CHAPTER V.

THE COURSE OF ENZYMATIC REACTIONS, AND ITS MATHEMATICAL THEORY.

A VAST amount of work has been done on the kinetics of reactions catalysed by enzymes. Much of it was done under conditions so complicated as to throw little light on the mechanisms involved. Thus whereas numerous authors had found that lipase action falls off with time, Rona and Lasnitzki [1924] and Rona and Ammon [1927], using respectively a manometric method measuring infinitesimal changes, and a titration method in which pH was kept constant, found the velocity to remain constant for long periods.

If an enzyme obeys the Michaelis equation, then at constant pH three cases may arise :—

(*a*) The substrate concentrations remain so high until very near the end of the experiment that the enzyme is fully saturated. This is the case with many enzymes of high affinity, including some lipases and oxidases.

(*b*) The substrate concentrations are so low that the amount of enzyme combined is proportional to the substrate concentration. This is true in many experiments with enzymes of low affinity.

(*c*) The substrate concentration may vary so that neither of these conditions is fulfilled.

If a be the initial substrate concentration, y the amount transformed in time t, and other symbols as in Chapter III., we have :—

(*a*) If the products of enzymatic action do not inhibit, $y = Vt$; if they inhibit, then the velocity falls off with time. Various equations have been constructed to meet such cases. If the affinities are low in comparison with that of the substrate, as is usually the case, the falling off is less than that characteristic of a unimolecular reaction.

(*b*) In this case, if the products do not inhibit $\frac{dy}{dt} = \frac{(a-y)V}{K_m}$,

$$\therefore t = \frac{K_m}{V}\log_e\left(\frac{a}{a-y}\right),$$

i.e. the reaction is unimolecular. If the products inhibit, the unimolecular constant falls off.

(*c*) In the absence of inhibition $\frac{dy}{dt} = \frac{V(a-y)}{a-y+K_m}$,

$$\therefore Vt = \int_a^y \frac{(a-y+K_m)dy}{a-y}$$

$$= y + K_m \log_e\left(\frac{a}{a-y}\right)$$

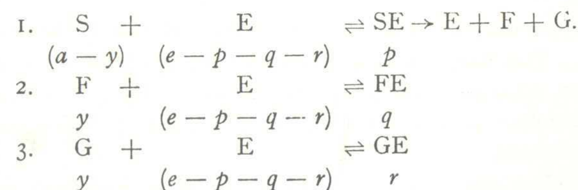
$$= y\left(1 + \frac{K_m}{a}\right) + y^2\frac{K_m}{2a^2} + \frac{y^3K_m}{3a^3} + \ . \ . \ .$$

This equation, first formulated by Henri [1903] gives a good approximation to the course of many hydrolyses. If a is large compared with K_m the logarithmic term is at first negligible, and the reaction proceeds uniformly, slowing down when y becomes an appreciable fraction of $\frac{a^2}{K_m}$.

The Course of Saccharose Hydrolysis.

A variety of equations have been produced to fit the course of such reactions as saccharose hydrolysis. All of them neglect the mutarotation which the products undergo, and the fact that in many cases there is non-competitive as well as competitive inhibition. When mutarotation is taken into account, a differential equation is reached which cannot be integrated in finite terms. If we neglect on the one hand mutarotation, and on the other, the non-competitive inhibition by α-glucose, we may make the following calculation (Michaelis and Menten).

The following reactions are considered, E representing enzyme, S saccharose, F fructose, G glucose, the molecular concentrations of each being written below. a is the initial saccharose concentration.



1.	S +	E	$\rightleftharpoons$ SE $\rightarrow$ E + F + G.	
	$(a-y)$	$(e-p-q-r)$	p	
2.	F +	E	$\rightleftharpoons$ FE	
	y	$(e-p-q-r)$	q	
3.	G +	E	$\rightleftharpoons$ GE	
	y	$(e-p-q-r)$	r	

It is supposed that in the first reaction the reactions $S + E \rightleftharpoons SE$ and $SE \rightleftharpoons S + E$ are very rapid compared with $SE \rightleftharpoons E + F + G$.

Then if K_m, K_f, K_g are the dissociation constants of SE, FE, and GE,

$$(a - y)(e - p - q - r) = K_m p$$
$$y(e - p - q - r) = K_f q$$
$$y(e - p - q - r) = K_g r.$$

Hence, eliminating q and r

$$p = \frac{e(a - y)}{a - y + K_m\left(1 + \frac{y}{K_f} + \frac{y}{K_g}\right)},$$

$$\therefore \frac{dy}{dt} = \frac{V(a - y)}{a - y + K_m\left(1 + \frac{y}{K_f} + \frac{y}{K_g}\right)},$$

where V is the initial velocity in a strong solution,

$$\therefore Vt = \left(1 - \frac{K_m}{K_f} - \frac{K_m}{K_g}\right) y + K_m\left(1 + \frac{a}{K_f} + \frac{a}{K_g}\right) \log_e \frac{a}{a - y}.$$

Clearly the same equation may be extended to a number of inhibitory products. It leads to a curve of the same nature as does Henri's equation, and gives a very fair fit to such curves as those of Fig. 13. Actually Nelson and Hitchcock [1921] found that the course of hydrolysis was best represented by the equation

$$kt = \log \frac{1}{1 - y} + \cdot 2642y - \cdot 0886y^2 - \cdot 1034y^3,$$

where y is the proportion of sucrose hydrolysed in a 10 per cent. solution. The last two terms represent deviations from the equations of Henri or Michaelis. They are probably, as Nelson and Bodansky [1925] point out, mainly due to mutarotation. However, non-competitive inhibition by α-glucose will account for the third term.

