[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE BELLEVUE HOSPITAL MEDICAL COLLEGE]

Invertase Action as a Heterogeneous Reaction

BY T. A. WHITE

Introduction

Catalysts of various sorts have been known and investigated for many years. In their efforts to determine the mechanisms by which these various agents can change the rate of a given reaction, investigators have been led to two points of view. With respect to enzymes, these points of view have resolved themselves into the question of whether the reaction is homogeneous or heterogeneous in nature. Since catalysts affect the rate of a reaction, many attempts have been made to determine the mechanism by means of reaction kinetics studies.

Most investigators in the field of enzyme action have applied the mass law to what is assumed to be a homogeneous system. This method of approach has led to an explanation of many observations that have been made by a multitude of investigators from time to time, and, consequently, has led a large group of workers to believe that enzyme reactions are homogeneous in nature. On the other hand, this method of approach has not led to a complete explanation of all the observed facts. For example, Nelson and Griffin[1] showed that not only was invertase active in solution, but it was also active while adsorbed on charcoal, demonstrating that the action may be of a heterogeneous nature. This, as well as the fact that the enzyme itself is of a colloidal nature, led Nelson and Vosburgh[2] to consider the action of invertase in hydrolyzing sucrose as a reaction of a heterogeneous rather than of a homogeneous nature. It should be mentioned, too, that Bayliss[3] also considered enzyme action as heterogeneous, and suggested that adsorption played a part in the reaction; but his views proved to be unsatisfactory. These various suggestions and methods of approach have not led to a picture which on the whole is as acceptable as that offered by Michaelis and Menten[4] from the homogeneous viewpoint.

This is due, to some extent at least, to the fact that equations which have been derived in the past from the heterogeneous viewpoint have not successfully explained the kinetics of enzyme reactions. In recent years, much progress has been made in understanding those gas reactions which take place at the surfaces of certain inorganic catalysts by recognizing that the seat of such reactions lies in the layer of adsorbed molecules on the surface of the catalyst. With invertase as an example and assuming the invertase–sucrose–water system to be heterogeneous in nature, it is the

(1) Nelson and Griffin, THIS JOURNAL, **38**, 1109 (1916).
(2) Nelson and Vosburgh, *ibid.*, **39**, 790 (1917).
(3) Bayliss, *Biochem. J.*, **1**, 175 (1906).
(4) Michaelis and Menten, *Biochem. Z.*, **49**, 333 (1913).

purpose of this paper to utilize these new ideas in showing that the experimental facts can be explained just as well from the heterogeneous as from the homogeneous point of view. Since the homogeneous view is so well known, and can be found discussed in full elsewhere,[5] the remainder of this paper will deal entirely with the presentation of the heterogeneous view.

Theoretical Introduction

While there is no need to give here a complete discussion of the ideas[6] that have developed around heterogeneous reactions, it may be useful to give at this time a brief survey of those principles which will later be employed in discussing the facts of invertase action. The active mass in a heterogeneous reaction is not the total amount of a given substance present, as it is in a homogeneous reaction, but is, according to the chemical adsorption theory, that part of the total mass which is actively adsorbed on the surface of the catalyst. Since only those molecules which are adsorbed by the catalyst react, the problem resolves itself into that of determining how much is adsorbed. If it be assumed that this can be more or less accurately determined by means of what is called the Langmuir[7] adsorption isotherm, which may be expressed in terms of concentration c, a constant b, and the fraction of the surface covered Θ, as

$$\Theta = \frac{bc}{1 + bc} \qquad (1)$$

a number of kinetic equations may be derived. Such an adsorption isotherm may, in the general case, be represented by a curve as is shown in Fig. 1. This shows that over the section A the catalyst is completely covered, according to the theory, with a unimolecular layer of adsorbed molecules, but less and less covered at B and C. Moreover, as long as the concentration of the reactant in the solution is at a sufficiently high level to cover the surface of the catalyst with adsorbed reactant, the rate of reaction should be constant. On the other hand, over the portion represented by C, the amount of reactant adsorbed, and consequently the active mass, is practically proportional to the total concentration of reactant. Under such conditions, the reaction

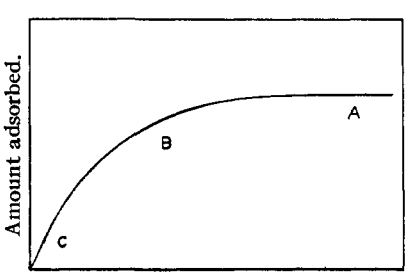

Fig. 1.

