performed in very dilute solutions of peroxide. As will be seen, the values of k_{uni} rise as the dilution of peroxide is increased, reaching a limiting value of 0.066 to 0.068. This behaviour is usually associated with adsorption processes.

Above 0.5 N, the velocity of decomposition commences to decrease with increasing concentrations of hydrogen peroxide. Similar behaviour has been observed in the case of other enzymes. Van Slyke and Cullen (21) find that solutions of urea more concentrated than 10 per cent depress the action of urease, and attribute this depressor action to the high osmotic pressure of the concentrated solutions of urea. The same behaviour is also found for invertase by Nelson and Larson (22). They attribute this behaviour with invertase to de-

creased adsorption of sucrose in concentrated solutions, basing their conclusion on the similarity of their curve to that obtained by von Schmidt-Walter (23) for the adsorption of acetic acid from aqueous solutions by charcoal. Morgulis, Beber and Rabkin (6) attribute the decrease in activity of catalase (as measured by the total amount of hydrogen peroxide which the catalase will liberate in infinite time) with increasing hydrogen peroxide concentration to be due to an oxidation of the enzyme by the excess peroxide. As will be seen from the results of Section IV, Table XII, the activity of the enzyme in concentrated solutions of hydrogen peroxide, as measured by the total amount of decomposition does not follow the same relation as the activity as measured by the initial velocity. Whereas the total amount of decomposition decreases with increased peroxide concentrations at all pH values, being slightly more pronounced on the alkaline side, the initial velocity decreases much more rapidly at pH 7.4, than at pH 6.8 or 6.2, where the velocity remains practically stationary. It is difficult to decide here whether the effect of the high concentrations of peroxide is due to a decrease in the activity of the enzyme surface as a catalyst or to a decrease in the adsorbed concentration at the surface. Both seem to be so intimately connected. This retardation or decrease in activity is accentuated in more alkaline solutions.

III.

The Decomposition by Liver Catalase of Hydrogen Peroxide in Dilute Solutions.

In this region of hydrogen peroxide concentration two simultaneous reactions occur, the enzyme being inactivated (induced inactivation) as the peroxide is catalytically decomposed. The kinetics of the whole process is represented by the expression already deduced, namely

$$k_3 = k_1 \cdot k_2 = \frac{1}{A \cdot t}\left(\log_e \frac{S_0}{S} - \log_e \frac{(S_0 + A)}{(S + A)}\right) \qquad (4)$$

It is of interest to obtain some information as to the variation of the rates of these two processes with the hydrogen ion concentration. The data of Bodansky (24) indicate that two simultaneous reactions affected in a different manner by pH are going on in the system. To

TABLE IV.

pH 4.0, temperature 15°C.

Time	Concentration of H_2O_2	k_3
min.	*mols/liter*	
0	0.05121	—
1	0.04991	(1.05)
2	0.04763	(1.51)
3	0.04660	1.38
4	0.04557	1.31
5	0.04402	1.42
7	0.04181	1.45
10	0.03961	1.38
12	0.03821	1.38
15	0.03682	1.32
20	0.03416	1.39

$A = -0.026$. Average $k_3 = 1.38$

For duplicate experiment $k_3 = 1.58$

Mean $k_3 = 1.48$

$k_1 \cdot E_0$ {(calculated) 0.035, (observed) 0.032} Average 0.0335. $\boldsymbol{k_1 = 0.0604}$ $\boldsymbol{k_2 = 24.50.}$

TABLE IV*a*.

pH = 8.0.

Time	Concentration of H_2O_2	k_3
min.	*mols/liter*	
0	0.05015	—
1	0.03179	(3.67)
2	0.02400	3.09
3	0.01716	3.14
4	0.01340	2.97
5	0.00964	3.07
6	0.00810	2.87
7	0.00596	2.95

$A = 0.084$. Average $k_3 = 3.01$

For duplicate experiment $k_3 = 3.31$

Mean $k_3 = 3.16$

$k_1 \cdot E_0$ {(calculated) 0.404, (observed) 0.40} Average 0.402. $\boldsymbol{k_1 = 0.721}$ $\boldsymbol{k_2 = 4.38.}$

TABLE IV*b*.

pH = 9.0.