Enzyme Destruction.

An enzyme may be destroyed or inactivated during the course of a reaction. This happens in all cases near the optimum temperature, and also at relatively low temperatures where H_2O_2 is a substrate (as with catalase and peroxidase) or a reaction product (as with xanthine oxidase).

The simplest case is when the enzyme is being destroyed by heat, the proportion destroyed per minute being constant, and the rate of catalysis by the remainder being independent of the falling substrate concentration. This is often nearly true with enzymes of

high affinity. If x be the amount of substrate at time t, V the original velocity,

$$\therefore -\frac{dx}{dt} = Ve^{-kt},$$

where k is a positive constant giving the rate of destruction of the enzyme. Then if a be the original substrate concentration,

$$x = a + \frac{V}{k}(e^{-kt} - 1).$$

Putting t infinite, $x = a - \frac{V}{k}$. Hence if $V \geq ak$ the reaction is completed, if $V < ak$ the reaction stops after a quantity $\frac{V}{k}$ of substrate has been converted. Hence the total amount of substrate changed varies as the amount of enzyme added, although the latter is a catalyst. Such cases have occurred, and led to considerable confusion. Thus Bach and Chodat [1904] found that the amount of pyrogallol oxidized by H_2O_2 under certain circumstances was proportional to the amount of peroxidase (an enzyme of very high affinity). Oppenheimer [1910] regarded this as contradicting the view that peroxidase is an enzyme. Similarly in the case of the Michaelis equation we have

$$a - x + K_m \log_e \frac{a}{x} = \frac{V}{k}(1 - e^{-kt}).$$

Hence the reaction always stops before it is complete, but if k is not too large it may be nearly completed.

For small values of k, $x_\infty = ae^{\frac{-V}{kK_m}}$ approximately.

Generally, however, either the substrate protects the enzyme, or destroys it. In the former case let E and a be the initial concentrations of enzyme and substrate, y and x their concentrations at time t, and p that of the enzyme-substrate compound. Let K_m be the Michaelis constant, k the velocity constant of the inactivation of the free enzyme, l that of the breakdown of enzyme-substrate compound which is measured, so that the initial velocity V at high substrate concentrations $= El$.

$$\therefore K_m p = x(y - p), \quad \frac{dx}{dt} = -lp, \quad \frac{dy}{dt} = -k(y - p),$$

$$\therefore \frac{dy}{dt} = -\frac{kK_m y}{K_m + x}$$

$$\frac{dx}{dt} = -\frac{lxy}{K_m + x} = \frac{lx}{kK_m} \cdot \frac{dy}{dt},$$

$$\therefore \log_e x = \frac{l}{kK_m} y + \text{constant}.$$

But when $$x = a, \quad y = \mathrm{E},$$

$$\therefore x = ae^{\frac{l(y-\mathrm{E})}{k\mathrm{K}_m}}$$

$$\therefore y = \mathrm{E} - \frac{k\mathrm{K}_m}{l}\log_e\frac{a}{x}.$$

Hence y vanishes and the reaction stops when

$$\log_e\frac{a}{x_\infty} = \frac{l\mathrm{E}}{k\mathrm{K}_m} = \frac{\mathrm{V}}{k\mathrm{K}_m}.$$

Putting

$$\frac{\mathrm{V}}{k\mathrm{K}_m} = q, \quad x_\infty = ae^{-q}.$$

Hence the reaction is never completed, and the proportion transformed when equilibrium is practically reached is $1 - e^{-q}$ or $1 - e^{\frac{l\mathrm{E}}{k\mathrm{K}_m}}$. It therefore increases with the amount of enzyme, being proportional to it when it is small, but is independent of the substrate concentration, a conclusion which has not, I believe, been verified experimentally. During the reaction

$$\frac{dx}{dt} = \frac{-lx}{x+\mathrm{K}_m}\left(\mathrm{E} + \frac{k\mathrm{K}_m}{l}\log_e\frac{x}{a}\right),$$

$$\therefore lt = \int_x^a \frac{(x+\mathrm{K}_m)dx}{x\left(\mathrm{E} + \frac{k\mathrm{K}_m}{l}\log_e\frac{x}{a}\right)}, \text{ or if } z = \frac{xe^q}{a},$$

$$k\mathrm{K}_m t = \int_{\frac{xe^q}{a}}^{e^q} \frac{(\mathrm{K}_m + aze^{-q})dz}{z\log_e z},$$

$$\therefore kt = \frac{a}{\mathrm{K}_m e^q}\left[\mathrm{E}i(q) - \mathrm{E}i\left(q\log_e\frac{x}{a}\right)\right] - \log_e\left[1 - \frac{\log_e\frac{a}{x}}{q}\right]$$

where

$$\mathrm{E}i(z) \equiv \int_{-\infty}^{z}\frac{e^u du}{u},$$

a function which is tabulated, and

$$q = \frac{\mathrm{V}}{k\mathrm{K}_m}.$$

In order to obtain a theoretical value for the optimum temperature for a given amount x of catalysis it would be necessary to differentiate this expression with regard to the temperature T, assuming V and k to be exponential functions of the temperature. This leads to a highly complicated formula from which, if the numerical data were known, the optimum temperature could be calculated in any given case.

Examples of reactions during which the enzyme is being destroyed are frequent in the literature, e.g. Ter Meulen [1905]. The velocity falls off more rapidly than in the case of a unimolecular reaction.

