THE KINETICS OF THE DECOMPOSITION OF PEROXIDE BY CATALASE.

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The study of the kinetics of catalase, as of most other enzymes, has led to contradictory results. It was found by Senter[1] that the reaction was monomolecular with dilute peroxide and low temperature, but that under other conditions the reaction could not be fitted to the monomolecular formula. Senter ascribed this anomaly to the destruction of the enzyme and limited his experiments to conditions under which the monomolecular formula held. Sörensen,[2] Michaelis and Pechstein,[3] Evans,[4] and others followed the reaction at higher temperature and with more concentrated peroxide, and confirmed Senter's results that under these conditions the reaction was not monomolecular. They were unable to derive any satisfactory mechanism for the reaction. Yamasaki[5] concluded that the enzyme was decomposing at a rate that was proportional to the rate of decomposition of the peroxide and could account for his results fairly well on this basis. He was unable, however, to give any mechanism for this relation. A thorough experimental study of the reaction has recently been made by Morgulis,[6] who also found that at 20°C. and high peroxide concentration the reaction was in general not monomolecular. Part of his results could be calculated by the bimolecular formula but a large part of them could not be accounted for. Morgulis' experiments showed that the enzyme

[1] Senter, G., *Z. physik. Chem.*, 1903, xliv, 257.
[2] Sörensen, S. P. L., *Biochem. Z.*, 1909, xxi, 131.
[3] Michaelis, L., and Pechstein, H., *Biochem. Z.*, 1913, liii, 320.
[4] Evans, C. A. L., *Biochem. J.*, 1907, ii, 133.
[5] Yamasaki, E., *Sc. Rep. Tohoku Imp. Univ.*, 1920, ix, 13.
[6] Morgulis, S., *J. Biol. Chem.*, 1921, xlvii, 341.

was being rapidly inactivated under the conditions of his experiments. In some experiments on the kinetics of trypsin the writer has found similar conditions and has been able to show experimentally that the inactivation of the enzyme is the cause of the anomalous results.[7] It has been found that the formulation derived for the trypsin experiments also agrees very well with the results of Morgulis and the present paper is a recalculation of Morgulis' results on this basis.

Experimental Conditions and General Results.

Morgulis followed the course of the reaction by noting the volume of oxygen given off. The reaction mixture was shaken to avoid supersaturation. The temperature was 20°C. The catalase was prepared from liver. Owing to the method of measurement, the total amount of oxygen was recorded and not the amount per unit volume of the solution, as is usually done. For use in this paper the results have been calculated to unit volume.

Effect of Varying the Concentration of Catalase.

The results of increasing the concentration of catalase with the same concentration of peroxide are given in Table I. It is evident that the time required to produce a constant amount of oxygen decreases much more rapidly than the concentration of enzyme increases. The value of Qt, therefore, decreases rapidly as the enzyme increases, which is contradictory to the result predicted for a catalyzed monomolecular reaction.

In Table II the amounts of oxygen liberated after equal time intervals with different concentration of catalase are given. The table shows that there is a direct proportionality between the amount of oxygen and the concentration of catalase. This result is the opposite of what is usually obtained with enzymes.

Effect of Varying the Peroxide Concentration.

A summary of the experiments in which the concentration of peroxide was varied, keeping the catalase constant, is given in Table

[7] Northrop, J. H., *J. Gen. Physiol.*, 1923–24, vi, 429.

TABLE I.

H_2O_2 = 0.32 M. (Morgulis, p. 359.)

Relative concentration of catalase.	Time to form 25 cc. O_2.	Qt
cc.	*min.*	
3	4.25	12.7
4	1.90	7.6
5	1.50	7.5
6	0.75	4.5
7	0.45	3.1

TABLE II.

Oxygen Evolved at Equal Time Intervals.

H_2O_2 = 0.20 M. (Morgulis, p. 359.)

Relative concentration of catalase.	O_2 after		$\frac{O_2}{\text{catalase}}$ (after 20 min.)
	10 min.	20 min.	
cc.	*cc.*	*cc.*	
6.75	80	102	15
5.63	69	91	16
4.5	55	76	17
3.75	47	63.5	17
3.0	33.6	46.4	16

TABLE III.