(5) See Chap. V, "The Course of Enzymatic Reactions," of "Enzymes" by Haldane (1930) for a discussion of the methods employed in deriving from the homogeneous viewpoint equations which are similar to those that will be derived and used here.

(6) For a fuller discussion of these principles, see, for example: (a) Hinshelwood, "Kinetics of Chemical Change in Gaseous Systems"; (b) Taylor, "Treatise on Physical Chemistry," Chap. XV, 2d ed.

(7) Langmuir, THIS JOURNAL, **38**, 2221 (1916).

should be unimolecular in nature. A reaction progressing along the section indicated by B should show an order which gradually changes from zero to unimolecular. Finally, if a reaction started in the region A, and was followed to completion, the reaction should give a constant rate, or zero order, at first, but decreasing constants thereafter, increasing unimolecular constants over the first and middle portions, and regular unimolecular constants during the last part of the reaction. In a general case, where a is the original amount of the reactant, x the amount of product at time t, k and b constants, the rate of such a reaction may be expressed as

$$\frac{dx}{dt} = \frac{k(a-x)}{1+b(a-x)}, \text{ or } k = 1/t \ln \frac{a}{a-x} + \frac{bx}{t} \tag{2}$$

But the above equation does not take into account the inhibiting action of the products of a reaction, and since the products do inhibit the hydrolysis of sucrose, equations should be derived to fit those conditions. The simplest case of this sort is the one that has an amount of adsorbed reactant which is proportional to the total concentration, namely, over section C of Fig. 1. Under such conditions, the amount of active mass of the reactant is equal to the product of that amount which would ordinarily be adsorbed (in this case, proportional to the total concentration of the reactant) and that fraction of the total surface which is not covered by the product. That fraction of the surface which is covered by the product is given by equation 1. The fraction of the surface which is available for the reactant is equal to

$$1 - \theta = 1 - \frac{bx}{1+bx} = \frac{1}{1+bx} \tag{3}$$

Therefore, the rate under such conditions is equal to

$$\frac{dx}{dt} = \frac{k_1(a-x)}{1+bx} \text{ or } k_2 = 1/t \ln \frac{a}{a-x} - \frac{k_3 x}{t} \tag{4}$$

Since the products inhibit the hydrolysis of sucrose with invertase, it should be expected that this equation would hold over the last part of the reaction, and it will be shown that it does.

At the beginning of the reaction, where the reactant is in sufficient concentration to cover all the surface left for it by the adsorbed product, the rate should be proportional to the product of the amount of surface available for the reactant and the amount (in this case, constant) of the reactant which would be adsorbed in the absence of any product. Thus, the rate is equal to

$$\frac{dx}{dt} = \frac{k_4}{1+bx} \text{ or } k_4 = \frac{x}{t} + \frac{bx^2}{2t} \tag{5}$$

It will be shown that this equation holds for a considerable fraction of the invertase reaction.

The final problem, however, is to obtain a single equation that will represent the rate of hydrolysis from the beginning to the end of the re-

action. In their studies of the adsorption of gas mixtures by silica, Markham and Benton[8] found that in general the fraction of the surface covered by one gas of a binary mixture followed an equation which is slightly different from equation 1. Considering the reactant and product as such a mixture, and using the same terms as before, the following equation expresses the fraction of the enzyme surface which is covered by the reactant.

$$\theta = \frac{b(a - x)}{1 + b(a - x) + cx} \tag{6}$$

Under such conditions, therefore, the rate is equal to

$$\frac{dx}{dt} = \frac{k_b(a - x)}{1 + b(a - x) + cx} \text{ or } k_b = \frac{1 + ac}{t} \ln \frac{a}{a - x} + \frac{(b - c)x}{t} \tag{7}$$

It will be noticed that equation 7 may be reduced to the same form as equation 2. Perhaps it is for this reason that various workers have found that the course of the reaction agreed well with equation 2, which was obtained by others from the homogeneous viewpoint as well as here without considering the products. Further, it should be pointed out that equation 7 reduces to equation 4 toward the end of the reaction, where the value of $b(a - x)$ becomes small in comparison with the other terms.

Experimental Methods

Before the actual results are presented, the methods and types of apparatus employed will be recorded. While no new or unusual methods were used, all recognized methods for obtaining accurate results were carefully observed as far as was possible. The details of such methods are as follows.