The Case of Catalase.

Here the enzyme is stabler in the absence than in the presence of substrate. Without H_2O_2 inactivation is inappreciable below 40° C. [Morgulis and Beber, 1928]. In presence of H_2O_2 inactivation is fairly rapid even at 10° C., the most satisfactory data on its catalytic activity being obtained at 0° C. Yamazaki [1921] and Nosaka [1928] concluded that in the case of the catalase of hæmolyzed blood, within certain limits, the rate of destruction both of H_2O_2 and of catalase were proportional to the product of the enzyme and substrate concentrations. If a be the initial H_2O_2 concentration, x its value at time t, b the initial catalase concentration, y its value at time t, we have Yamazaki's equations,

$$\dot{x} = -kxy, \; \dot{y} = k'xy,$$

$$\therefore \quad k'x - ky = ak' - bk.$$

If this quantity is positive, then at the end of the reaction H_2O_2 is left over, and the amount at any given time is given by

$$\frac{x}{a} = \frac{ak' - bk}{ak' - e^{(bk-ak')t}bk},$$

the final amount being $x = a\left(1 - \frac{bk}{ak'}\right)$. Hence the total amount of H_2O_2 destroyed is $\frac{bk}{k'}$, i.e. is proportional to the amount of catalase. This fact led many authors to doubt whether catalase was really a catalyst.

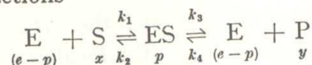
If $bk > ak'$ the H_2O_2 is all destroyed, and

$$\frac{x}{a} = \frac{bk - ak}{bke^{(bk-ak')t} - ak}.$$

Nosaka [1928 1, 2] found that k (the rate of H_2O_2 destruction) has a maximum at pH 7·0 while k' is little affected by pH. The temperture coefficient of enzyme destruction is much larger than that of H_2O_2 destruction. The rather inadequate data concerning peroxidase can probably be interpreted along similar lines.

Reversible Reactions.

Consider the reactions



$$\underset{(e-p)}{E} + \underset{x}{S} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{p}{ES} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{(e-p)}{E} + \underset{y}{P}$$

where the total amount of enzyme is e, of substrates $x + y = a$, and the velocity constants of the various reactions are written above or below the arrows.

Then (as on p. 40) we have

$$\frac{dp}{dt} = k_1(e-p)x + k_4(e-p)y - k_2p - k_3p = 0,$$

$$\therefore p = \frac{(k_1x + k_4y)e}{k_2 + k_3 + k_1x + k_4y},$$

the observed reaction velocity,

$$v = k_3p - k_4(e-p)y$$
$$= \frac{e(k_1k_3x - k_2k_4y)}{k_2 + k_3 + k_1x + k_4y}.$$

Hence equilibrium is reached when

$$\frac{x}{y} = \frac{k_2k_4}{k_1k_3},$$

i.e. the above quantity is equal to the equilibrium constant of the reaction, which depends only on the free energies of S and P, and not on the catalyst.

Putting

$$y = 0, \quad v = \frac{k_3ex}{x + \frac{k_2 + k_3}{k_1}}.$$

Hence for the reaction

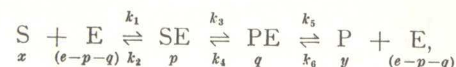
$$S \rightarrow P, \quad V = k_3e, \quad K = \frac{k_2 + k_3}{k_1}$$

where V is the maximum velocity, K the Michaelis constant. Similarly for the reaction

$$P \rightarrow S, \quad V' = k_2e, \quad K' = \frac{k_2 + k_3}{k_4}.$$

Now this is not in accordance with the facts. For example, if S is β-methyl-*d*-glucoside, and P glucose, the rate of the back reaction V′ is roughly proportional to the concentration of methyl alcohol in the solution. Clearly this cannot affect the rate of breakdown of the glucoside-enzyme compound into enzyme and glucoside, i.e. k_2. We must therefore develop a completer theory.

Consider the reactions



$$\underset{x}{S} + \underset{(e-p-q)}{E} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \underset{p}{SE} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \underset{q}{PE} \underset{k_6}{\overset{k_5}{\rightleftharpoons}} \underset{y}{P} + \underset{(e-p-q)}{E},$$

where the six velocity constants are k_1, k_2, etc., and the concentrations of the various reactants as above. The above equation may be applied to such a case as the reversible hydrolysis of β-methyl glucoside, where S represents glucoside, P glucose, provided the concentrations of water and methyl alcohol are little altered during the reaction. In this case k_3 should be roughly proportional to the concentration of H_2O, k_4 to that of CH_4O.

We have then

$$\frac{dp}{dt} = k_1x(e-p-q) + k_4q - (k_2 + k_3)p = 0,$$

$$\frac{dq}{dt} = k_6y(e-p-q) + k_3p - (k_4 + k_5)q = 0.$$

Solving these equations for p and q,

$$\frac{p}{e} = \frac{k_1(k_4 + k_5)x + k_4k_6y}{k_2k_4 + k_2k_5 + k_3k_5 + k_1x(k_3 + k_4 + k_5) + k_6y(k_2 + k_3 + k_4)}$$

$$\frac{q}{e} = \frac{k_1k_3x + k_6(k_2 + k_3)y}{k_2k_4 + k_2k_5 + k_3k_5 + k_1x(k_3 + k_4 + k_5) + k_6y(k_2 + k_3 + k_4)}$$

$$\therefore v = k_3p - k_4q,$$
$$= \frac{(k_1k_3k_5x - k_2k_4k_6y)e}{k_2k_4 + k_2k_5 + k_3k_5 + k_1x(k_3 + k_4 + k_5) + k_6y(k_2 + k_3 + k_4)}$$
$$= \frac{VK'x - V'Ky}{KK' + K'x + Ky}$$

where

$$V = \frac{k_3k_5e}{k_3 + k_4 + k_5}, \quad V' = \frac{k_2k_4e}{k_2 + k_3 + k_4},$$

$$K = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_1(k_3 + k_4 + k_5)}, \quad K' = \frac{k_2k_4 + k_2k_5 + k_3k_5}{k_6(k_2 + k_3 + k_4)}.$$