Concentration of H_2O_2.

Catalase constant (5 cc.). (Morgulis, p. 359.)

Concentration of H_2O_2.	Time to form 25 cc. O_2.	O_2 liberated in 10 min.
M	*min.*	*cc.*
0.16	1.2	81.6
0.20	1.55	85.6
0.24	1.70	82.5
0.28	1.85	81.6
0.32	1.80	81.6
0.36	1.85	82.9
0.40	2.35	69.4

III. The results show that the time required to liberate a constant amount of oxygen increases slightly as the peroxide increases, whereas the amount of oxygen liberated in a constant time interval is independent of the peroxide concentration over a considerable range. This result confirms the statement made above that the rate of reaction is independent of the peroxide concentration in this range and that the enzyme is decreasing as a function of time and not as a function of the amount of oxygen produced. This result was obtained experimentally by Michaelis and Pechstein, who found that the reaction as followed by the decrease in peroxide was identical whether or not the oxygen was removed as formed or allowed to accumulate in the solution. The fact that the reaction was independent of the peroxide concentration in concentrations over about 0.1 M was noted by Senter. This is a common phenomenon in enzyme reactions and may be accounted for either by assuming that the enzyme forms a compound with the substrate or that there is an equilibrium between the substrate and the solvent, so that the "active" concentration of substrate does not increase as does the apparent concentration. Yamasaki has also been able to account for this effect in the case of urease by considering the reaction as consisting of three consecutive steps. The same phenomenon is known in trypsin hydrolysis and the writer has been able to show experimentally that it is not due to the formation of a compound between the enzyme and substrate.[8]

Relation between the Concentration of Catalase and the Total Amount of Oxygen Liberated.

The results of the experiments in which the concentration of catalase was varied are given in Table IV. The table shows that the total amount of oxygen liberated is directly proportional to the concentration of catalase (provided, of course, that there is an excess of peroxide). This result is the exact opposite of that expected for a catalytic reaction, since the end-result should be independent of the concentration of catalyst.

[8] Northrop, J. H., *J. Gen. Physiol.*, 1923–24, vi, 239, 337.

Concentration of Peroxide and Total Amount of Oxygen Liberated.

The relation between the concentration of peroxide and the total amount of oxygen liberated is shown in Fig. 1. It shows that the total amount of oxygen liberated increases with the concentration of peroxide up to about 0.32 M and then decreases and that there is no constant percentage decomposition. This result also applies only to the case where there is excess peroxide.

TABLE IV.

(Morgulis, p. 368.)

H_2O_2 ⇌ 112 cc. O_2.			H_2O_2 ⇌ 181 cc. O_2.		
Catalase.	Total O_2.	$\frac{O_2}{\text{cc. catalase}}$	Catalase.	Total O_2.	$\frac{O_2}{\text{cc. catalase}}$
cc.	*cc.*		*cc.*	*cc.*	
6.75	112	17	7	180.7	25.8
5.63	104	18	6	158.0	26.3
4.5	88	17	5	122.7	24.5
3.75	70	19	4	106.0	26.5
3.0	57	19	3	71.0	23.9

Summary of General Results.

The results of the foregoing experiments may be summarized as follows:

Peroxide Constant and in Excess, and Catalase Varied.

1. The amount of oxygen liberated after a given time is directly proportional to the concentration of catalase

2. The total amount of oxygen liberated is directly proportional to the concentration of catalase.

3. There is no simple relation between the time required to liberate a constant amount of oxygen and the concentration of catalase. The time decreases much more rapidly than the catalase concentration increases.

Catalase Constant and Peroxide Varied. Peroxide Always in Excess.

1. The amount of oxygen liberated after a constant time interval is independent of the peroxide concentration within a wide range.