Time	Concentration of H_2O_2	k_3
min.	*mols/liter*	
0	0.05115	—
2	0.03387	(4.98)
3	0.03078	4.31
4	0.02790	4.07
5	0.02504	4.01
7	0.02143	3.89
10	0.01693	3.95
12	0.01480	4.00
15	0.01244	4.05
20	0.00950	4.28
25	0.00826	4.06
30	0.00604	3.82

A = zero. Average k_3 = 4.04

For duplicate experiment k_3 = 4.44

$k_1 \cdot E_0$ {(calculated) 0.21 / (observed) 0.25} Average 0.23. $\boldsymbol{k_1 = 0.414}$ $\boldsymbol{k_2 = 10.24.}$

TABLE V.

Temperature 15°C.

pH	k_1	k_2	k_3
4.0	0.0604	24.50	1.48
5.0	0.202	15.01	3.15
5.6	0.590	5.66	3.33
6.0	0.757	5.15	3.90
7.0	0.856	3.00	2.56
8.0	0.721	4.38	3.16
8.4	0.590	4.51	2.66
9.0	0.414	10.24	4.44

TABLE VI.

Temperature 20°C.

pH	k_1	k_2	k_3
4.0	0.0991	22.7	2.25
5.0	0.261	8.0	2.09
6.0	0.870	4.63	4.03
7.0	0.901	4.22	3.80
8.0	0.784	5.04	3.95
8.4	0.420	6.04	2.54
9.0	0.353	10.40	3.67

investigate the variation in the velocities of the two reactions in dilute solutions of hydrogen peroxide, with change in pH, experiments were performed with hydrogen peroxide solutions 0.05 to 0.055 N, and catalase of strength 1.17 at pH values from 4.0 to 9.0, the pH being attained by the use of Kolthoff's buffer mixtures. The experiments were performed at 15° and 20°C. The results of three typical experiments are given in Tables IV, IV*a* and IV*b*.

It will be seen that in Table IV, A happens to be negative because of the pH value chosen. Tables IV*a* and IV*b* are examples where the value of A is positive and zero respectively.

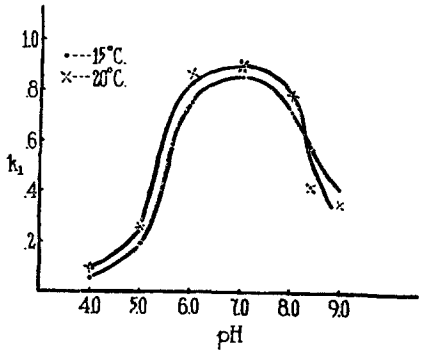

FIG. 4. Variation of k_1 with pH at 15° and 20°C.

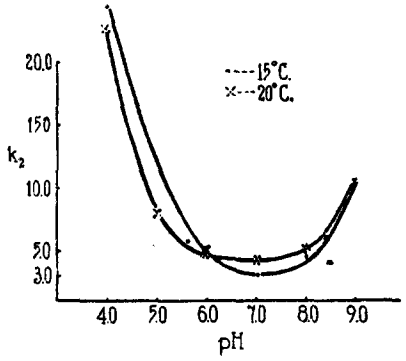

FIG. 5. Variation of k_2 with pH at 15° and 20°C.

The results of the series of experiments, of which Tables IV, IV*a* and IV*b*, are different typical examples, are summarized in Tables V and VI, and plotted in Figs. 4 and 5. Each result is the mean value of two concordant duplicate experiments.

From the results shown in Tables V and VI, it is seen that the activity of catalase given by k_1 rises on both sides to a maximum at about pH 7.0 in agreement with the position of the optimum pH found by others by a different method, while at the same time the value of k_2, the velocity constant of induced inactivation of the enzyme, passes through a minimum at the same pH. It is difficult to state whether the intersection of the curves in Figs. 4 and 5 is a real effect or due to the inherent errors introduced by the rather long and indirect method of calculating the results. It will be seen that except between pH 6.6

and 7.2, there is only a very small difference between the values of k_2 at the two temperatures, *i.e.*, the temperature coefficient or more accurately the critical increment of the induced inactivation of catalase is very small.

In the above cases summarised in Tables V and VI, the initial concentration of hydrogen peroxide, namely 0.05 N, and the catalase concentration were such that equation (4) in its complete form had to be employed. We will now work with a still more dilute solution of H_2O_2, namely 0.03 N, where in the presence of excess of catalase, the

TABLE VII.