If

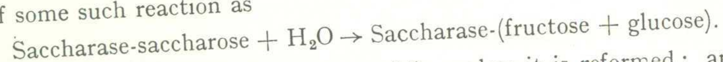
$$y = 0, \quad v = \frac{Vx}{K + x},$$

hence V is the maximum velocity of the reaction S→P in absence of P, K its Michaelis constant, V′ and K′ the same constants for the reverse reaction. We cannot calculate even the ratios of the six k's from these four quantities. However, it is in some cases an observed fact that the velocity v of the reaction varies with influences

which can only be supposed to affect k_3 and k_4. Thus Josephson [1925, 2] found that the rate of synthesis of β-methyl glucoside was proportional to the concentration of methyl alcohol, provided this did not exceed 20 per cent. In other words, V′ is proportional to k_4. This can only be the case if k_4 is small compared with k_2, and similarly it is probable that k_3 is small compared with k_5. Probably k_3 and k_4 are smaller than the other constants. If we can take this to be the case we have approximately

$$V = k_3 e, \quad V' = k_4 e, \quad K = \frac{k_2}{k_1}, \quad K' = \frac{k_5}{k_6}.$$

Hence the Michaelis-Menten assumption is applicable, except that the limiting velocity is given, not by the rate of breakdown of the enzyme-substrate compound, but by the rate of its transformation into a compound of the enzyme and another substrate. In the case of sucrose hydrolysis k_4 is infinitesimal, and k_3 is the velocity constant of some such reaction as



$$\text{Saccharase-saccharose} + H_2O \rightarrow \text{Saccharase-(fructose + glucose)}.$$

The latter complex breaks down rapidly, unless it is reformed; and since $k_5 > k_3$, the amount of it present is negligible unless fructose and glucose are present in the solution in appreciable amounts. It must not, of course, be assumed that k_3 and k_4 are always relatively small. It is difficult to imagine that this is the case with peroxidase.

Perhaps, however, the most important result of the above investigation is that the equation

$$v = \frac{VK'x - V'Ky}{KK' + K'x + Ky},$$

represents the course of the reaction no matter what are the relative values of the velocity constants. When equilibrium is reached

$$\frac{x}{y} = \frac{V'K}{VK'} = \frac{k_2 k_4 k_6}{k_1 k_3 k_5}.$$

So this quantity is equal to the equilibrium constant, which depends on the free energies of S and P, and is independent of the catalyst. This relation is borne out by the facts. Euler and Josephson [1924, 2] calculated the ratio of the affinities of β-glucosidase for glucose and β-methyl glucoside as 4·0 from the velocity constants and Bourquelot's [1914] equilibrium data. The Michaelis constants calculated from their then data gave a ratio 3·3, but Josephson's [1925] data (see p. 49) give K_m for glucose 0·1775 (mean of six different methods), for β-methyl glucoside 0·71. The ratio is therefore exactly 4·0 as expected.

If a is the total amount of substrate S + P, we may write $y = a - x$, and if b is the amount of S at equilibrium, putting $z = x - b$, i.e. the departure from the equilibrium value, we have

$$b = \frac{V'Ka}{V'K + VK'},$$

$$v = \frac{(VK' + V'K)z}{KK'\left[1 + \frac{(V + V')a}{VK' + V'K}\right] + (K - K')z} = -\frac{dz}{dt}.$$

Hence Henri's equation describes the change of z with time. z may be positive or negative, so if z is plotted against t the curve consists of two parts approaching the same asymptote. If K = K′, both are logarithmic curves. Otherwise one may have an approximately straight portion, but if so the other cannot. Examples of such pairs of curves obtained in dilute solutions are given by Woolf [1929]. Most of the reversible reactions so far followed have occurred in such strong solutions that their kinetics are relatively complicated.

Bi-molecular Reactions.

The full discussion of these reactions is very complicated. We consider only the case of an irreversible reaction where the enzyme-substrate compound is transformed at a velocity negligible compared with those involved in its formation and breakdown. We have then four equilibria,

$$E + A \rightleftharpoons EA, \quad E + B \rightleftharpoons EB, \quad EA + B \rightleftharpoons EAB \text{ and } EB + A \rightleftharpoons EAB.$$

Let K_1, K_2, K_3, K_4 be their dissociation constants, so that $K_1K_3 = K_2K_4$.

Let k be the velocity constant of the reaction EAB → E + C.

Let e be the total amount of enzyme, p the amount of EAB.

$$\therefore p = \frac{exy}{K_1K_3 + K_3x + K_4y + xy},$$

$$\therefore v = \frac{kexy}{K_1K_3 + K_3x + K_4y + xy}.$$

Hence if y is constant and x varied,

$$V = \frac{key}{K_3 + y}, \quad K_m = \frac{K_1K_3 + K_4y}{K_3 + y},$$

i.e. if y is small compared with K_3,

$$V = \frac{key}{K_3}, \quad K_m = K_1,$$

and if y is large compared with K_3,

$$V = ke, \qquad K_m = K_4.$$

Hence the Michaelis constant of one of the two substrates may vary with the concentration of the other.

