ON THE RATE OF REACTION BETWEEN ENZYME AND SUBSTRATE.

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"The function of enzymes, as catalysts, being to change the rate of reaction, it follows that the study of their action . . . . , consists essentially in the investigation of the velocity of reaction."

W. M. Bayliss

The investigation reported in this paper is the outgrowth of some experiments which dealt with the antitryptic titre of the blood serum, in certain pathological conditions. In these researches we utilized the method developed by Northrop (1) of gauging enzyme action by viscosity changes of the substrate. We soon reached the conviction that no precise interpretation of our results, or any results derived by similar methods, was possible, without some prior knowledge of the quantitative laws that connected the viscosity changes observed with the enzyme action that brought them about. Accordingly, we inaugurated a series of experiments which looked to the ultimate possibility of stating, in mathematical language, the functional relationship obtaining among all the important variables concerned in the method.

Our first objective was a quantitative description of the rate of change of viscosity as the reaction proceeded from beginning to completion. This we believe we have satisfactorily attained, and our results constitute the content of this report.

Since the viscosity, we suppose, is governed by the size of the gelatin molecule, changes in its value correspond to the hydrolysis of the protein by the enzyme, and the question is equivalent, therefore, to one concerned with the rate of hydrolysis.

Such questions have attracted the attention of investigators for many years.[1] Moore (2) states that O'Sullivan and Tompson were the first observers who studied the velocity of the action of an enzyme, quantitatively, throughout the course of the reaction. Their work (3) was published in 1890, when, employing invertase and cane sugar, they found that the action was monomolecular, following the mass action law, and giving a logarithmic curve. Henri, however, who later worked on the same subject, obtained contradictory results, and found that the percentage rate of change, which is constant in a monomolecular reaction, increased with the progress of hydrolysis. Tammann (4), in a series of researches, investigated not only the action of invertase on cane sugar, but also of emulsin on different glucosides. He found that the velocity was retarded in increasing amount as the reaction proceeded. Duclaux (5) found that with the concentration of the enzyme and sugar fixed, the amount hydrolyzed up to the point at which 20 per cent had been inverted, was simply proportional to the time, so that the curve representing the progress of the reaction up to this point was a straight line and not a logarithmic function. At a later stage, the curve began to obey the logarithmic law. Henri showed afterward that even in this position, Duclaux's results do not give a logarithmic curve, the percentage rate of change all the time increasing with the progress of the reaction.

Henri (6), from an extensive series of experiments on the inversion of cane sugar by invertase, formulated the following equation that predicted the time t, required for a change x, from an original concentration a:

$$K(1 + \epsilon) = \frac{1}{t}\left[\log \frac{a}{a - x} + \log\left(1 + \epsilon \frac{x}{a}\right)\right] \tag{1}$$

ϵ = a factor that refers to the active fraction of the enzyme.

K = a factor that refers to the rate of reaction.

Another formula he developed, supposed to be more generally applicable, is:

$$K = \frac{a}{t}\left[(m - n)\frac{x}{a} + n \log\left(\frac{a}{a - x}\right)\right] + \frac{1}{t}\log\left(\frac{a}{a - x}\right) \tag{2}$$

These equations will be discussed, together with similar ones, in a later part of our paper.

Barendrecht (7), working with invertase and lactose, assuming a certain distri-

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[1] We are indebted to Van Slyke and Cullen (9) for an excellent review of the work done on this subject.

bution of enzyme energy between substrate and products, formulated the following equation:

$$t = \frac{an}{m} \log \left(\frac{a}{a - x} \right) + \left(\frac{1 - n}{m} \right) x \tag{3}$$

Mlle. Filoche (8) found that the action of maltase follows the equation:

$$t = \frac{1}{K} \left(2x + a \log \frac{a}{a - x} \right) \tag{4}$$

Brown and Glendinning have noted in the action of diastase on starch a time curve, which, according to Van Slyke and Cullen (9), is given by the equation:

$$t = \frac{1}{c} \log \left(\frac{a}{a - x} \right) + \frac{x}{a} \tag{5}$$

Abderhalden and Michaelis (10) have plotted the course of the cleavage of *d*-alanyl-*d*-alanine by yeast juice from the results of Abderhalden and Kolliker, and give the following equation as descriptive of the course of reaction:

$$t = \frac{1}{K} \left(\log \frac{a}{a - x} + \frac{e}{K} x \right) \tag{6}$$

Finally, Van Slyke and Cullen (9), in a carefully argued and exhaustive examination of this question, decided in favor of the equation:

$$t = \frac{1}{\epsilon} \left(\frac{1}{c} \log \frac{a}{a - x} + \frac{x}{a} \right) \tag{7}$$

We may now present our own formulation.

The method of experiment employed follows that of Northrop (1). Gelatin was used as a substrate, and a solution of commercial pancreatin added in an Ostwald viscosimeter maintained at a temperature of 34.5°C. Changes in viscosity were then followed, by noting at successive intervals the time required for the mixture to flow, under the action of gravity, between two points. We call the duration of the fall v, and the time at which the observation is made t. The goal of the inquiry is, then, to discover the mathematical relationship of v and t.

In agreement with previous observers, we found, when low concen-

trations of enzyme were used, that the changes in reaction rate, as reflected in the changes of v, could be represented adequately as an exponential function of t. With stronger solution of enzyme, this was not so; the percentage rate of change departed the more from constancy, the longer the reaction proceeded.

It was experience such as this by previous workers that was the starting point for the development of several of the formulas presented above. The interpretation they made was that the reaction contained different phases, each dominated by a different principle, and equations were constructed on the plan of representing the total action algebraically as the sum of two such elementary processes.