2. The total amount of oxygen liberated increases up to a concentration of peroxide of 0.32 M and then decreases.

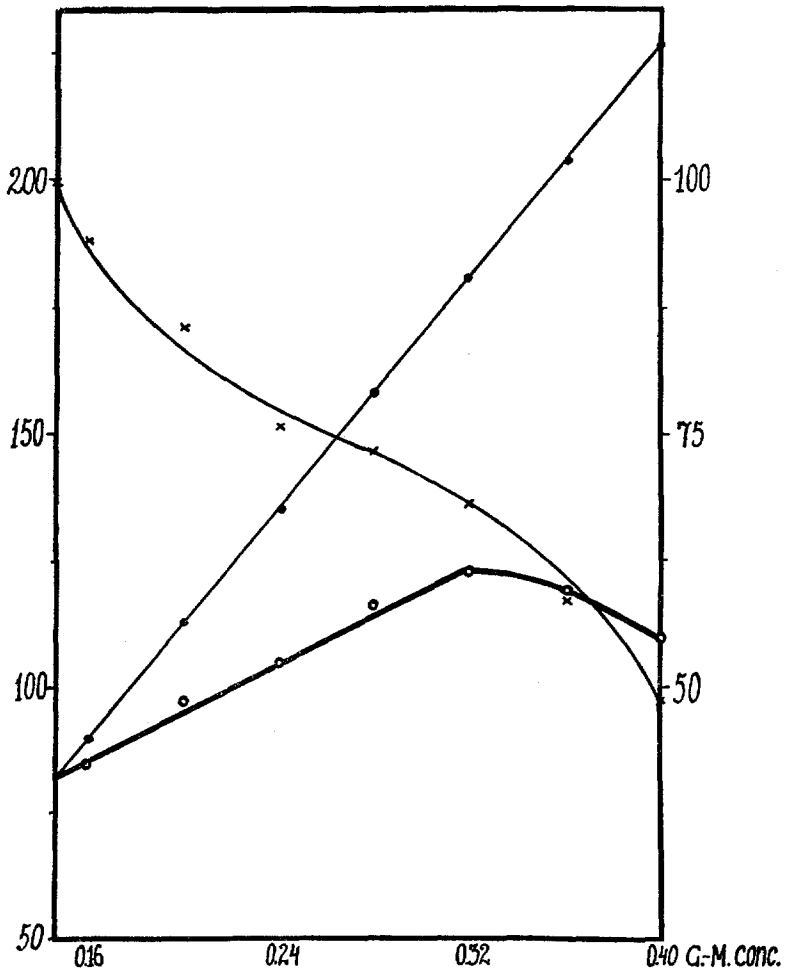

FIG. 1. Effect of increasing quantities of hydrogen peroxide on the catalase reaction. ●—● cc. of oxygen available. o—o cc. of oxygen liberated. ×—× per cent of hydrogen peroxide decomposed. (After Morgulis.)

3. The time required to liberate a constant amount of oxygen increases as the peroxide increases.

These results show definitely that under these conditions the reaction is not monomolecular with respect to the peroxide and catalyzed by the enzyme, since in that case the end-result would be independent of the concentration of enzyme and not directly proportional to it as is the experimental result. They also show that the reaction is not bimolecular nor does the catalase enter into it in any stoichiometric way, since the total amount of oxygen formed with a constant amount of catalase varies with the concentration of peroxide.

Formulation of the Reaction.

According to classical physical chemistry the general formula for the rate of reaction between any two molecules is

$$-\frac{dS}{dt} = KES$$

in which E is the concentration of (in this case) catalase at the time t, and S is the concentration of peroxide at the time t. If the catalase behaves as a typical catalyst its concentration will not change during the course of the reaction and E becomes a constant. The equation then becomes $-\frac{dS}{dt} = K'S$ or, if x is the amount of substrate decomposed, $\frac{dx}{dt} = K'(S_0 - x)$ which is the ordinary monomolecular formula. This formula was found to hold for the present reaction at low temperatures and low concentration of peroxide by Senter. The results of Morgulis, however, show that (1) the concentration of enzyme is rapidly decreasing as a function of the time, and (2) the reaction rate is independent of the initial peroxide concentration. The formula therefore becomes

$$\frac{dx}{dt} = kSE$$

in which k is the velocity constant of the reaction, S is a constant representing the "active" concentration of peroxide (and independent of the concentration used provided the latter is above 0.1 M), and E is the concentration of catalase at time t. The simplest assumption