If the fact that the enzyme has united with one substrate does not influence its affinity for the other, then

$$K_4 = K_1, \quad K_3 = K_2, \quad \text{and} \quad v = \frac{kexy}{(x + K_1)(x + K_2)},$$

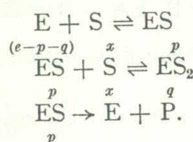
i.e. the Michaelis constants do not vary in this way. Josephson [1925, 2] has shown that this is roughly true for prunase. Where the enzyme-substrate combination depends on molecular charge, as with the proteases, it is rather unlikely that K_1 and K_4 should be equal. Otherwise the expectation is not unreasonable as a first approximation.

Attempts have been made to calculate the Michaelis constant of a molecule from those of its products of hydrolysis. They have not so far been very successful, perhaps because the importance of H_2O as a reactant has not been sufficiently stressed.

Inhibition by Excessive Substrate Concentrations.

This phenomenon is fairly common, a good example being furnished by the lipases, cf. Fig. 16. The following theory will fit the observations in this case, though not in that of xanthine oxidase.

Consider the reactions



$$\underset{(e-p-q)}{E} + \underset{x}{S} \rightleftharpoons \underset{p}{ES}$$

$$\underset{p}{ES} + \underset{x}{S} \rightleftharpoons \underset{q}{ES_2}$$

$$\underset{p}{ES} \rightarrow E + P.$$

The molecular concentration of each reactant species is written below it. Let K_1, K_2 be the Michaelis constants of the first two reactions, k the velocity constant of the third, supposed to be relatively slow.

$$\therefore (e - p - q)x = K_1 p, \quad px = K_2 q,$$

$$\therefore p = \frac{ex}{K_1 + x + \frac{x^2}{K_2}},$$

$$\therefore v = \frac{kex}{K_1 + x + \frac{x^2}{K_2}}.$$

The reaction proceeds at a maximum velocity

$$\frac{ke\sqrt{K_2}}{2\sqrt{K_1} + \sqrt{K_2}}$$

when $x = \sqrt{K_1 K_2}$. This is a fraction

$$\frac{1}{1 + 2\sqrt{\frac{K_1}{K_2}}}$$

of the maximum possible if EA_2 were not formed.

Since

$$\frac{v}{ke} = \frac{1}{1 + \frac{K_1}{x} + \frac{x}{K_2}},$$

it is clear that when velocity is plotted against the logarithm of substrate concentration, we should obtain the same familiar bell-shaped curve as when the dissociation residue of an ampholyte is plotted against pH. No work has been done in concentrations high enough to verify the symmetry of the curve, but it is at least nearly symmetrical for sheep's liver lipase, for which in the case of ethyl-*l*-mandelate, $K_1 = \cdot 00166$, $K_2 = \cdot 166$ approximately. In this case the nature of the compound ES_2 may be readily imagined. Whereas in EA the enzyme is united to the ester at two spots (see Chap. X.), one on the alcohol, the other on the acid residue, in EA_2 each molecule is only once united, and hence the strain producing hydrolysis does not arise.

In these cases Bamann and Schmeller [1929] have shown that the velocity increases, sometimes by as much as 27 per cent., during the course of the hydrolysis, in spite of possible back reactions. The equation for the amount x left from an initial concentration a after time t is

$$Vt = a - x + \frac{a^2 - x^2}{2K_2} + K_1 \log_e \frac{a}{x}.$$

When $a - x$ is plotted against t the curve is at first concave upwards, as in an autocatalytic reaction. In relatively high initial concentrations the velocity goes on increasing till the reaction is almost complete.

Competing Substrates and Asymmetrical Hydrolysis.

This case has assumed importance with the investigation by Willstätter, Kuhn and Bamann [1928], and Weber and Ammon

[1929] of the hydrolysis of *d*, *l*, and *dl*-esters by lipases. The theory here given is an extension of theirs. Consider the hydrolysis of a mixture of two substrates S_1 and S_2. Let the initial concentrations be a_1, a_2, the maximal velocities for each substrate alone V_1, V_2, the Michaelis constants K_1, K_2. Let x_1, x_2 be the concentrations of substrate at any moment, p_1, p_2 the concentrations of enzyme combined with each, e the total concentration of enzyme. Consider the case of S_1. We have the reactions

$$E + S_1 \rightleftharpoons ES_1 \rightarrow E + S_1',$$

where S_1' stands for the product of reaction. Then the concentration of free enzyme is $e - p_1 - p_2$, that of ES_1 is p_1, that of S_1 being x_1.