We adopted a different hypothesis as a guiding idea. We assumed that the process was governed throughout by one uniform principle, expressible in a single differential equation containing no summation element. The appearance at times of linearity, and, at others, of an exponential character, we took to signify that the real equation tended to degenerate to these forms under particular circumstances. If the changes described by the equation were minimal, the differences which would distinguish one form from another would become small, and might be obscured by random experimental variation. Our search, then, was for such a mathematical function as would tend to equilibrium with the progress of the reaction, and which would, further, approximate exponentiality and linearity as the process became slower. Several general forms having these characters were exhaustively investigated. We finally decided in favor of the following:

$$V_t - d = \frac{K_1}{1 - K_2 e^{-rt}} \tag{8}$$

V_t = duration of flow of the mixture at time t.

t = the time at which the observation v is made.

r = a parameter reflecting the "intrinsic" rate and depending on the concentration.

K_1 = a parameter equal to the distance between two asymptotes.

K_2 = a parameter determined by d, K_1, and the initial value of v, v_0.

d = a parameter which represents a shifting of the x axis d distance in the y direction.

Diagrammatically the equation is represented in Fig.1.

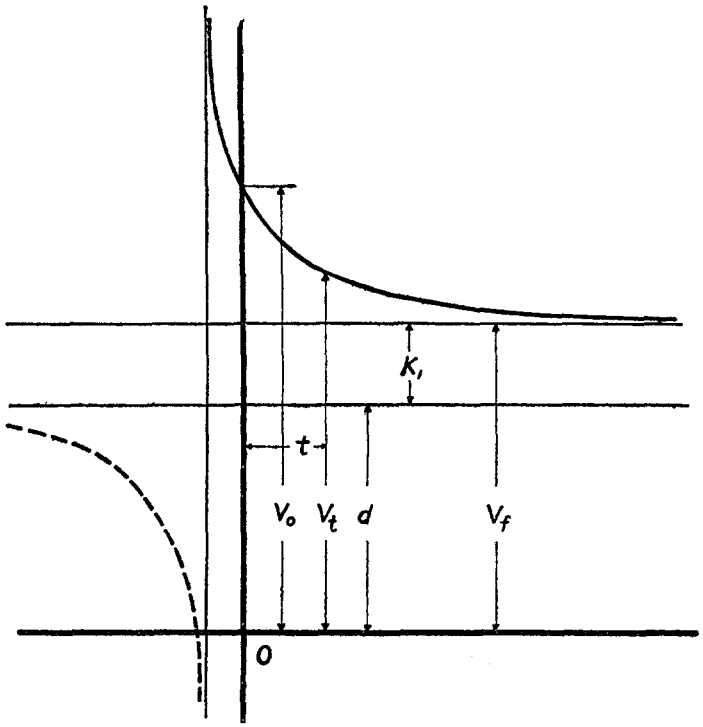

FIG. 1. $v_f = v$ after the proteolysis is complete.

If the logarithm of (8) be derived, we obtain

$$\log\left(\frac{v_t - d - K_1}{v_t - d}\right) = \log K_2 - r \log e \cdot t \tag{9}$$

From this we see that $\log\left(\frac{v_t - d - K_1}{v_t - d}\right)$ plotted against t should give a straight line, the slope of which is $r \log e$. We utilize this fact below, for the evaluation of r.

In order to test the applicability of the equation to our observations, it is first necessary to evaluate the constants, K_1, K_2, and d, in terms of our data. If d were known, this could be accomplished from the following consideration: K_1 = the upper asymptote minus d. By allowing the gelatin to digest until no further change can be noted, the asymptotic value of $v = v_f$ is observed directly, and by subtracting

d, K_1 is obtained. K_2 can then be derived from K_1, d, and the initial reading v_0. The possibility of testing the equation, therefore, turns upon the determination of the quantity d.

By reference to the place that d has in equation (8), it is seen that it is a constant, which, when it is subtracted from each of the observed v's, leaves a residuum, that changes with t. It suggests itself at once that d is the time required for the flow of some constituent of the gelatin mixture inert to the enzyme, and therefore remaining constant through the course of the reaction. We concluded, on the basis of certain of our experimental results, that this was none other than the water itself. Further investigation corroborated this. With d thus established the equation becomes:

$$v_t - v_w = \frac{v_f - v_w}{1 - \frac{v_0 - v_f}{v_0 - v_w} e^{-rt}} \tag{10}$$

in which:

t = time at which the observation is made.
v_t = the duration of flow at any time t.
v_w = v for water.
v_f = the final v after proteolysis is complete.
v_0 = the initial observation of v at $t = 0$.
r = the "intrinsic" exponential rate of increase, obtained from the slope of the line $\log \frac{v - v_f}{v - v_w}$ vs. t
e = the constant 2.71 . . . .

We now apply the formula to two series of our observations, comparing values of v observed, with those calculated from the equation. (Experiments I and II.)

Experiment I.