The sucrose used in these experiments was prepared by recrystallizing ordinary cane sugar at room temperature by adding enough absolute ethyl alcohol to a saturated water solution of cane sugar to make the concentration of alcohol about 75% by volume. The precipitated sucrose was filtered off, washed several times with absolute alcohol, and dried at room temperature. Fresh solutions were prepared from this preparation just before a hydrolysis was begun by adding to the necessary amount of sugar 25 cc. of buffer and enough distilled water to make the volume 500 cc.; 400 cc. (half quantities were sometimes used) of this solution was placed in a glass-stoppered bottle and allowed a half hour to come to temperature equilibrium in a water thermostat, accurate to $\pm 0.02°$, before the reaction was started by pipetting a given quantity of enzyme into the reaction solution. Twenty cc. samples were pipetted from the reaction mixture and run into 5 cc. of 0.2 *M* sodium carbonate at room temperature in order to stop the reaction and to hasten mutarotation. Two drops of 40% sodium hydroxide were used instead of the sodium carbonate in the case of 3% sugar solutions. The delivery time of the pipet was ten seconds, and the mean of this value was taken as the time of taking the sample.

Two buffer solutions were used: P_H 4.7 was obtained with a 0.1 *N* sodium acetate–acetic acid solution, and P_H 6.4 and 7.4 were obtained with Clark and Lubs'[9] acid potassium phosphate–sodium hydroxide buffer solutions. The invertase was prepared from yeast according to the method suggested by Morrow,[10] and a 2.6% solution was used.

(8) Markham and Benton, THIS JOURNAL, **53**, 497 (1931).

(9) See Clark, "The Determination of Hydrogen Ions," 1928, 3d ed., p. 192.

10) Morrow, "Biochemical Laboratory Methods," 1927, p. 281.

All readings were taken at room temperature in a 2-dm. tube. A high grade, triple-field Schmidt and Haensch polarimeter, calibrated to 0.01°, was employed. While the room temperature varied from day to day by as much as four degrees, such changes were followed, and it is believed that they did not produce trends in the readings, for all readings were generally made within a narrow temperature range. A *Lab Arc*, with a Corning Glass filter to give the 5461 Å. line, was used as a light source. Rotations due to strains in the cover glasses were avoided by rotating the tube between readings. Readings were always taken until mutarotation was complete, as indicated by the constancy, within 0.01°, of polarimetric readings over a period of ten minutes. The initial rotation was synthetically determined by taking 20 cc. of a mixture of the original sugar solution with a proportionate amount of water to correspond to the amount of invertase which was added to the reaction solution. The invertase had no measurable optical activity. After allowing the reaction to run for one or two days, the rotation at the end of that time was considered as the final value. These values agreed within the experimental error with the theoretical amounts. The initial concentration of the sucrose, in terms of degrees of rotation, was taken as the initial rotation minus the final rotation; and the concentration of the sucrose at any time t was taken as the rotation at that time minus the final rotation.

Experimental Results

The Beginning of the Reaction.—It was found, as so many investigators have found, that the initial part of the hydrolysis proceeded with a constant rate, and continued to do so up to about ten per cent. hydrolysis. If the products did not inhibit the reaction, one would expect the rate to remain constant so long as the amount of sucrose adsorbed by the invertase is constant; but with the products inhibiting the reaction, the rate, under normal conditions, should gradually fall off. Equation 5 was derived to fit these conditions, and the following values in Table I show that the equation holds over a considerable portion of the reaction. Other values will be found in column 6 of Table IV.

TABLE I

RESULTS FOR 25° AND P_H 4.7

Run 9: 1.0 cc. enzyme per 400 cc. of 6% sucrose				Run 11: 1.0 cc. enzyme per 400 cc. of 10% sucrose			
Time, min.	x °Rot.	% Hydro.	$\frac{x}{t} + \frac{0.045x^2}{t} = k$	Time, min.	x °Rot.	% Hydro.	$\frac{x}{t} + \frac{0.013x^2}{t} = k$
0	0.00	0.0	...	0	0.00	0.0	...
10	1.26	12.9	0.133	15	1.70	10.5	0.116
20	2.42	24.8	.134	30	3.37	20.8	.117
40	4.44	45.4	.133	60	6.50	40.2	.117
60	6.10	62.3	.130	90	9.06	56.0	.113
90	7.82	79.9	.128	120	11.14	68.9	.106
∞	9.79	..	...	∞	16.19	..	...