Hence (as on p. 39)

$$x_1(e - p_1 - p_2) = K_1 p_1.$$

Similarly,

$$x_2(e - p_1 - p_2) = K_2 p_2.$$

$$\therefore p_1 = \frac{K_2 x_1 e}{K_2 x_1 + K_1 x_2 + K_1 K_2},$$

$$\therefore \frac{\frac{1}{x_1}\frac{dx_1}{dt}}{\frac{1}{x_2}\frac{dx_2}{dt}} = \frac{V_1 K_2}{V_2 K_1},$$

$$\therefore \log\frac{a_1}{x_1} = \frac{V_1 K_2}{V_2 K_1}\log\frac{a_2}{x_2}.$$

Or if

$$a_1 = a_2, \quad \frac{\log x_1}{\log x_2} = \frac{V_1 K_2}{V_2 K_1}.$$

Weber and Ammon show that in the case of Willstätter, Kuhn, and Bamann's hydrolysis of *dl*-ethyl mandelate with pig's liver lipase, where $x_1 + x_2$ is given by the degree of total hydrolysis, $x_1 - x_2$ by the polarimetric reading, the values of $\frac{\log x_1}{\log x_2}$ only vary between 2·9 and 3·1, and that this value agrees fairly well with that of 2·0 calculated from the V and K values, which, however, have a considerable uncertainty.

$$\text{Log}\frac{x_2}{a_2} = \frac{V_2 K_1}{V_1 K_2}\log\frac{x_1}{a_1},$$

$$\therefore x_2 = a_2\left(\frac{x_1}{a_1}\right)^k, \text{ where } k = \frac{V_2 K_1}{V_1 K_2},$$

$$\therefore -\frac{dx_1}{dt} = \frac{V_1 K_2 x_1}{K_2 x_1 + K_1 a_2\left(\frac{x_1}{a_1}\right)^k + K_1 K_2},$$

$$\therefore V_1 t = \int_{x_1}^{a_1}\left[1 + \frac{K_1}{x} + \frac{K_1 a_2 x^{k-1}}{K_2 a_1^k}\right]dx,$$

$$\therefore t = \frac{a_1 - x_1}{V_1} + \frac{a_2}{V_2}\left[1 - \left(\frac{x_1}{a_1}\right)^k\right] + \frac{K_1}{V_1}\log\frac{a_1}{x_1}.$$

This is Henri's equation with an extra term which is clearly small if k is small, and large if k is large. Hence if V_1K_2 and V_2K_1 are very different, the more rapidly destroyed component of the mixture will disappear almost according to Henri's equation. The more slowly destroyed will, under certain circumstances, be destroyed slowly at first, and then reach a maximum rate of destruction. Let A_1 be more slowly destroyed, so that $k > 1$. Then the maximum rate of transformation of A_1 occurs when

$$\frac{x_1}{a_1} = \left(\frac{K_2}{ka_2 - a_2}\right)^{\frac{1}{k}}.$$

So the velocity only increases with time if $a_2 > \frac{K_2}{k-1}$. In a racemic mixture $a_1 = a_2$, and the optical rotation of the products of hydrolysis is proportional to $x_1 - x_2$, or

$$x_1 - a\left(\frac{x_1}{a}\right)^k.$$

This is a maximum when

$$1 - k\left(\frac{x_1}{a}\right)^{k-1} = 0,$$

$$\therefore x_1 = ak^{\frac{1}{1-k}}, \quad x_2 = ak^{\frac{k}{1-k}}.$$

The maximum rotation is, therefore, proportional to

$$k^{\frac{1}{1-k}} - k^{\frac{k}{1-k}}, \text{ or } k^{\frac{1}{1-k}}\left(1 - \frac{1}{k}\right).$$

When $k = 1$, this is of course zero. As k increases or decreases it gradually increases towards unity, reaching a value $\frac{1}{2}$ when $k = 4·5$ or ·2̇, i.e. in order to obtain during hydrolysis a rotation equal to half that which would be reached were the enzyme absolutely specific, k must be as large as 4·5, or as small as $\frac{2}{9}$. In the case quoted above $k = 3$ approximately, and the maximum rotation should have been over 38·5 per cent. of that theoretically possible with an absolutely specific enzyme. The maximum found was 35·4 per cent.

The proportion of the substrate un-hydrolysed at the time of maximum rotation is

$$\frac{1}{2}\left(k^{\frac{1}{1-k}} + k^{\frac{k}{1-k}}\right).$$

Now when $k = 1$, this $= e^{-1}$ or $\cdot 368$, and it only increases to $\cdot 401$ when $k = 5$ or $\cdot 2$, and to $\cdot 5$ when k is 0 or ∞. Hence the maximum rotation should always occur at a hydrolysis of between 63·2 per cent. and 50 per cent. of the racemic mixture. Rona and Ammon [1927] in relatively rough experiments, found maximum rotations at percentage hydrolyses varying between 33 and 55 per cent., but Willstätter, Kuhn, and Bamann in very careful experiments found a maximum rotation at 50·5 per cent. hydrolysis, and Bamann [1929] at 52 per cent. They extracted the mandelic acid at different stages of hydrolysis, and took polarimetric readings. The specific rotation of the mandelic acid was plotted against the degree of rotation. The curve so obtained was independent of the pH and enzyme concentration. It should also be independent of the initial substrate concentration, but this was not varied. Its theoretical equation is $1 - x + xy = (1 - x - xy)^k$ where x is the fraction hydrolysed, y the rotatory power of the mandelic acid divided by that of the pure acid. The agreement with prediction was not exact, the rotations observed being too low at both ends of the curve. The rotation of the mandelic acid should be maximal at first, being $\frac{k - 1}{k + 1}$ of that of *d*-mandelic acid, and then falling.

Actually it rose from 60·1° to 70·0°, corresponding to $k = 2 \cdot 6$. This initial rise can perhaps be explained by the partial failure of the Michaelis equation actually observed at high substrate concentrations. The fall at high degrees of hydrolysis is not at present explicable. Bamann [1929] found no effect of altering the initial substrate concentration in the case of pig's liver lipase, but a large effect with human and rabbit preparations. The acid liberated by them at high substrate concentrations was dextro-rotatory, at low concentrations lævo-rotatory.