0.5 cc., 0.15% commercial pancreatin sol. + 10 cc. 3% gelatin—by viscosity method.
Temperature 34.5°C.
v_0 = 59.5 sec. observed.
v_w = 29.5 " "
v_f = 35.5 " "
r = 0.006518 per min., calculated from slope of

$$\log \frac{v - v_f}{v - v_w} \text{ vs. } t$$

From (10) the equation becomes for this case:

$$v_t = \frac{6}{1 - 0.800\, e^{-0.006518\, t}} + 29.5 \qquad (11)$$

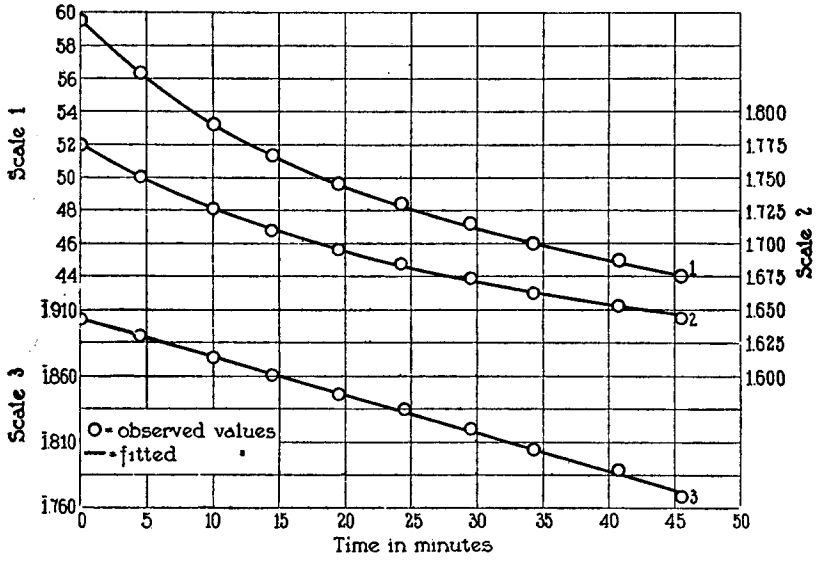

FIG. 2. Experiment I. 0.5 cc. 0.15% pancreatin + 10 cc. 3% gelatin.

1. Arithmetic graph, v vs. t
2. Exponential " $\log v$ vs. t
3. Autocatalytic " $\log \frac{v - K_1}{v}$ vs. t

v_f = 35.5 sec., v_w = 29.5 " } observed.
$K_1 = v_f - v_w = 6.0$
r = 0.006518 from slope.

t	v_t	v_t
	Observed	Calculated from (11)
min.	*sec.*	*sec.*
0	59.5	59.5
4½	56.3	56.4
10°	53.2	53.4
14½	51.3	51.5
19½	49.6	49.8
24½	48.4	48.4
29½	47.2	47.1
34½	46.0	46.1
40¾	45.0	45.0
46½	44.4	44.1

Standard deviation calculated from observed = ±0.15 sec.
Coefficient of variation = ±0.3%

Experiment II.

0.5 cc., 0.25% commercial pancreatin sol. + 10 cc. 3% gelatin — by viscosity method.

Temperature 34.5°C.

v_0 = 51.6 sec. observed.

v_w = 30.5 " "

v_f = 36.2 " "

r = 0.0092876 per min., calculated from slope of

$$\frac{v_t - v_f}{v_f - v_w} \text{ vs. } t$$

The equation becomes for this case:

$$v_t = \frac{5.7}{1 - 0.72986\, e^{-0.0092876\, t}} + 30.5 \qquad (12)$$

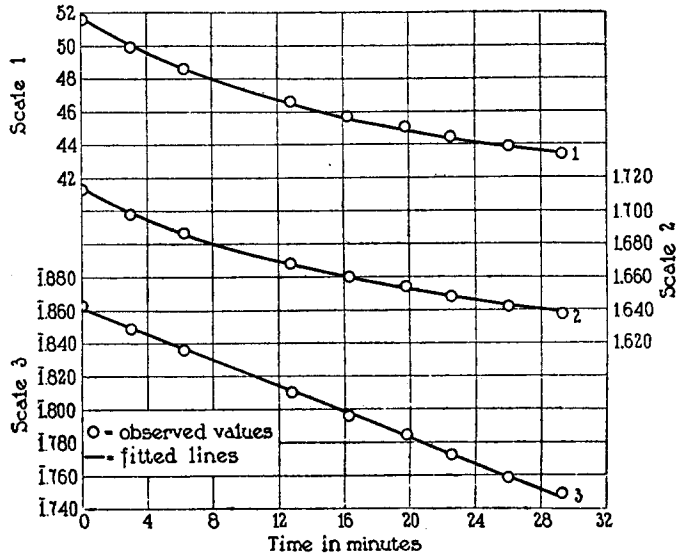

FIG. 3. Experiment II. 0.5 cc. 0.25% pancreatin + 10 cc. 3% gelatin.

1. Arithmetic graph, v vs. t — v_f = 36.2, v_w = 30.5 } observed.
2. Exponential " log v vs. t
3. Autocatalytic " $\log \frac{v - K_1}{\quad}$ vs. t — $K_1 = v_f - v_w = 5.7$, r = 0.0092876 from slope.

	v_t	v_t
	Observed	Calculated from (12)
min.	*sec.*	*sec.*
0	51.6	51.6
3°	49.9	50.1
6¼	48.6	48.8
12¾	46.6	46.8
16¼	45.7	45.6
19¾	45.1	45.0
22½	44.5	44.5
26°	43.9	43.9
29¼	43.5	43.4

Standard deviation calculated from observed = ± 0.13 sec.
Coefficient of variation = ± 0.3%

Such tests of the equation have now been made by us upon many series of observations and the precision of prediction shown in the above experiments is typical.