Different Amounts of Enzyme.—Figure 2 gives a series of curves which shows how the unimolecular constant, as calculated from $k_m = 1/t \log a/(a - x)$, varies as the reaction progresses. It will be noticed that the constants increase until about 93.5% of the sucrose has been hydrolyzed, and then decrease over the remainder of the reaction. Since the tempera-

ture, P_H, sucrose concentration, and water concentration were the same, and only the amount of enzyme was varied in these experiments, it must be concluded that the maxima, which come at approximately 93.5% hydrolysis in all the cases, are independent of the amount of enzyme present.

The well-recognized fact that the rate of hydrolysis for a given concentration of sucrose is proportional to the concentration of enzyme present is also illustrated in the curves. This would be expected, for the enzyme will adsorb twice as much of the reactant as half that amount of enzyme will adsorb under the same conditions, and, since the rate is proportional to the amount of reactant adsorbed by the catalyst, the rate of hydrolysis should be twice as great in the former as in the latter case.

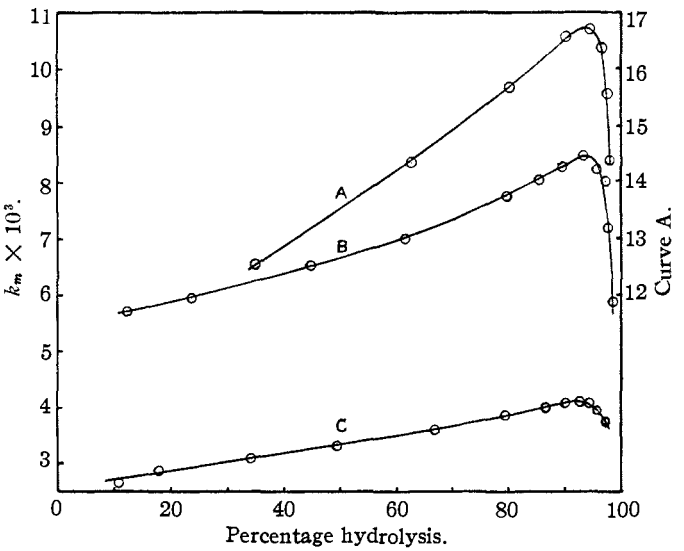

Fig. 2.—400 cc. of 6% sucrose, P_H 4.7, 25°, with varying amounts of enzyme: Curve A, run 10. 2.0 cc. of invertase; Curve B, run 13, 1.0 cc. of invertase; Curve C, run 12, 0.5 cc. of invertase.

Different Amounts of Sucrose.—It will be noticed that the curves of Fig. 3 show the same general properties as those of Fig. 2. On the other hand, the additional fact is brought out that while the unimolecular constant goes through a maximum at a definite per cent. hydrolysis for a given initial sucrose concentration, irrespective of the amount of enzyme present, each sugar solution gives a maximum which depends on the initial concentration of the sugar. The maximum comes at 96% hydrolysis for 10% sucrose, at 93.5% hydrolysis for 6% sucrose, and at about 90% hydrolysis for 3% sucrose. These values indicate that the unimolecular constant goes through the transition period when the amounts of sucrose present in the solution are 0.40, 0.39, and 0.30%, respectively.

Different P_H Values.—Besides the very interesting fact that the rate of hydrolysis markedly decreases with an increase in the P_H of the solution from 4.7 to 7.4, the curves of Fig. 4 also show that the unimolecular constant goes through a maximum at different percentages of hydrolysis for different values of P_H. For 6% sucrose solutions, the maximum occurs at 93.5% hydrolysis for P_H 4.7, at 91.5% hydrolysis for P_H 6.5, and at 86.5% hydrolysis for P_H 7.4. From these values, therefore, it must be concluded that besides decreasing the amount of sucrose adsorbed by the invertase, as indicated by a decrease in rate, an increase in P_H from 4.7 to 7.4 also modifies the general shape of the adsorption isotherm so that the isotherm reaches its saturation value at higher sucrose concentrations with higher values of P_H. It is not known how the surface of the enzyme particles varies with P_H, but it seems reasonable to suppose that the active surface varies considerably with P_H; and hence contributes to the above mentioned variations.