This cannot be explained in terms of any theory so far put forward. It is clear that our conceptions of enzyme-substrate affinity are still in a primitive stage. The theory given above should hold good, as regards the relation between hydrolysis and optical activity, though not as regards kinetics, even if we allow for inhibition by high substrate concentrations as on page 84.

Consecutive Reactions.

Consider a chain of irreversible catalysed reactions, $A \to B \to C \to D$, etc., each reaction alone being subject to the Michaelis-Menten

conditions. Such examples are frequent in nature. They include the hydrolysis of proteins and triglycerides in dilute solutions, and probably alcoholic fermentation by zymin in presence of excess of phosphate. We will confine ourselves at first to the reactions $A \to B \to C$, where the velocity of the first reaction alone is given by $v_1 = \frac{V_1 x}{x + K_1}$, of the second by $v_2 = \frac{V_2 y}{y + K_2}$; x, y, and z being the concentrations of A, B, and C, respectively. Let a be the initial concentration of A. The differential equations for the system are clearly

$$\frac{dx}{dt} = \frac{-V_1 x}{x + K_1}, \quad \frac{dy}{dt} = \frac{V_1 x}{x + K_1} - \frac{V_2 y}{y + K_2},$$

provided A, B, and C are not competing for the same enzyme.

These are not in general integrable except numerically, however they can be integrated provided K_1 and K_2 are small or large compared with a. Four cases arise :—

1. K_1 and K_2 are large. In this case the individual reactions are nearly quasi-unimolecular, and the case is the familiar one of two consecutive reactions, or of the decay of radium emanation.

The two velocity constants are $\frac{V_1}{K_1}$ and $\frac{V_2}{K_2}$.

2. K_1 and K_2 are small. Clearly the rate of destruction of A and production of B is steady till near the end of the reaction, and equal to V_1. Two cases arise :—

(*a*) $V_1 > V_2$. In this case y increases steadily with time at a rate $V_1 - V_2$ until A is nearly exhausted. It reaches a maximum value just less than $\left(1 - \frac{V_2}{V_1}\right) a$, and then falls at a rate V_2, until near the end of the reaction. Its graph consists of two straight lines; z increases at a constant rate V_2 till near the end of the reaction. There is, of course, a small latent period in the production of C.

(*b*) $V_2 > V_1$. The velocity of the first reaction is now the limiting factor. After a short initial lag the value of y reaches the small value $\frac{V_1 K_2}{V_2 - V_1}$, and remains there till almost the end of the reaction.

The rate of production of C is equal to V_1 throughout. This probably represents roughly the state of affairs in many metabolic processes, where the intermediary metabolites are rapidly destroyed, but the rate of the total reaction is fairly steady.

3. K_1 is small, K_2 is large. In this case A diminishes at a constant rate, so that

$$\frac{dx}{dt} = -V_1, \quad \frac{dy}{dt} = V_1 - \frac{V_2 y}{y + K_2}.$$

Two cases arise :—

(*a*) $V_1 > V_2$. $\therefore (V_1 - V_2)t = y - \frac{V_2 K_2}{V_1 - V_2} \log_e \left[1 + \frac{(V_1 - V_2)y}{V_1 K_2}\right]$,

y increases fairly steadily with time, reaching a maximum less than $\left(1 - \frac{V_2}{V_1}\right) a$ when A is nearly exhausted; z increases slowly at first, then more rapidly, and its rate then slows down again. Its graph is nowhere linear.

(*b*) $V_2 > V_1$. Again the velocity of the first reaction is the limiting factor.

$$\therefore (V_2 - V_1)t = y + \frac{V_2 K_2}{V_2 - V_1} \log_e \left[1 - \frac{(V_2 - V_1)y}{V_1 K_2}\right],$$

y comes very near to the value $\frac{V_1 K_2}{V_2 - V_1}$ (unless this is nearly as large as a), and remains there till A is almost exhausted. This value is not necessarily very small. Until this time C is produced at a constant rate V_1, after the lag period is over. On plotting z against t a graph is obtained with a linear central portion, but appreciably bent at both ends.

4. K_1 is large, K_2 small.

$$\therefore \frac{dx}{dt} = \frac{-V_1 x}{K_1}, \quad \therefore x = ae^{\frac{-V_1 t}{K_1}},$$

$$\frac{dy}{dt} = \frac{-dx}{dt} - V_2 \quad \therefore y = a - ae^{\frac{-V_1 t}{K_1}} - V_2 t,$$

x decreases according to Henri's equation, y increases, reaching a maximum value of

$$a - \frac{V_2 K_1}{V_1}\left[1 + \log_e \frac{V_1 a}{V_2 K_1}\right],$$

provided that V_2 is not too large. It soon falls from this maximum again; however, the rate of increase of z is linear and equal to V_2 as long as y is several times as large as K_1. If V_2 is very large y is always less than K_2, and the transformation of A into C almost instantaneous.

In a series of more than two reactions matters are more complicated. When, however, all the reactants have a high affinity for the catalysts concerned, the velocity of the whole is equal to the slowest of the V constants. If we consider these in order V_1, V_2, V_3, etc., it is clear that intermediate products cannot accumulate after the slowest reaction has been accomplished; before this they do so whenever a reaction proceeds more slowly than any of the preceding reactions.

Schutz's Law.