The examples here referred to are ones in which the rate of hydrolysis is rapid. When the concentration of the enzyme used is low, and the velocity of reaction correspondingly slow, the equation describing the process approaches the exponential and it becomes impossible to differentiate it experimentally from one which is a simple monomolecular. This may be shown theoretically as follows:

Using differential expressions, the value of v determined from the exponential would be:

$$v_t = r \int_0^t v \, dt \tag{13}$$

where $v_t = v$ at the time t, r = rate constant. Determined from (8) it would be:

$$v_t = \frac{r}{K_1} \int_0^t v(v - K_1) \, dt \tag{14}$$

The difference between the values thus determined would, therefore, be:

$$\text{Diff.} = r \left[\int v dt + \frac{1}{K_1} \int v(v - K_1) dt \right]_0^t \tag{15}$$

It is seen that the difference diminishes in proportion as r decreases, *i.e.* when the reaction is a slow one. We may expect, therefore, that

as we use an enzyme solution of greater dilution the reaction will appear logarithmic. This proves to be the case experimentally.

In illustration, if we examine a series of observations made with a mixture of gelatin and dilute pancreatin it will be found that they can be well represented by a logarithmic curve. But it is equally possible to fit a curve of form (8) to them and we present such a fit for the same experiment. The explanation is simply that in this instance r is so small, that the difference between the two functions is of the order of the experimental error, and so is inappreciable.

Experiment III.

0.5 cc., 0.025% pancreatin plus 10 cc. 3% gelatin — by viscosity method.
Temperature 34.5°C.
r = 0.0032 per min., from exponential graph, log v vs. t.
v_0 = 66.4 sec. observed.
Exponential equation is, therefore:

$$v_t = v_0^{-rt} = 66.4\, e^{-0.0032t} \tag{16}$$

v_w = 27.1 sec. observed.
K_1 = 12.8, K_2 = 0.674 } calculated.[2]
r = 0.0032 per min. from exponential function.
Autocatalytic equation is therefore from (8):

$$v_t = \frac{12.8}{1 - 0.674\, e^{-0.0032\, t}} + 27.1 \tag{17}$$

t	v_t From exponential function (16)	v_t From autocatalytic function (17)	v_t Observed
min.	*sec.*	*sec.*	*sec.*
0	66.4	66.4	66.4
4½	65.5	65.3	65.4
8½	64.6	64.3	64.4
13½	63.6	63.3	63.3
18¼	62.6	62.3	62.6
22¾	61.7	61.4	61.7
27⁰	60.9	60.6	60.7
31½	60.1	59.9	60.0
36⁰	59.1	59.2	59.6
40¼	58.3	58.5	58.6

[2] V_f could not in this case be directly observed because of the deterioration of the pancreatin itself during the long period required for complete hydrolysis.

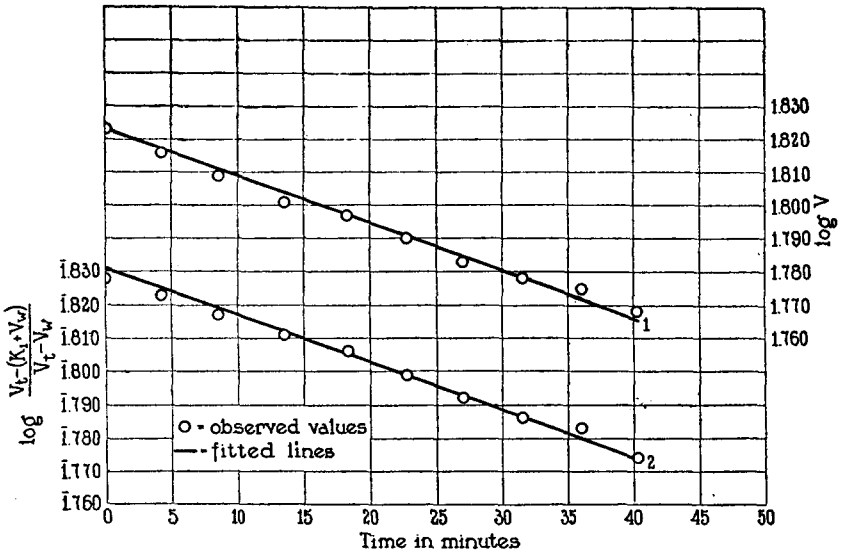

FIG. 4. Experiment III. 5 cc. 0.025% pancreatin + 10 cc. gelatin. Viscosity method.

1. Exponential function, log v vs. t. $r = 0.0032$ per min. from slope.
2. Autocatalytic function, $\log \frac{v_t - (K_1 + v_w)}{v_t - v_w}$ vs. t

$v_w = 27.1$ sec., observed.
$K_1 = 12.8$, $K_2 = 0.674$ } calculated.
$r = 0.0032$ per min. from slope.

On the basis of the results reviewed and many others of similar character, we believe that an equation of form (10) is a true quantitative description of the viscosity changes in a mixture of gelatin and pancreatin. Further, we are convinced by these results, and by evidence about to be considered, that it refers to the proteolytic process and, indeed, is necessarily consequent to the laws that govern the velocity of enzyme reactions in general. The grounds for this conviction will now be considered.

If equation (8) be differentiated, the following is obtained:

$$\frac{dv}{dt} = \frac{r}{K_1}(v)(v - K_1) \tag{18}$$

This states that the rate of change of viscosity (as measured by change in duration of flow) is proportional at any instant, to the value at that instant of v, and v minus a constant K_1. Now it can easily be shown that this is identical in general form with the differential equation obtained by Ostwald (11) to describe a process which he called "autocatalytic." Characteristic of such a reaction, is the fact that a change in the amount of substrate is accompanied by a change (in his case an increase) in the amount of enzymatic activity.