Temperature 15°C.

pH	k_1	pH	k_1
6.2	.836	7.0	.884
6.4	.878	7.2	.872
6.6	.896	7.4	.848
6.8	.896		

TABLE VIII.

Temperature 20°C.

pH	k_1	pH	k_1
6.2	.938	7.0	.980
6.4	.991	7.2	.950
6.6	.997	7.4	.925
6.8	1.003		

reaction gives a unimolecular velocity constant, namely k_1 (which refers to the catalytic decomposition of hydrogen peroxide), for under these conditions equation (4) reduces to $k_1 = \frac{1}{E_0.t} . \log_e \frac{S_0}{S}$. This latter form is arrived at as follows: $A = \frac{E_0}{k_2} - S_0$. Since E_0 is in excess, E_0 will be much greater than $k_2 S_0$, and A will correspondingly be much greater than S_0 and S so that the term $\log_e \left(\frac{S_0 + A}{S + A}\right)$ of

equation (4) becomes zero and since $A = \frac{E_0}{k_2}$, equation (4) becomes

$k_3 = k_1k_2 = \frac{k_2}{E_0.t} . \log_e \frac{S_0}{S}$, that is $k_1 = \frac{1}{E_0.t} \cdot \log_e \frac{S_0}{S}$.

Experiments performed under such conditions are summarised in Tables VII and VIII. The concentration of H_2O_2 solutions was 0.03 N and the catalase solution of strength 0.81. The experiments were performed at 15° and 20°C.

The pH values were obtained with the aid of Clark and Lubs' KH_2PO_4 – NaOH mixtures. From Tables VII and VIII, it will be seen that the optimum pH for catalase is at pH 6.8, which agrees very well with that obtained under the different conditions to which Tables V and VI refer. This is in agreement with the values given by Sörensen (2) and Morgulis (5). At this optimum pH two effects coincide, namely the maximum catalytic activity of catalase and the minimum induced inactivation. It will be seen in the next section that the same behaviour is met with in more concentrated solutions of hydrogen peroxide.[2]

IV.

The Decomposition by Catalase of Hydrogen Peroxide in Concentrated Solutions.

Morgulis and his coworkers (5, 6) have studied the action of catalase on hydrogen peroxide in solutions 0.2 N to 18 N and have noted several interesting features. The results obtained by the writer in general confirm the results of these workers.

In the deduction of equation (4) which fitted the results of the previous section, the fundamental postulate already indicated by Yamasaki and for which further experimental evidence has been offered was that the rates of inactivation of the enzyme and the decomposition of hydrogen peroxide by catalase are connected by the relationship $dE/dt : dS/dt$ = a constant (equation (3)). Furthermore, the induced inactivation was regarded as being due to the product of reac-

[2] Theoretically, the values of k_1 in Tables VII and VIII should be identical with the corresponding values at the same pH and temperature in Tables V and VI. The difference is due to the use of different buffers.

tion, oxygen, *while still in the activated state* immediately following its production from the peroxide. On this basis, the constant of equation (3) becomes identified with k_2, the velocity constant of induced inactivation. (This interpretation of the constant differs from that of Yamasaki.) It is possible by applying these considerations to the case of concentrated hydrogen peroxide solutions to arrive at an expression identical in form with that deduced by Northrop (25) to fit the results of Morgulis (5).

It has been shown in Section II that the velocity of decomposition of hydrogen peroxide may be taken as approximately independent of the concentration of peroxide in the more concentrated solutions, the divergence being greatest in alkaline solutions. So that we may write

$$-\frac{dS}{dt} = k_1 \cdot E \cdot S_l = k'_1 \cdot E \tag{1a}$$

where S_l is the concentration of hydrogen peroxide at which the velocity becomes independent of concentration, S_l will be referred to as the limiting concentration of hydrogen peroxide.

$$\text{Also we have } -dE/dt = -k_2 \cdot dS/dt \tag{3}$$
$$\text{As before } E = k_2 \cdot (S + A) \text{ where } A = (E_0/k_2 - S_0).$$

Substitution in (1a) and integration gives

$$k'_2 = k'_1 \cdot k_2 = \frac{1}{t} \cdot \log_e \frac{(S_0 + A)}{(S + A)} \tag{4a}$$