in regard to the catalase would be that it was decomposing monomolecularly itself. The concentration E at any time would then be $E_t = E_0 e^{-Kt}$ in which e is the base of the natural logarithms and K is the constant for the decomposition of the enzyme. The formulation of the entire reaction then becomes

$$\frac{dx}{dt} = kSE_0 e^{-Kt}$$

On integrating this equation it becomes

$$x = \frac{SkE_0}{K}(1 - e^{-Kt}) \tag{1}$$

It may be seen that this equation predicts qualitatively the experimental results obtained by Morgulis, and summarized on page 377. For instance, if $t = \infty$, $x = \frac{SkE_0}{K}$, that is the total amount of oxygen formed is proportional to the amount of catalase taken.

If S and t are the same in two experiments, $\frac{x}{x'} = \frac{E}{E'}$, *i.e.* the amount of oxygen produced in a given time is proportional to the amount of catalase taken.

If E_0 and t are the same, but the peroxide concentration is varied, the same amount of oxygen will be liberated since there is no term in the equation for the original concentration of peroxide.

If E is varied and the time noted to liberate x cc. of oxygen, *i.e.* $x = x'$, the time will decrease more rapidly than the enzyme increases, since the formula then becomes

$$\frac{E'}{E} = \frac{1 - e^{-Kt}}{1 - e^{-Kt'}}$$

This type of result has been taken as evidence that the rate of reaction is some exponential function of the enzyme concentration and that therefore the enzyme is adsorbed by the substrate or *vice versa*. The above derivation shows that it is simply the result of the fact that the enzyme is decreasing as a function of time and not as a function of the products of the reaction.

As will be seen below the value of K varies with the original concentration of peroxide. The mechanism of this effect and of the fact that the reaction rate is independent of this value is not taken into account in the above formula.

In order to apply the formula to the time curves of the reaction it may be further simplified as follows:

Since SkE_0 and K are constant for any one experiment, the term $\frac{SkE_0}{K}$ will be constant and may be made equal to A; substituting in (1) $x = A - Ae^{-Kt}$ or

$$K = \frac{1}{t} 1n \frac{A}{A - x}$$

which is again the form of a monomolecular reaction. It must be noted, however, that A does not refer to the peroxide but to the enzyme. It is proportional to the total amount of oxygen liberated, therefore, and not, as would ordinarily be the case, to the total amount of substrate present. K is the constant of decomposition of the enzyme and not the reaction constant. It may vary, therefore, with the peroxide concentration but will be independent of the enzyme concentration which is just the opposite of the behavior of the ordinary monomolecular constant.

Morgulis' experiments have been recalculated with this formula and found to agree very well. The value of A has been taken directly in the cases where a maximum value was reached. In other cases it was obtained by graphic extrapolation; which is of course a rather uncertain method. The results with varying pH, catalase concentration, and peroxide concentration are given in Tables V to VII. It will be seen in every case that the value of K is constant within the experimental error.

Effect of Various Factors on the Value of K.

The values obtained for K, which is the measure of the rate of inactivation of the enzyme, at different degrees of acidity with the same concentration of enzyme and peroxide, are given in Tables V and VIII. Apparently the effect of the pH is not on the main reaction but on the inactivation of the enzyme.

TABLE V.

Rate at Different pH.

H_2O_2 = 0.31 M. Total O_2 = 173 cc. 5 cc. catalase. (Morgulis, pp. 345–346.)