We are able to prove that an equation of form (18) results from assuming a reaction between gelatin and pancreatin of such nature, that the change of given amount of gelatin is accompanied by the inactivation of a fixed quantity of enzyme.

$$n_1 \text{ gelatin}_1 + n_2 \underset{\text{active}}{\text{enzyme}} \underset{\longleftarrow}{\longrightarrow} n_3 \text{ gelatin}_2 \cdot \underset{\text{inactive}}{\text{enzyme}} \tag{19}$$

Let E_0 = initial concentration of the enzyme.
e_a = the concentration of active enzyme at any moment.
e_i = " " " inactive " " " "
G_0 = the initial concentration of gelatin.
g_1 = the concentration of unchanged gelatin.
g_2 = " " " proteolyzed "
n_1, n_2, n_3 = stoichiometric factors.

We make the following assumptions:

(1) That the change in the direction $g_1 \leftarrow g_2$ is negligible so that the reaction $g_1 \rightarrow g_2$ is practically irreversible.

(2) That the velocity is governed by the law of mass action.

(3) That v is proportional at any moment to the concentration of unchanged gelatin.

We have, then:

$$\frac{dg}{dt} = -k_1 (g_1)(e_a) = -k_1 (g_1)(E_0 - e_i) = -k_1 (g_1)\left[E_0 - \frac{n_2}{n_3}(G_0 - g_1)\right]$$

$$= -\frac{n_2}{n_3} k_1 (G_1)\left(G_1 - \frac{n_2 G_0 - n_3 E_0}{n_2}\right) \tag{20}$$

$\frac{n_2}{n_3} k_1$ and $\frac{n_2 G_0 - n_3 E_0}{n_2}$ are constants and if we suppose v proportional to the unchanged substrate we have:

$$\frac{dv}{dt} = -C_1 (v)(v - C_2) \tag{21}$$

Equation (21) is identical with (18) and is the differential equation of (8) and (10). We have been able to show, therefore, that equation (8), which is found to agree precisely with our observations, follows from the assumption of a simple bimolecular reaction between pancreatin and substrate. How widely applicable are these conclusions?

Although we have experimented with gelatin and pancreatin alone, and utilized only the viscosity method of gauging the progress of the reaction, we are of the opinion that enzyme changes in general follow fundamentally the same process, and that this would be supported by other methods of estimating their action, *e.g.* surface tension, electric conductivity, polarimetric method,[3] etc. The results of many experimenters employing a wide variety of substrates and methods have been examined. We have not, in general, been able to make a direct test of the applicability to them of equation (8) for want of certain measurements necessary to the determination of the required constants. But no instance has come to our notice in which the form of changes observed could not be explained on the assumption of an autocatalytic reaction defined by (8).[4] Happily, in a few cases, the circumstances of the experiment, and the manner of presentation of the data, permit a direct test, and for these the results follow.

The data tested comprise:

(1) Three series of observations by Van Slyke and Cullen (9) in which urea and urease were used and the amount of NH_3 produced determined by titration.

(2) One series of observations by Brown and Glendinning (12) in which starch and diastase were employed, and the changes followed by determining the cupric reducing power of the products.

(3) One series of Abderhalden and Michaelis (10) in which the splitting effect of yeast juice on polypeptid was measured by the polarimetric method.

In each of these, we found, the results did not agree with an autocatalytic equation such as fitted our own observations. This, however, was a limited example, and applied to the case when the enzyme

[3] Experiments to test this are planned.

[4] We mean, in this statement, that the constants be permitted plus or minus signs.

was decreasing. If we assume instead, that the enzymatic activity is increasing the corresponding equation becomes:

$$x' = \frac{K_1}{1 + K_2 e^{rt}} \tag{22}$$

x' = amount of substrate remaining at time t.
t = time at which the observation is made.

If we begin the experiment at the moment of greatest possible velocity it may be shown that K_1 equals twice the original value of x' and K_2 equals 1, and we have

$$x' = \frac{2 \cdot x_0}{1 + e^{rt}} \tag{23}$$

Experiment IV.

Van Slyke and Cullen (9).
0.02 mol. sol. urea plus 0.03% enzyme—by titration of NH_3 produced.
$K_1 = 2 \times 40 = 80$
$r = 0.0033492$ from slope of $\log\left(\frac{K_1 - x'}{x'}\right)$ vs. t
From (23) the equation for this case becomes:

$$x' = \frac{80}{1 + e^{0.0033492\, t}} \tag{24}$$

x' = units of NH_3 combined as urea $\left(1 \text{ cc.} \frac{N}{10} NH_3 \text{ per } 10 \text{ cc. sol.} = 1 \text{ unit}\right)$
t = time of observation, min.

Observed by Van Slyke and Cullen		Calculated from equation (24)
t	x'	x'
min.	*units* NH_3	*units* NH_3
60	35.80	36.01
120	31.60	32.08
186	27.90	27.93
240	25.16	24.74
300	21.92	21.44
360	18.90	18.44
420	16.30	15.74

Standard deviation observed from calculated = ±0.43 min.
Coefficient of variation = ±1.7%

Experiment V.