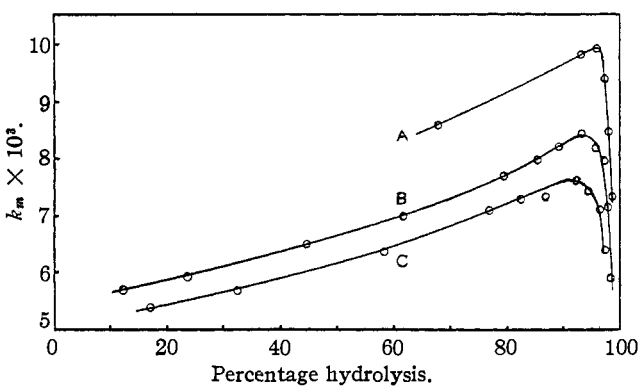

Fig. 3.—400 cc. of sucrose, P_H 4.7, 25°, with varying sucrose concentrations: Curve A, run 17, 10% sucrose, 2.0 cc. of invertase; Curve B, run 13, 6% sucrose, 1.0 cc. of invertase; Curve C, run 15, 3% sucrose, 0.5 cc. of invertase.

Effect of Temperature.—It is a well-recognized fact that the higher the temperature the higher the concentration at which an adsorption isotherm reaches its saturation value. In view of this fact one would expect that, for a given initial sucrose concentration and at the same P_H, the maximum of the unimolecular versus percentage hydrolysis curve would come at a lower percentage of hydrolysis the higher the temperature. The curves of Fig. 5 show that this is actually the case; for, if the curves of Fig. 5 are compared with those of Fig. 4, it will be noticed that with an initial sucrose concentration of 6% the maximum is shifted from 93.5% hydrolysis to 89.0% hydrolysis at P_H 4.7, and from 86.5% hydrolysis to 75.0% hydrolysis at P_H 7.4, for a ten degree rise in temperature. It should be pointed out that, since the dispersion of colloids is affected by temperature, some of this change is probably due to a change in the enzyme surface.

The End of the Reaction.—Throughout the results that have been given, it has been assumed that the variations of the unimolecular constant

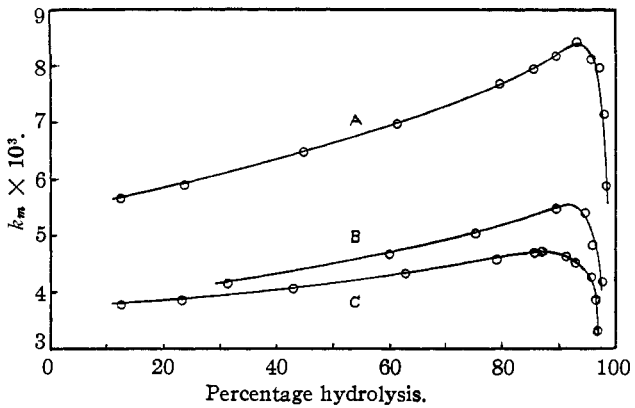

Fig. 4.—400 cc. 6% sucrose, 25°, at various P_H values: Curve A, run 13, P_H 4.7, 1.0 cc. of invertase; Curve B, run 19, P_H 6.5 1.0 cc. of invertase; Curve C, run 21, P_H 7.4, 4.0 cc. of invertase.

with percentage hydrolysis are real and that they are the result of the reaction itself. Previous investigators[11] have obtained one or a few unimolecular constants which showed a decreasing tendency toward the end

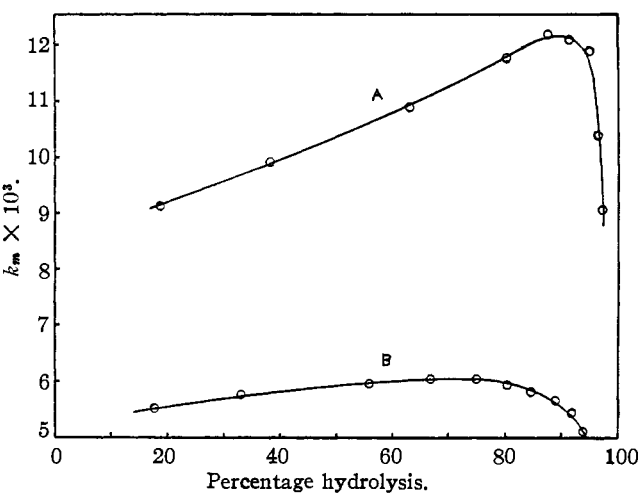

Fig. 5.—400 cc. 6% sucrose, 35°: Curve A, run 25. P_H 4.7, 1.0 cc. of invertase; Curve B, run 24, P_H 7.4, 4.0 cc. of invertase.

of the reaction, but they have neglected them as due to a destruction of the enzyme by means of heat or as due to experimental error. The fact that

(11) Auden and Dawson, *Biochem. J.*, **25**, 1909 (1931).

the maximum in the unimolecular constants for a given initial sucrose concentration comes at the same percentage hydrolysis, irrespective of the velocity of conversion, indicates that the enzyme is not destroyed at the temperatures used in this investigation. On the other hand, while it must be said that the experimental error toward the end of the reaction is larger than at the beginning, it is felt that a much larger error than was actually present would have to be assumed in order to change the general trend of the curves. The most striking part of the curves, however, is the definiteness with which the unimolecular constant decreases toward the end of the reaction. It is for this last part of the reaction that equation 4 was derived to fit Due to the fact that only a few points could ordinarily be obtained within this range of hydrolysis, only a few constants could be calculated.