Schutz [1885] found that the amount of peptone produced in a given time by pepsin from egg albumin varied as the square root of the amount of pepsin. This was confirmed by J. Schutz [1900] and others, provided that not more than half the albumin was digested. The reason for a falling off of peptic activity with pepsin concentration was shown by Northrop (see Chap. III.) to be the combination of pepsin with an inhibitory substance. Arrhenius [1907] extended this rule to the tryptic digestion of gelatin and casein, and also to the course of the reaction. He found that over a considerable range the amount of protein digested could be represented by the formula $K\sqrt{et}$ where e is the enzyme concentration, t the time. This formula gives for the velocity of the reaction $v = K\sqrt{\frac{e}{2t}}$. It is at once clear that this formula cannot hold when t is large or small. For the amount digested becomes infinite when t is infinite, the velocity infinite when t is zero. Northrop [1924, 3] investigated the matter thoroughly and found, as was to be expected, that Schutz's law, as modified by Arrhenius, gave too high values when t is small, too low when t is large. Nevertheless, it gives a good approximation to fact over a fairly wide range. But it must be regarded rather as a convenient rule than as a law throwing light on the nature of enzyme action.

The equation $y = k\sqrt{t}$ has been successfully applied to lipase action in unbuffered solutions by Herzog [1913] and Kuhn [1925, 2], who attributes the slowing down to enzyme destruction, the equation $v \propto \sqrt{e}$ to lipase action by Goldschmidt [1925].

General Considerations.

The question often arises as to what deductions can be made from the observed kinetics of an enzyme reaction. If the solutions are unbuffered very little can be deduced unless the substrate and reaction products are non-electrolytes. If the velocity is constant during the utilization of an appreciable fraction of the substrate, we may conclude that the enzyme is all combined with substrate, i.e. that K_m is

considerably less than the substrate concentration of the experiment. Moreover, the reaction products cannot unite with appreciable quantities of enzyme under the circumstances of the experiment.

When the velocity falls off any of the following may be occurring :—

1. Inadequate saturation of the enzyme as the substrate concentration falls.

2. Reversible union of part of the enzyme with reaction products.

3. Reversible union of part of the enzyme with substrate to form an inactive compound.

4. Irreversible destruction of the enzyme.

5. Changes of pH.

6. Changes in the state of aggregation of the substrate, as in the hydrolysis of colloids and emulsions.

7. Changes in the reaction measured, as when a lipase catalyses the successive hydrolysis of tripalmitin, dipalmitin, and monopalmitin.

8. Changes in the molecular concentration of H_2O and other effects found in strong solutions. These are generally marked when enzymes are being used for synthesis.

Now in many cases a number of these factors are operating simultaneously. Thus in the hydrolysis of an oil emulsion by a lipase such as that of castor-oil beans which is destroyed by water all except perhaps (3) are possible, and several occur. In peptic digestion of solid proteins (1), (2), (5), (6), and (7) are all prominent. It therefore appears futile to give any but an empirical equation in such cases.

Occasionally, however, a reaction is found to follow the equation $kt = \log \frac{a}{a-x}$ of a unimolecular reaction with great accuracy. In such a case, especially where processes (5), (6), (7), and (8) can be ruled out, as in disaccharide hydrolyses, it is very likely that the reaction is proceeding according to Henri's equation, and the substrate concentration is well below the Michaelis constant of the enzyme. Such a conclusion is not, however, certain. Thus, if in the equation for sucrose hydrolysis (p. 76) we suppose that one product of the reaction has the same affinity as the substrate for the enzyme, i.e., put K_g infinite, $K_f = K_m$, $\therefore \frac{dy}{dt} = \frac{V(a-y)}{a+K_m}$. Hence the course of the reaction is unimolecular, though the initial velocity is not proportional to the substrate concentration.

To sum up, the nature of the reaction is better deduced from several rough curves representing its course under different conditions than by a single accurate curve representing it under only one set of conditions.

CHAPTER VI.

SPECIFICITY.

By specificity is meant the manner in which catalysis by an enzyme is influenced by the chemical structure of its substrates or possible substrates. We may compare either different substrates, or different linkages in the same substrate. For example, saccharase hydrolyses sucrose but not maltose, and trypsin hydrolyses some, but not all, of the peptide linkages in a protein. It may perhaps be legitimate to extend the term so as to cover the difference between the ways in which different enzymes attack the same substrate. Thus yeast zymase breaks up glucose into alcohol and carbon dioxide, muscle "zymase" into lactic acid; pancreatic amylase hydrolyses starch into α-maltose, malt amylase into β-maltose. In this chapter, however, we shall mainly confine ourselves to the first type of specificity.

Criteria for the Unity of an Enzyme.

When the facts of specificity were first discovered there was a tendency among some workers to postulate a separate enzyme for every substrate. Others went too far in the other direction. For example, it was believed that the same enzyme in emulsin hydrolysed β-glucosides and β-galactosides. The following criteria for the identity of the enzymes acting on several substrates have since been employed :—

1. They cannot be separated by fractional precipitation and adsorption. Thus yeast saccharase can readily be separated from maltase, but not from raffinase, with which it is apparently identical.

2. When one is destroyed by heat or chemical reagents so is the other. Thus the lactase (β-galactosidase) of emulsin is readily destroyed at a temperature of 45°, according to Armstrong, Armstrong and Horton [1908], and by trypsin or dialysis according to Ohta [1914]. None of these procedures destroy the β-glucosidase. This criterion must of course be applied with care where a coenzyme is present. Thus if papain-HCN is heated, the HCN is driven off, thus lowering the specificity of the papain.