Van Slyke and Cullen (9).
0.02 mol. sol. urea plus 0.1% enzyme—by titration of NH_3 produced.
K_1 = 80 as in Experiment IV.
r = 0.011585 from slope of $\log\left(\frac{K_1 - x'}{x'}\right)$ vs. t

Equation (23) becomes:

$$x' = \frac{80}{1 + e^{0.011585\,t}} \tag{25}$$

Observed by Van Slyke and Cullen		Calculated from equation (25)
t	x'	x'
min.	*units* NH_3	*units* NH_3
15	36.20	36.52
30	32.76	33.12
45	29.48	29.99
60	26.72	26.63
75	23.70	23.63
90	21.10	20.86
105	18.50	18.29
120	16.60	15.95
135	14.64	13.85
150	12.46	11.97
165	10.42	10.30
180	8.70	8.84
195	7.20	7.57
225	5.46	5.72

Standard deviation observed from calculated = ±0.38 units.
Coefficient of variation = ±2.0%

Experiment VI.

Van Slyke and Cullen (9).
0.02 mol. sol. urea plus 0.3% enzyme—by titration of NH_3 produced.
K_1 = 80 as in Experiment IV.
r = 0.036406 from slope of $\log\left(\frac{K_1 - x'}{x'}\right)$ vs. t

From (23) the equation for this case becomes:

$$x' = \frac{80}{1 + e^{0.036406\,t}} \tag{26}$$

Observed by Van Slyke and Cullen		Calculated from equation (26)
t	x'	x'
min.	*units* NH_3	*units* NH_3
20	25.92	26.04
30	19.78	20.09
40	14.94	15.12
51	10.80	10.81
60	8.02	8.09
75	4.88	4.90
90	3.10	2.91
105	2.00	1.71
120	1.00	1.00
135	0.46	0.58

Standard deviation observed from calculated = ± 0.168 units.
Coefficient of variation = ± 1.9%

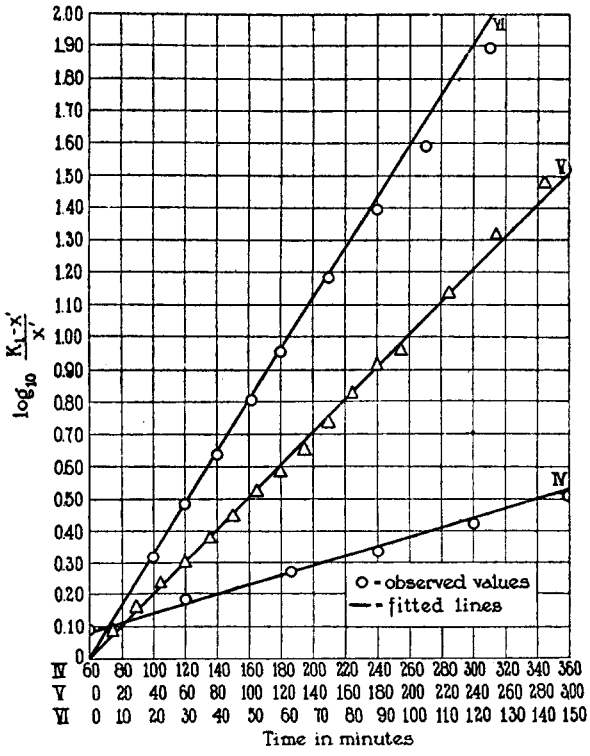

FIG. 5. Autocatalytic graphs. $\log_{10} \frac{K_1 - x'}{x'}$ vs. t.

Experiment IV. 0.03% enzyme. r, from slope = 0.0033492.
" V. 0.1% " r, " " = 0.011585.
" VI. 0.3% " r, " " = 0.036406.
From data of Van Slyke and Cullen. Urea and urease, by titration.

Experiment VII.

Brown and Glendinning (12).
3% starch sol. plus 0.25% malt extract—by cupric reducing power of products.
$K_1 = 2 \times 1 = 2$
$r = 0.023337$ from $\log\left(\frac{K_1 - x'}{x'}\right)$ vs. t
Equation (23) for this case becomes:

$$x' = \frac{2}{1 + e^{0.023337\,t}} \tag{27}$$

x' = units of unchanged starch.
t = time at which observation was made, min.

Observed by Brown and Glendinning		Calculated from equation (27)
t	x'	x'
min.	*units*	*units*
10	0.892	0.884
20	0.775	0.771
30	0.665	0.664
40	0.565	0.565
50	0.465	0.475
60	0.385	0.396
70	0.320	0.327
80	0.262	0.268
90	0.220	0.218
100	0.185	0.177
110	0.150	0.142
120	0.120	0.115
130	0.097	0.092
140	0.078	0.073
150	0.060	0.053
160	0.050	0.046

Standard deviation calculated from observed = ±0.0064 units.
Coefficient of variation = ±2.0%

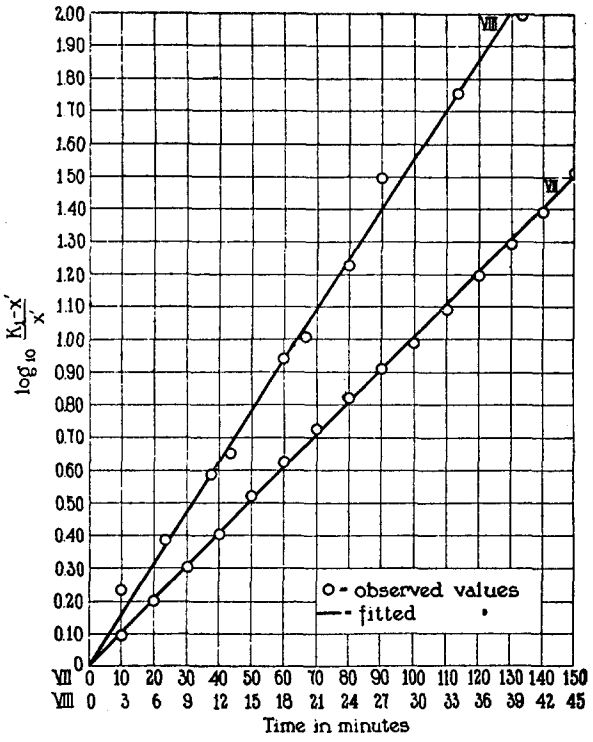

FIG. 6. Autocatalytic graphs. $\log_{10} \frac{K_1 - x'}{x'}$ vs. t.