TABLE II

RESULTS FOR 25° AND *P*H 4.7

Run 12: 0.5 cc. enzyme per 400 cc. 6% sucrose				Run 17: 2.0 cc. enzyme per 400 cc. 10% sucrose			
Time, min.	$a - x$ °Rot.	$k_m = 1/t \log \frac{a}{a-x}$	$k = k_m - \frac{0.05x}{t}$	Time, min.	$a - x$ °Rot.	$k_m = 1/t \log \frac{a}{a-x}$	$k = k_m - \frac{0.069x}{t}$
0	9.79	.....	.....	0	16.21	.....	.....
15	8.95	0.00260	.....	60	5.19	0.00859	.....
30	8.04	.00285	.....	120	1.08	.00980	.....
60	6.41	.00307	.....	140	0.66	.00993	0.00234
90	4.96	.00328	.....	170	.41	.00939	.00299
135	3.21	.00356	.....	210	.27	.00847	.00324
180	2.00	.00383	.....	270	.17	.00733	.00323
220	1.30	.00399	.....				
250	0.95	.00405	.....				
280	.70	.00409	0.00247				
310	.54	.00406	.00257				
350	.42	.00391	.00257				
410	.29	.00373	.00257				

TABLE III

RESULTS FOR 35°

Run 24: *P*H 7.4; 4.0 cc. enzyme per 400 cc. 6% sucrose				Run 25: *P*H 4.7; 1.0 cc. enzyme per 400 cc. 6% sucrose			
Time, min.	$a - x$ °Rot.	$k_m = 1/t \log \frac{a}{a-x}$	$k = k_m - \frac{0.033x}{t}$	Time, min.	$a - x$ °Rot.	$k_m = 1/t \log \frac{a}{a-x}$	$k = k_m - \frac{0.089x}{t}$
0	9.73	.....	.....	0	9.85	.....	.....
15	8.05	0.00549	.....	10	7.99	0.00909	.....
30	6.54	.00575	.....	21	6.10	.00991	.....
60	4.28	.00594	.....	40	3.63	.01084	.....
80	3.22	.00600	.....	60	1.95	.01172	.....
100	2.43	.00603	.....	75	1.21	.01214	.....
120	1.90	.00591	0.00376	90	0.81	.01205	0.00310
140	1.50	.00578	.00384	110	.49	.01185	.00428
170	1.07	.00564	.00396	140	.35	.01035	.00431
200	0.80	.00543	.00396	180	.23	.00906	.00431
240	.60	.00504	.00378				

However, the values in Tables II, III and IV show that equation 4 holds remarkably well.

Summary of Results.—In the preceding sections, it has been pointed out that the velocity of hydrolysis is constant for about the first 10% hydrolysis, but later decreases; that the unimolecular constant increases to a maximum and then decreases as the reaction progresses; that this maximum value changes in a regular manner with P_H, temperature, and initial concentration of sucrose, but not with the amount of enzyme present; and, finally, the various equations which were derived in the theoretical introduction have been applied to the data. As a final summary of the results, however, Table IV shows how those equations hold for a single hydrolysis. It will be noticed that equation 7 holds fairly well over most of the reaction, that equation 4 holds over the last part of the reaction, and that equation 5 holds well over the first half of the reaction. In other words, each equation holds over that part of the reaction which it is theoretically supposed to fit.