Now the concentration of hydrogen peroxide at any time t is found from (4a) to be:

$$S_t = \frac{E_0}{k_2} \cdot e^{-k'_2 t} - A.$$

When t is infinity, *i.e.* the reaction is completed, the first term on the dexter side is zero and $S_t = -A$. If A is negative, *i.e.* E_0 is less than $k_2.S_0$, hydrogen peroxide being in excess, the reaction will stop with a residual concentration of hydrogen peroxide in solution numerically equal to A. This condition is fulfilled in all the experiments quoted in this section. If we call the total amount of hydrogen peroxide decomposed B, it is seen that,

$$B = (S_0 + A) = \frac{E_0}{k_2} \tag{5}$$

If the amount of hydrogen peroxide decomposed after a time t is x, then it follows that the term $(S + A)$ becomes $(B - x)$ so that equation (4*a*) becomes

$$k_3' = \frac{1}{t} \cdot \log_e \frac{B}{(B - x)} \tag{4b}$$

which is identical in form with that deduced by Northrop (25).

In Northrop's expression, deduced on the assumption that the enzyme is inactivated in an unimolecular manner with respect to itself, the final constant obtained is the inactivation constant of the enzyme.

TABLE IX.

Catalase solution strength 0.10. Temperature 10°C.

pH	k_1'	k_2	k_3'
5.6	0.0693	0.1095	.00759
5.8	0.0849	0.0862	.00732
6.0	0.0970	0.0757	.00734
6.5	0.1303	0.0593	.00773
6.6	0.1386	0.0541	.00750
6.8	0.1483	0.0539	.00799
7.0	0.1328	0.0575	.00764
7.2	0.1225	0.0618	.00757
7.4	0.1120	0.0652	.00730

Here k_3' is the product k_2, the constant of the inactivation process, k the constant of the catalytic decomposition of hydrogen peroxide and S_l, the limiting concentration of peroxide. The same difference is noted between the expression of the writer and Northrop as was found in the previous case between Yamasaki and the writer, the difference resting on the significance given to the relation $dE/dt : dS/dt$ = a constant. From the experimental results the value of k_3' is readily obtained. As will be seen from (1*a*) the initial tangent to the concentration time curves for the experiments will give the value of $k_1 \cdot S_l \cdot E_0$. The catalase concentration being known, in terms of the unit previously defined, the value of $k_1 \cdot S_l$ may be calculated, from which $\frac{k_3'}{k_1 S_l}$ gives the value of k_2. The value of the limiting concentra-

tion (S_i) of hydrogen peroxide on catalase is difficult to determine except approximately. It seems to vary with pH between the values 0.09 and 0.14 mols per liter.

Tables IX, X and XI contain a summary of the results obtained with 0.2 to 0.25 N solutions of hydrogen peroxide at different pH and

TABLE X.

Catalase solution strength 0.13. Temperature 15°C.

pH	k_1'	k_2	k_3'
5.6	0.1346	0.0929	.0125
6.0	0.1515	0.0766	.0116
6.2	0.1496	0.0929	.0139
6.6	0.1670	0.0664	.0111
6.8	0.1696	0.0696	.0118
7.2	0.1468	0.0776	.0114
7.4	0.1384	0.0932	.0129
7.8	0.1309	0.0879	.0115

TABLE XI.

Catalase solution strength 0.10. Temperature 20°C.

pH	k_1'	k_2	k_3'
5.6	0.1468	0.217	.0319
6.0	0.1616	0.202	.0326
6.2	0.1696	0.212	.0360
6.6	0.1793	0.204	.0366
6.8	0.1858	0.200	.0372
7.2	0.1709	0.197	.0336
7.4	0.1583	0.184	.0292
7.8	0.1538	0.180	.0277

temperatures. In calculating the values of k_3', it was found best to calculate them using the second reading as the basis rather than the initial. It appears here that some initial disturbance takes place which adversely affects the value of k_3'. The pH values were attained with the aid of Clark and Lubs' KH_2PO_4 – NaOH mixtures. The values of k_1' and k_2 and k_3' are given in Tables IX to XI. The

results of these tables are plotted in Figs. 6 and 7. As will be seen, these results show a similar behaviour to those quoted in the previous section for dilute solutions of hydrogen peroxide. The values for k_1' show a maximum at pH 6.8 for the three temperatures. The constant of the inactivation process (k_2) shows a minimum at pH 6.8 for 10° and 15°C., while for 20°C., the value of k_2 is nearly constant, decreasing slightly with increase in pH.