Experiment VII. From data of Brown and Glendinning. Starch and diastase. r, from slope = 0.023337.

Experiment VIII. From data of Abderhalden and Michaelis. Dipetid and enzyme. r, from slope = 0.11827.

Experiment VIII.

Abderhalden and Michaelis (10).

1.45 units of dipeptid plus 6 cc. enzyme—by polarimetric method.

$K_1 - 2 \times 1.45 = 2.90$

$r = 0.11827$ from slope of $\log \left(\frac{K_1 - x'}{x'} \right)$ vs. t

Equation (23) for this case becomes:

$$x' = \frac{2.9}{1 + e^{0.11827\, t}} \tag{28}$$

x' = the amount of unchanged dipeptid in units of optical rotation.
t = time at which observation was made, min.

Observed by Abderhalden and Michaelis		Calculated from equation (28)
t	x'	x'
min.	*units*	*units*
0	1.45	1.45
3	1.20	1.06
7	0.88	0.84
11	0.62	0.61
13	0.51	0.53
18	0.31	0.30
20	0.25	0.26
24	0.16	0.16
27	0.11	0.09
34	0.05	0.05
40	0.03	0.03

Standard deviation observed from calculated = ±0.017
Coefficient of variation = ±3.7%

In all these experiments, as was the case with our own, the deviations of observed from calculated values from the autocatalytic equation is of the order of the experimental error involved in the method used.

Comparison with Other Formulas and Discussion of the Autocatalytic Equation.

A review of the more important equations that have been formulated to represent the reaction between enzyme and substrate has been made above. They may be analyzed to be of three kinds. First, those equating the time for a change x to a logarithmic function of x. Second, those equating the time to the sum of a linear and logarithmic function of x. Third, the first formula of Henri, which stands by itself and equates the time to the sum of two logarithmic functions of x.

The first is the ordinary exponential form, and is the one corresponding to a monomolecular reaction. It was abandoned by all experimenters who worked over considerable ranges of concentration of enzyme, on the grounds that it did not accord with observed results. We may discard it, as an unsatisfactory form for general application, with the note that in our experience with low concentrations of enzyme, as with other experimenters, working in the realm of slowly proceeding changes, this form was found to apply satisfactorily. An illustration of this was given in our Experiment III.

The second group, that giving the time as the sum of a logarithmic and an arithmetic function of x, is the largest and warrants more careful analysis. We may generalize the experience of those workers who were persuaded to formulate this kind of equation, somewhat as follows:

They found that in certain reactions in their entirety, and in portions of others, the changes proceeded at a sensibly constant rate, giving a linear relationship between x and t. In other situations the relationship was curvilinear, the rate of changes varying rapidly. In the case where a reaction showed both features, neither linear nor exponential was applicable throughout. An exponential equation fitted to the rapidly changing portion did not suit the linear part and any rule that described the straighter region, broke down when applied to that with greater curvature. Then they noted that a function consisting of the sum of two parts, a linear and logarithmic, would suit all the observations more or less, the constants being so chosen that the linear part of the function applied to the constant part of the reaction and the logarithmic became important when the reaction rate varied.

We think this gives a fair picture of the methodology involved in all the formulas under discussion. They differ among themselves according to the mode of choice and interpretation of the constants.

Now, while these formulas will, if the proper constants are inserted, give good approximate fits, this of itself is no vindication of their form. We have compared, where feasible, predictions made by means of them, and by our own, and in each case tested ours gives significantly better values. But the important distinction rests not in the greater precision of prediction so much as in the simplicity of form of the autocatalytic and in the economy and character of the constants used.

In our equation (23) which is comparable with (7) of Van Slyke and Cullen, there are two as against four constants. Further, if we consider the more general form (10) used in our own experiments, each of the constants involved has definite physical meaning and with the exception of r^5 is determined by an experiment quite independent of the series to which it is later applied. These considerations lead to the further fact that the differential equation is of a simple form, and requires a minimum of assumptions for its derivation. In contradistinction, the differential equation derived from the sum of an arithmetic and logarithmic function, requires a complexity of assumptions for its establishment and has resulted in the construction of involved theories respecting the nature of enzyme action that have no independent corroboration.

The remaining equation to be considered, (1) that of Henri, may be shown to reduce to an autocatalytic form when certain of the constants are combined. It was, in fact, derived from Ostwald's differential equation for just this reaction. The form elaborated by Henri is to be criticized for the introduction of constants which are not necessary and which destroy the virtue of simplicity of the original.

The autocatalytic form utilized by us, we believe, gives an adequate quantitative description of the velocity changes in the reaction between enzyme and substrate. The generality of its application is reconciled with the exponential and linear changes that other experimenters and we have noted in some instances, by the fact that it tends to approximate these forms when r assumes small values. It can be deduced from the simple assumption of a bimolecular reaction, obeying the mass law. In every case tried the prediction made on the basis of it, deviates from observation by no greater amounts than involved in the experimental error. This expresses the fact that it is as close an approximation to the truth as is permitted to us by the available methods of measurement. It is what characterizes a law; and if the experimental results presented above should be verified for enzymes in general, it would establish the equation as the law governing such reactions.