pH	t	$A-x$	K	pH	t	$A-x$	K
	min.	*cc.*			*min.*	*cc.*	
5.2	0	98		7.2	0	133	
	5	47	0.064		5	72.7	0.053
	10	23	0.063		10	43.1	0.049
	15	9	0.068		15	24.8	0.048
	20	4	0.069		20	13.6	0.049
	23	2.7	0.068		25	6.8	0.051
					31	3.0	0.053
5.8	0	122		7.5	0	138	
	5	59.5	0.062		5	77.9	0.051
	10	31.8	0.058		10	48.4	0.045
	15	16.8	0.058		15	30.0	0.045
	20	8.7	0.058		20	17.4	0.048
	25	4.9	0.054		25	10.0	0.045
	31	2.3	0.057		30	5.2	0.047
					35	2.8	0.049
6.4	0	134		8.3	0	138	
	5	71.0	0.055		5	78.9	0.048
	10	40.4	0.0523		10	51.4	0.043
	15	20.6	0.054		15	30.2	0.044
	20	9.8	0.057		20	18.0	0.044
	25	5.7	0.054		25	10.4	0.049
	29	2.8	0.057		30	5.0	0.048
	33	1.3	0.060		35	1.9	0.053
7.0	0	140					
	5	74.9	0.054				
	10	44.7	0.049				
	15	25.7	0.049				
	20	14.8	0.049				
	25	9.1	0.052				
	29	6.1	0.047				
	34	3.9	0.046				

Tables VII and IX show the relation of the peroxide concentration to the value of K. Increasing the peroxide up to 0.4 M decreases the rate of inactivation of the enzyme.[9] It is evident, there-

TABLE VI.

Varying Concentrations of Catalase.

$H_2O_2 \Rightarrow$ 112 cc. O_2. (Morgulis, p. 368.)

Catalase.	t	$A-x$	K	Catalase.	t	$A-x$	K
cc.	*min.*	*cc.*		*cc.*	*min.*	*cc.*	
6.75	0	113		3.75	0	71	
	5	56.4	0.060				
	10	33.0	0.053		10	23.9	0.047
	15	19.0	0.052				
	20	10.9	0.051		20	7.5	0.048
	25	6.7	0.050				
	30	4.2	0.047		30	1.8	0.053
	40	1.2	0.050		40	1.0	0.046
5.63	0	105		3.0	0	59	
	5	56.0	0.054				
	10	35.7	0.047		10	24.2	0.0387
	15	22.9	0.045				
	20	12.5	0.046		20	12.6	0.034
	25	7.5	0.045				
	30	4.0	0.047		30	5.8	0.033
	40	1.3	0.053		40	2.6	0.034
					45	1.8	0.034
4.5	0	89					
	5	51.6	0.046				
	10	33.9	0.042				
	15	21.6	0.041				
	20	12.9	0.042				
	25	8.3	0.041				
	30	5.3	0.041				
	35	3.2	0.040				
	45	1.0	0.043				

fore, that, as Morgulis stated, the enzyme is not oxidized by the peroxide. Further evidence that this is the case is shown by the

[9] In some unpublished experiments kindly furnished the writer by Professor Morgulis the rate of inactivation was found to be independent of the peroxide concentration. Professor Morgulis also kindly furnished the cut for Fig. 1.

fact that the value of K for any one reaction is constant. The concentration of peroxide is, however, decreasing. The inactivation of

TABLE VII.

Varying Concentrations of Peroxide.

Catalase 5 cc. Total volume 50 cc. (Morgulis, p. 364.)

Concentration of H_2O_2.	t	$A-x$	K	Concentration of H_2O_2.	t	$A-x$	K
M	*min.*	*cc.*		M	*min.*	*cc.*	
0.16	0	90		0.32	0	127	
	5	25.6	0.109		5	70.5	0.051
	10	8.4	0.104		10	45.4	0.045
	12	5.0	0.103		15	27.5	0.044
					20	16.4	0.039
					25	9.0	0.060
					30	5.0	0.043
0.20	0	100		0.36	0	125	
	5	36.9	0.087		5	69	0.051
	10	14.4	0.084		10	42	0.048
	15	4.9	0.087		15	24.5	0.053
	18	3.0	0.085		20	14	0.0475
					25	8	0.047
					30	6	0.043
0.24	0	106		0.40	0	115	
	5	47.5	0.070		5	67.3	0.046
	10	23.5	0.065		10	45.6	0.040
	15	9.5	0.069		15	28.4	0.040
	20	2.7	0.078		20	17.2	0.041
	24	1.5	0.077		25	9.8	0.043
					30	5.9	0.043
					33	5.3	0.041
0.28	0	120					
	5	63.3	0.056				
	10	38.4	0.050				
	15	20.5	0.051				
	20	9.6	0.055				
	25	4.8	0.056				
	27	4.1	0.054				

the enzyme, therefore, cannot be due to the peroxide. It seems probable that the stabilizing effect noted is due to an impurity pres-

ent in the peroxide. In higher concentration, however, the inactivation is increased rapidly and is here probably due to direct oxidation. Under these conditions the constants drop.