It has already been stated that Morgulis measures the activity of catalase by the total amount of oxygen which it liberates from hydrogen peroxide. A comparison of the behaviour of the initial velocity of decomposition of hydrogen peroxide, used by the writer as a measure

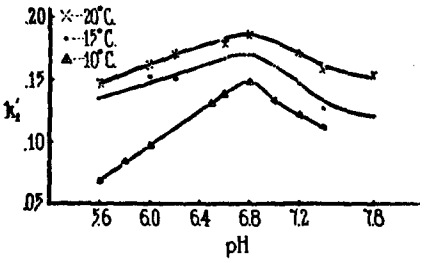

FIG. 6. Variation of k_1 with pH at 10°, 15° and 20°C.

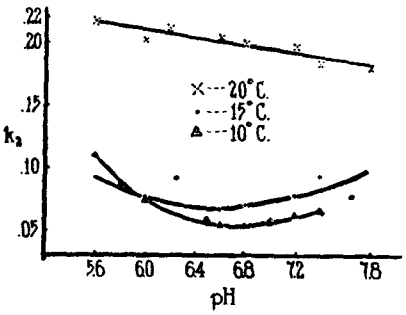

FIG. 7. Variation of k_2 with pH at 10°, 15° and 20°C.

of the activity of catalase ($k_1' \cdot E_0$) and the total amount of decomposition with change in pH is given in Table XII. In each case the relative activity of the enzyme in the least concentrated solution is taken as unity. The same applies to the relative amount of hydrogen peroxide decomposed. The value of k_2 (also relative) is obtained by dividing k_3' by the relative activity of the catalase expressed by the relative initial velocity at that concentration of hydrogen peroxide.

From Table XII, it will be seen that the relative activity ($k_1' \cdot E_0$) and the relative amount of decomposition decrease as the hydrogen peroxide concentration is increased above 0.5 N. Further, whilst the total amount of decomposition decreases with increased hydrogen peroxide concentration at all pH values, the decrease in initial velocity becomes more marked in the more alkaline solutions. On the other hand, the value of k_3' and k_2 at any given pH remain sensibly constant

TABLE XII.

pH 5.8, temperature 20°C.

pH	Concentration of H_2O_2	Relative initial velocity	Relative amount of decomposition	k_3'	Relative k_2
	mols/liter				
	0.1973	1.0	1.0	0.029	0.029
	0.3962	1.375	1.037	0.031	0.023
	0.5894	1.49	0.941	0.036	0.024
	0.7820	1.27	0.898	0.034	0.027
	0.9306	1.28	0.852	.025–.04	.02 –.031
	1.2986	1.29	0.762	.02 –.042	.016–.03
	1.86	1.22	0.707	.02 –.04	.016–.033
6.2	0.1871	1.0	1.0	0.028	0.028
	0.3834	1.284	0.974	0.032	0.024
	0.5600	1.33	0.908	0.033	0.025
	0.7292	1.14	0.803	0.028	0.0245
	0.9136	1.16	0.763	0.027	0.023
	1.3184	1.16	0.649	0.033	0.028
	1.8860	1.0	0.607	.025–.039	.025–.039
6.6	0.1997	1.0	1.0	0.032	0.032
	0.4056	1.0	0.951	0.029	0.029
	0.5860	1.008	0.909	0.028	0.028
	0.7958	1.0	0.879	0.031	0.031
	0.9642	0.926	0.830	0.030	0.032
	1.3532	0.886	0.757	0.025	0.028
	1.9272	0.839	0.628	0.031	0.036
7.0	0.1957	1.0	1.0	0.028	0.028
	0.3900	1.068	0.980	0.027	0.025
	0.5910	1.022	0.946	0.028	0.027
	0.7646	0.914	0.882	0.024	0.026
	0.9076	0.884	0.819	0.022	0.025
	1.2784	0.782	0.770	0.024	0.031
	1.8310	0.742	0.616	0.020	0.027
7.4	0.1960	1.0	1.0	0.021	0.021
	0.3928	1.020	1.063	0.026	0.025
	0.5864	1.045	0.925	0.029	0.028
	0.7744	1.010	0.798	0.022	0.022
	0.9622	0.936	0.686	0.022	0.024
	1.3616	0.852	0.592	0.020	0.024
	1.9460	0.663	0.539	0.020	0.030