TABLE IV

RUN 2 AT 25° AND P_H 4.7: 0.5 CC. OF ENZYME PER 400 CC. OF 6% SUCROSE

Time, min.	$a-x$ °Rot.	% Hydro	$k_m = 1/t \log \frac{a}{a-x}$	$k = \frac{1}{t_2-t_1} \log \frac{a-x_1}{a-x_2}$	$k = \frac{x}{t} + \frac{0.0048x^2}{t}$	$k = k_m + \frac{0.035x}{t}$	$k = k_m - \frac{0.0095x}{t}$
0	9.82	0.0	.....	.....	....	.....	.....
5	9.46	3.7	0.00324	0.00324	0.0732	0.00576	.....
15	8.76	11.8	.00331	.00337	.0743	.00579	.....
30	7.78	20.8	.00337	.00333	.0747	.00575	.....
40	7.23	26.4	.00343	.00318	.0729	.00570	.....
60	6.08	38.1	.00347	.00376	.0735	.00565	.....
90	4.55	53.4	.00371	.00419	.0734	.00576	.....
120	3.33	66.1	.00391	.00452	.0710	.00580	.....
180	1.67	83.0	.00427	.00483	.0656	.00586	.....
240	0.86	91.4	.00441	.00464	....	.00572	0.00406
270	.63	93.5	.00438	.00451	....	.00561	.00405
300	.48	95.2	.00437	.00393	....	.00546	.00407
330	.38	96.2	.00428	.00338	....	.00528	.00401

General Discussion

The Role of Water.—The foregoing results are in agreement with the recognized fact that the initial velocity of hydrolysis increases to a maximum as the initial sucrose concentration is increased to about 6%, and declines as the sucrose concentration is increased above that amount. Nelson and Schubert[12] showed that the velocity of hydrolysis for strong sucrose solutions decreased as the water concentration decreased, and stated "that the concentration of water is a factor in determining the magnitude of hydrolysis of sucrose by invertase," but they did not show how the water is a determining factor. While Henri[13] regarded the enzyme as containing

(12) Nelson and Schubert, THIS JOURNAL, **50**, 2188 (1928).

(13) Henri, *Z. physik. Chem.*, **51**, 27 (1905).

some of the water, it seems that water, as a controlling factor in determining the velocity of hydrolysis, has been rather generally neglected. Moreover, following the views of Michaelis and Menten,[4] it has become customary to explain the increase in initial velocity up to about 6% sucrose concentration as due to increasing amounts of sucrose combined with the enzyme. On the other hand, investigators seem to have forgotten the fact that the initial velocity during this range of sucrose concentration is zero order, and that the velocities can only be of that order when the enzyme is saturated with respect to sucrose, which, according to the views of Michaelis and Menten, is the case only at considerably higher sucrose concentrations.

From the chemical adsorption view, which requires that both the water and the sucrose must be adsorbed before the enzyme can influence the velocity of the combination, water occupies a special position. Except in solutions with high sucrose concentrations, water is always present in large excess. Consequently, the amount of water used up in a given reaction is negligible, and, hence, the amount of water adsorbed by the enzvme remains essentially constant. Therefore, for a given hydrolysis, water plays no important part in the manner in which the velocity changes as the hydrolysis proceeds. On the other hand, since the amount of water present does vary from one initial sucrose concentration to another, water does play an important part in determining the velocity with which the hydrolysis will go at different initial sucrose concentrations.

If it be assumed that water is adsorbed in preference to sucrose, the facts can be explained in this manner. Water molecules will then be adsorbed and will cover the surface of the invertase according to their concentration; and sucrose molecules will saturate, even at quite low sucrose concentrations, that part of the surface which is not covered by water. At low (less than 6%) initial sucrose concentrations, where the water concentration is high, more water is adsorbed than sucrose; but at high (greater than 6%) initial sucrose concentrations, where the water concentration is lower, more sucrose and less water are adsorbed. The maximum velocity would occur at an optimum ratio of adsorbed sucrose to adsorbed water, and the velocity should fall off from this value for both higher and lower initial sucrose concentrations. Furthermore, on the basis of such a mechanism, it is possible for the amount of adsorbed sucrose to reach a saturation value for practically any initial sucrose concentration, and, hence, give a constant rate at the beginning of each hydrolysis.

Inhibition.—The manner in which invert sugar inhibits the hydrolysis deserves special mention. It has been pointed out that the reaction proceeds as a zero order reaction for about the first 10% of hydrolysis. This indicates that the products of the reaction are not adsorbed by the invertase and do not inhibit during that time, for they could only inhibit the reaction by occupying some of the enzyme surface which would ordi-

narily be active. At least three factors contribute to this delay in the inhibition of the reaction: first, the mutarotated or β-forms of the invert sugar inhibit to a greater degree than the α-forms;[14] second, the slow rate of transformation of the α-form to the β-form produces at a given time a smaller quantity of the inhibiting substances than the equilibrium amount;[15] third, the inhibiting substances may not only be slowly adsorbed, but there may be an induction period before adsorption occurs.[16] All of these factors would be more noticeable at the beginning of the reaction, because there the velocity of the hydrolysis reaction is at its maximum, mutarotational velocities are slowest, and the products (in the presence of large quantities of sucrose) would have more difficulty in being adsorbed by the invertase.