[5] Correlated with concentration of enzyme—see note following this paper.

CONCLUSIONS.

1. An equation of the form:

$$v_t - v_w = \frac{v_f - v_w}{1 - \frac{v_0 - v_f}{v_0 - v_w} e^{-rt}} \tag{10}$$

in which v_t is the time of flow of the mixture, v_w the time of flow for water, v_f the time of flow of the mixture when proteolysis is complete, v_0 the time of flow at the beginning of the experiment, t the time of observation, and r a constant, has been found to describe accurately the course of change of viscosity in a mixture of gelatin and pancreatin.

2. An equation of the same general form has been found to apply similarly to the reaction between other enzymes and other substrates.

3. The equation may be derived theoretically from assuming a bimolecular reaction between enzyme and substrate obeying the mass action law.

We wish to thank Dr. Wilburt C. Davison, of the Department of Pediatrics, under whom this investigation was conducted, for the interest and encouragement he has given our work, and for the generous permission granted at the cost of much personal inconvenience, to use his laboratory and instruments.

To Dr. Lowell J. Reed, of the Department of Biometry and Vital Statistics, is acknowledged great indebtedness for advice sought by us throughout the investigation, respecting the mathematical problems that arose. His suggestions have been incorporated in the paper at many points, though we alone remain responsible for any errors in the final statement.

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APPENDIX.

A NOTE ON THE RELATIONSHIP OF r TO THE ENZYME CONCENTRATION.

The authors are pursuing researches to establish the functional relationships of the rate parameter r of the autocatalytic equation to other variables. They have not yet completed either the analysis of the questions involved or the experimental work necessary. But they believe they have sufficient evidence to warrant the tentative conclusion that as respects the relationship of r to the concentration of the enzyme used, the correlation is linear.

In substantiation, we present, in graphic form, the results of four series of experiments, as follows:

Series A.—Concentration against r; five of our experiments with gelatin and trypsin, using viscosity, in which the reaction was slow, and the r calculated from the exponential function.

Series B.—Ditto A, the reaction rapid and r calculated from the autocatalytic function.

Series C.—Three experiments of Van Slyke and Cullen, using urea and urease, in which the data were taken from their article[6] and r calculated in each case from the autocatalytic function.

Series D.—Four experiments of Abderhalden and Michaelis,[7] using dipeptid and enzyme in which the data were taken from their article, and r calculated from the autocatalytic function.

[6] Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1914, xix, 141.
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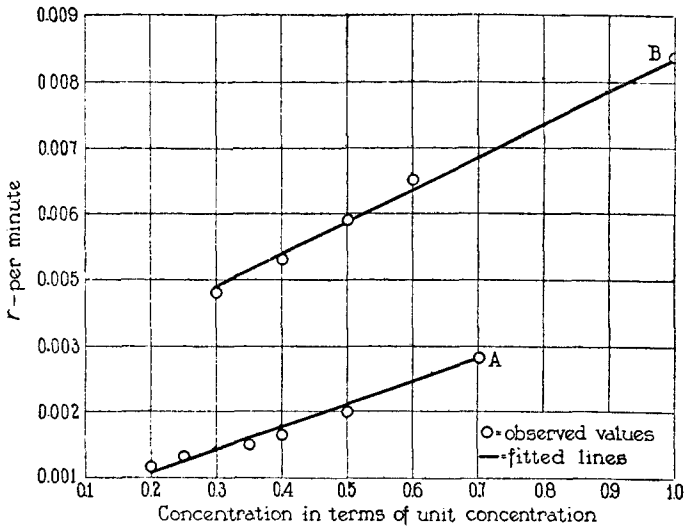

FIG. 7. Concentration of pancreatin vs. r. Gelatin and pancreatin. Viscosity method.

Series A. r from exponential function about 0.4% pancreatin = unity.
Series B. r " autocatalytic " " 0.5% " = "

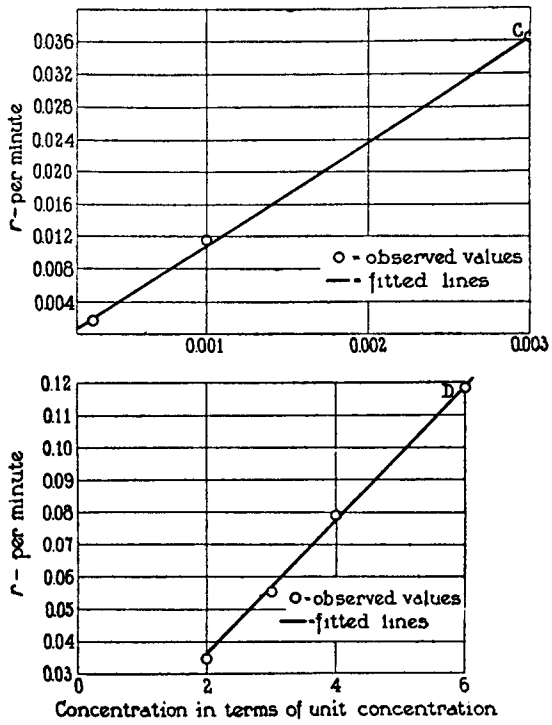

FIG. 8. Concentration vs. r.

Series C. Data of Van Slyke and Cullen. Urea plus urease. 20% extract soy bean = unit concentration urease. r = from autocatalytic function.

Series D. Data of Abderhalden and Michaelis. Dipetid plus enzyme. r = from autocatalytic function.