as the concentration of hydrogen peroxide is changed, whereas it was to be expected that since the total amount of hydrogen peroxide decomposed is given by E_0/k_2 (*cf.* equation (5)), k_2 should *increase* in the same proportion as the total amount of decomposition *decreases*. Morgulis, Beber and Rabkin (6) report that their constant corresponding to k_3' remained constant over the range of peroxide concentration to which Table XII refers. It has been noted that in the evaluation of k_3', the second reading is taken as the basis of calculation. This procedure was adopted because of the initial disturbance which takes place. Morgulis, Beber and Rabkin have also noted this initial "outburst" in oxygen evolution, after which the reaction settles down to a steady course. In very concentrated solutions of peroxide, they find that the reaction is entirely confined to this initial stage, lasting a few minutes. It follows that the constant k_3', and hence k_2, refers to this steady course. Before it has been reached, however, the enzyme has been inactivated to a great extent in the concentrated solution of peroxide, as much as 50 to 60 per cent of the enzyme's activity (as measured by the instantaneous velocity of decomposition of hydrogen peroxide) having been lost, so that it is the rate of loss of this remaining 40 to 50 per cent of the activity of the enzyme which is given by k_2, and which remains sensibly constant over the range of hydrogen peroxide concentration considered. The total amount of decomposition however includes both the initial disturbance and the steady course. The discrepancy between the behaviour of k_2 and the total amount of decomposition may be due to the destruction of the enzyme in the initial stage of the reaction.

Quite apart from the question of the oxygen "outburst" effect there is a point to which attention may be drawn in comparing the results of Morgulis and his collaborators with those of the present writer. As already stated, Morgulis measures the activity of the catalase by the total amount of oxygen liberated, the hydrogen peroxide being in excess. The writer on the other hand measures the activity of the catalase by the initial velocity.[3]

[3] Clearly the method of total amount of oxygen liberated would be inapplicable to those conditions wherein the catalase is in excess whilst the initial velocity method would be applicable. Morgulis and his collaborators are concerned however only with relatively concentrated solutions of peroxide and naturally do not suggest that the total oxygen liberated is elsewhere applicable.

Morgulis and Beber find that on the basis of the total oxygen liberated that catalase shows a maximum activity at 2°C., the activity of enzyme (thus measured) decreasing as the temperature rises according to an empirical relation discovered by them. These workers attribute this decrease in activity to destruction of the catalase by oxidation.

The optimum at 2°C. is not to be confused with the more usual optimum temperature found for enzymes in general which is much higher, and is usually attributed to the opposing influence of true enzyme (catalytic) effect upon the substrate and the "spontaneous" inactivation of the enzyme as a consequence of temperature. The 2°C. optimum is ascribed on the other hand to the opposing influence of true enzyme (catalytic) effect upon the substrate and the induced inactivation (peculiar to the catalase-peroxide system) brought about by the presence of the peroxide or as the writer prefers to regard it by the product (oxygen) of the reaction.

On employing the initial velocity as the measure of the enzyme activity, we do not find any maximum at 2°C., the value of the initial velocity increasing with temperature over the range examined (compare values of k_1' at 10°, 15° and 20°C. in Tables IX, X and XI). By adopting this measure of the enzyme activity, we obtain a quantity which is not influenced by the induced inactivation effect. It would therefore seem to be a simpler measure of the enzyme effect than that adopted by Morgulis and his coworkers.

V.

The Effect of Temperature on the Action of Catalase.

The net effect of temperature on the reaction between catalase and hydrogen peroxide will depend on the effect of temperature on each of the two reactions, namely the catalytic decomposition of the peroxide and the induced inactivation of catalase, which takes place simultaneously. The critical increments of the two processes have been calculated from the data of the preceding tables by the aid of the Marcelin-Rice equation, and are given in Tables XIII to XV. E_1 is the critical increment of the catalytic reaction and is given by $E_1 =$

TABLE XIII.