On the other hand, it must not be forgotten that these factors should also be considered during the entire reaction in determining the correct concentration of products which are responsible for the inhibiting action at a given time. In view of the many factors controlling the amounts of products which are adsorbed by the enzyme, it is somewhat surprising that equations 4 and 5 hold as well as they do. However, over a short portion of the hydrolysis, and especially toward the end of the reaction where the rate factors are favorable, the amounts of inhibiting substances which are active are probably more or less proportional to the total quantity of products present. While equation 7 does not fit over the entire reaction, it does express the course of the reaction remarkably well. It is quite possible that the Langmuir isotherm does not fit over the entire range, and, hence, is the root of the difficulty; but it is more probable that most of the trouble is due to an unaccountable variation in the inhibiting action of the various products. Moreover, it is absolutely necessary to know what the concentrations are of those products which inhibit at any given time during the hydrolysis in order to develop for the reaction a theoretical equation that will fit from the beginning to the end of the reaction.

Summary

1. Assuming that sucrose and water are adsorbed by invertase and that the seat of the reaction is in this adsorbed phase, a theoretical treatment of the kinetics of the reaction has been given from that point of view.

2. It has been shown that the unimolecular constants increase at first as the reaction progresses, and then decrease toward the end of the reaction. The influence of the amount of enzyme, P_H, initial sucrose concentration, and temperature on this maximum is shown.

3. An explanation of the role water plays in influencing the initial velocity of hydrolysis has been advanced.

(14) For example, see Nelson and Bodansky, THIS JOURNAL, **47**, 1624 (1925).

(15) Compare with Pennycuick, *J. Chem. Soc.*, **125**, 2049 (1924).

(16) The recent results of Benton and White [THIS JOURNAL, **54**, 1820 (1932)] with hydrogen and iron indicate that an induction period is possible in the adsorption process.

4. The inhibiting action of the products and its importance in obtaining a general kinetic equation for the reaction has been discussed.

BELLEVUE HOSPITAL MEDICAL COLLEGE
NEW YORK CITY

RECEIVED AUGUST 8, 1932
PUBLISHED FEBRUARY 9, 1933

[CONTRIBUTED FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF NORTH CAROLINA]

The Debye-Hückel Ion Size Parameter in Terms of Individual Ionic Radii. The Activity Coefficient of Lead Chloride in Solutions of Cadmium Nitrate[1]

BY H. D. CROCKFORD AND HENRY C. THOMAS

In the past the ion size parameter of the Debye–Hückel theory of solutions of strong electrolytes has been determined as a mean value for the ions of a given solution. If the parameter "a" could be calculated from the individual ionic radii and the result of this calculation verified experimentally, the physical meaning of the quantity "a" would take on added significance. It is the purpose of this paper to present this calculation and to give a preliminary test of its result.

Theoretical Calculation.—Consider a solution containing ions of kinds

$$1 \ldots i \ldots s$$

Let a_{ij} be the mean distance of closest approach for the ions of the i^{th} and j^{th} kinds, *i. e.*, the mean collision distance between the centers of the ions considered as spheres. Now if f_{ij} is the time average, or frequency, of collisions between the ions of the i^{th} and j^{th} kinds, then

$$\text{"}a\text{"} = \Sigma f_{ij} a_{ij} / \Sigma f_{ij} \qquad (1)$$

is the mean distance of closest approach for all ions in the solution, the summations being taken over all combinations of values of i and j. On the average for a large number of random collisions between any two ions the distances from the points of contact to their centers may be considered as the radii of spheres surrounding the ions; so that

$$a_{ij} = a_i + a_j$$

The quantities a_i and a_j depend presumably only upon the nature of the individual ions.

The following calculation of the frequency of collisions between the ions is based on the fundamental assumption of the Debye–Hückel theory: the effects between the ions may be attributed wholly to the electrical charges on them. Consider an ion of the i^{th} kind in a solution of

$$n_1 \ldots n_i \ldots n_s$$

moles of ions with valences

$$z_1 \ldots z_i \ldots z_s$$

(1) The material for this paper was taken from a thesis submitted by Henry C. Thomas to the Faculty of the University of North Carolina in partial fulfilment of the requirements for the degree of Master of Science.