TABLE VIII.

Effect of pH on Value of K.

pH	K
5.2	0.068
5.8	0.057
6.4	0.055
7.0	0.049
7.2	0.049
7.5	0.049
8.3	0.047

TABLE IX.

Rate of Decomposition and Concentration of H_2O_2.

(Morgulis, p. 364.)

Original concentration of H_2O_2.	K	K × concentration H_2O_2
0.16	0.105	0.0168
0.20	0.086	0.0172
0.24	0.072	0.0172
0.28	0.053	0.0148
0.32	0.048	0.0153
0.36	0.048	0.0172
0.40	0.042	0.0168
2.4	0.7–0.5	

TABLE X.

(*Cf.* Table VI.)

Relative concentration of catalase.	K
6.75	0.051
5.63	0.048
4.5	0.042
3.75	0.048
3.0	0.035

Table X shows that the rate of inactivation is nearly independent of the enzyme concentration as it should be if the decomposition of the enzyme is really monomolecular.

The mechanism upon which the equation used is based furnishes, therefore, a simple explanation for the experimental results obtained by Morgulis. The reaction proceeds in accordance with the laws of mass action under these conditions just as under the conditions studied by Senter. In the former case, however, the reaction rate is determined almost entirely by the destruction of the enzyme, whereas in Senter's experiments it was determined by the decomposition of the peroxide. It is evident that between the two extreme conditions there will be a zone where neither formula will hold accurately but where both substrate and enzyme would have to be considered as variables. A case similar to this was considered by Tammann working with emulsin, and has been found by the writer to be true for trypsin.[10] If the catalase is just sufficient to decompose nearly all the peroxide the reaction will be monomolecular with respect to each, *i.e.* $\frac{dx}{dt} = C\,(A - x)\,(B - x)$, in which A is the concentration of catalase, and B is the concentration of peroxide. Since under these conditions $A = B$, the reaction follows the bimolecular course as Morgulis pointed out.

The experiments of Yamasaki were made with a vegetable catalase and differ somewhat from those considered here. They cannot be calculated by the present formula since the enzyme does not decompose monomolecularly, but becomes progressively more stable. Yamasaki has taken this into account by setting the rate of inactivation equal to the substrate concentration. This method leads to an equation which fits the experiments quite well, but which contains several constants. It seems possible that the difficulty is due to the fact that Yamasaki did not regulate the pH of the solution.

Michaelis and Pechstein's, and Sörensen's results are in general agreement with those discussed here except that they did not use such high concentrations of peroxide. The variation in the peroxide concentration must therefore be considered.

SUMMARY.

It has been shown that the experimental results obtained by Morgulis in a study of the decomposition of hydrogen peroxide by

[10] Northrop, J. H., *J. Gen. Physiol.*, 1923–24, vi, 439.

liver catalase at 20°C. and in the presence of an excess of a relatively high concentration of peroxide are quantitatively accounted for by the following mechanisms.

1. The rate of formation of oxygen is independent of the peroxide concentration provided this is greater than about 0.10 M.

2. The rate of decomposition of the peroxide is proportional at any time to the concentration of catalase present.

3. The catalase undergoes spontaneous monomolecular decomposition during the reaction. This inactivation is independent of the concentration of catalase and inversely proportional to the original concentration of peroxide up to 0.4 M. In very high concentrations of peroxide the inactivation rate increases.

4. The following equation can be derived from the above assumptions and has been found to fit the experiments accurately.

$$K = \frac{1}{t} \log \frac{A}{A - x}$$

in which x is the amount of oxygen liberated at the time t, A *is the total amount of oxygen liberated* (not the total amount available), and K is the inactivation constant of the enzyme.