Critical Increments Calculated from the Data of Tables V and VI.

pH	E_1 .15–20°C.	E_2 15–20°C.
4.0	16,730	—
5.0	8,820	—
6.0	4,700	—
7.0	1,730	11,530
8.0	2,830	4,740
8.4	—	9,870

TABLE XIV.

Critical Increments from the Data of Tables VII and VIII.

pH	E_1 .15–20°C.	pH	E_1 15–20°C.
6.2	3,870	7.0	3,460
6.4	4,100	7.2	2,830
6.6	3,630	7.4	2,960
6.8	3,830		

TABLE XV.

Critical Increments Calculated from the Data of Tables IX to XI.

pH	E_1 .10–15°C.	E_1 .15–20°C.	E_2 .10–15°C.	E_2 .15–20°C.
5.6	21,600	2,940	—	28,670
6.0	14,540	2,180	383	32,770
6.2	—	4,240	—	27,260
6.6	6,070	2,405	6,680	37,930
6.8	4,380	3,080	8,330	35,670
7.2	5,910	5,090	7,425	31,480
7.4	7,450	4,535	11,650	22,990
7.8	—	5,450	—	23,450

$RT^2 . \frac{d \ln k_1}{dT}$. E_2 is the critical increment of induced inactivation and is given by $E_2 = RT^2 . \frac{d \ln k_2}{dT}$.

From the data in Tables XIII to XV, it is seen that the critical increment of the catalytic decomposition of hydrogen peroxide by cata-

lase is fairly constant over the range 15° to 20°C. and of the order of 3000 calories. This is in agreement with the value calculated from the data of Yamasaki (4), namely, 2700 to 3150. Senter (1) and Nordefeldt (26) obtained a value of 6000 calories. The value of E_1 appears to pass through a minimum in the neighbourhood of the optimum pH.

The values for the critical increment of induced inactivation are more irregular. For the dilute solutions of peroxide, the value seems to be low. The most surprising abnormality is noticed in the values calculated for the experiments performed in the more concentrated solutions of peroxide (Table XV). The value rises from about 8000 at 10 to 15° to 30,000 at 15° to 20°C. This behaviour is similar to that reported by Morgulis, Beber and Rabkin (6) who find that the critical increment (which applies to the inactivation reaction and not to the "catalase reaction" as they state) varies with concentration of hydrogen peroxide, and increases with temperature even up to 40,000 calories. This sudden change in the value of the critical increment may mean that the nature of the induced inactivation reaction changes in the more concentrated solution at the higher temperature, by the intervention of some factor which was previously of minor importance. Increase in temperature has also a large effect on the initial stage of the reaction. As the temperature is raised, the decomposition tends to become more limited to this initial stage where the decomposition of hydrogen peroxide is immeasurably fast and the enzyme is quickly inactivated. This effect is seen in the rapid decrease in the amount of hydrogen peroxide a given amount of catalase will decompose as the temperature rises.

SUMMARY.

1. The velocity of decomposition of hydrogen peroxide by catalase as a function of (*a*) concentration of catalase, (*b*) concentration of hydrogen peroxide, (*c*) hydrogen ion concentration, (*d*) temperature has been studied in an attempt to correlate these variables as far as possible. It is concluded that the reaction involves primarily adsorption of hydrogen peroxide at the catalase surface.

2. The decomposition of hydrogen peroxide by catalase is regarded as involving two reactions, namely, the catalytic decomposition of

hydrogen peroxide, which is a maximum at the optimum pH 6.8 to 7.0, and the "induced inactivation" of catalase by the "nascent" oxygen produced by the hydrogen peroxide and still adhering to the catalase surface. This differs from the more generally accepted view, namely that the induced inactivation is due to the H_2O_2 itself. On the basis of the above view, a new interpretation is given to the equation of Yamasaki and the connection between the equations of Yamasaki and of Northrop is pointed out. It is shown that the velocity of induced inactivation is a minimum at the pH which is optimal for the decomposition of hydrogen peroxide.

3. The critical increment of the catalytic decomposition of hydrogen peroxide by catalase is of the order 3000 calories. The critical increment of induced inactivation is low in dilute hydrogen peroxide solutions but increases to a value of 30,000 calories in concentrated solutions of peroxide.

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