THERMAL ANALYSIS OF ENZYME REACTIONS*.

Report I. Invertase Action.

By Takekazu Kôsaki.

The invertase action has long been studied by many investigators, as invertase is relatively stable and obtained in a large amount. Willstätter, Euler, Nelson and others have already reported various results of the investigation. All of them have employed the method of measuring the change of rotating power during the inversion or the reducing power of monoses formed for the observation of the reaction velocity. Though the dilatatometric method of measuring the volume increase of the reaction system during the inversion has been proposed, yet there is no report on the invertase action employing the method. In those methods, except the dilatatometric method, a part of the reaction system must be taken out each time to observe the reaction velocity, so it is difficult to make continued observation always under the same condition.

The writer attempted to study the invertase action employing the thermo-analytical method originated by Prof. Shinkichi Horiba[1]. He measured the reaction velocity, observing the rise of the temperature of the reaction system due to the inversion heat by means of a simple glass calorimeter[2]. This thermo-analytical method is expected to make up for the defects of the above mentioned methods. Moreover, he attempted to measure the inversion heat of sucrose accurately and to accomplish thermal analysis of various enzyme reactions, especially of those having no perfect method for its measurement, and so he selected at first invertase.

(I) Materials.

(1) The Invertase Solution. Yeast whose time value had been made relatively small by cultivation was for long hours left to stand without being diluted for neutral autolysis. The autolytic liquid thus obtained was purified twice by the alcohol precipitation method and then being adsorbed with non-treated raw kaolin it was desorbed with dilute ammonia. By such a simple procedure as this, considerably large quantity of relatively pure invertase solution

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* This paper is the English translation of the some article which appeared in this *Journal*, **9**, 151 (1934).

1) S. Horiba and T. Ichikawa, *Rev. Phys. Chem.*, 1, 145 (1927); 4, 1 (1930).
S. Horiba and H. Baba, *Rev. Phys. Chem.*, **6**, 47 (1932).

2) S. Horiba and K. Sato, *Rev. Phys. Chem.*, **6**, 16 (1932).

whose time value was 0.25 could be prepared.

(2) Sucrose. Sucrose was dissolved in water to remove impurities by filtration, and made syrupy by vacuum distillation. Alcohol being added to 60%, the precipitate formed was separated. Sucrose thus treated, being washed with alcohol for several times and then with ether, was completely dried. Repeating this treatment two days before the experiment, and, on the preceding day, ascertaining that the rotating power of the preparation was $[\alpha]_D^{20}=66.6°$ without any reducing power, it was used.

Method of Measurement.

The thermal analysis of a chemical reaction is a method to know the reaction velocity from the following equation by observing the temperature change of the reaction system $\frac{dT'}{dt}$ due to a chemical reaction:

$$\frac{dT}{dt}-\frac{dT'}{dt}=\frac{Q}{W}\frac{dx}{dt}. \qquad (1)$$

The cooling velocity $\frac{dT'}{dt}$, which is characteristic of the reaction vessel and is caused by the temperature difference between the reaction system and its surrounding medium, the water equivalent W of the system, including the reaction vessel, were measured and further the reaction heat Q was determined. $\frac{dT}{dt}$ of the liquid system can be measured directly by a simple glass calorimeter, different from that of the gaseous system. $\frac{dT'}{dt}$ can be known from the observation of $\frac{dT}{dt}$ in the case when $\frac{dx}{dt}=0$, i.e. the cooling curve. $\frac{dT'}{dt}$ obeys Newton's law as ascertained by Horiba and his co-workers and also by the present author:

$$-\frac{dT'}{dt}=k\Delta T, \qquad (2)$$

where k is a constant and ΔT is the temperature difference between the reaction system and its surrounding medium.

To determine W, $\frac{dT}{dt}$ was observed by giving constantly a certain quantity of heat q' per unit time to the reaction system with the electric current after the reaction, and W was calculated from the equation:

$$\frac{dT}{dt}+k\Delta T=\frac{q'}{W}. \qquad (3)$$

The result of this measurement was also compared with that obtained with water.

The reaction heat Q was determined directly as follows. Equation (1) can be written as

$$W\left(\frac{dT}{dt}+k\Delta T\right)=\frac{dQ}{dt}=q. \qquad (4)$$

If we find the relation between the time t and the quantity of heat produced by the reaction system per unit time $\frac{dQ}{dt}$ from this equation, and then calculate $\int_{t_0}^{t}dQ$ from the beginning of the reaction t_0 to its termination t by the graphical integration, Q can be obtained. In case the reaction is not complete, measure the amount of the substance which has reacted

during the time interval from t_0 to t_1 by the observation of the rotating power of the reaction system after t_1 from the beginning, and Q will be obtained.

For the observation of $\frac{dT'}{dt}$, which is the fundamental element of the present research, such a calorimeter as shown in Fig. 1. was employed.

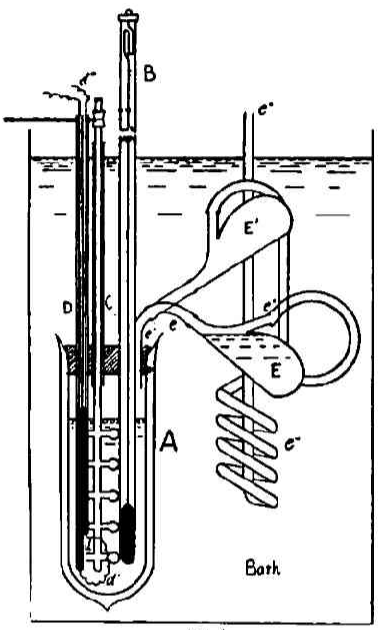

Fig. 1

A is a Dewar vessel about 17 cm. in depth, B a Beckmann thermometer, C a stirrer, D a heater, and the platinum wire d' (radius 0.1 mm., length 20.4 cm.) attached to the end of the glass tube d is connected with the conducting wire d'' by the intermediation of mercury in d. E is a glass vessel, which holds about 25 c.c. of water, and communicates with the Dewar vessel by the rubber tube e and the glass tube e' and also with open air by aid of e''. If E is brought to the position E', the contents of E can be removed into the Dewar vessel almost instantaneously. The whole apparatus is sunk in a thermostat as shown in the figure.

The Dewar vessel was filled with 100.0 c.c. of sucrose solution of a certain concentration prepared with the buffer solution and E also with 20.2 c.c. of a dilute enzyme solution. Leaving it for about 5 hours in the bath to let the temperature of the content of A become equal to that of the bath, the temperature change of the contents of A after the transfer of the enzyme solution into A was observed. The time elapsing from the transfer of the enzyme solution to the beginning of the elevation of the temperature was always 15 seconds in every experiment and the time from the closing or the cutting of the circuit in the case of electric heating to the beginning of the change of the temperature was also 15 seconds[3]. So the time when the temperature began to rise, namely 15 seconds after the transfer, was taken as the zero-point of the time of observation.

k and W' were measured after the observation of $\frac{dT'}{dt}$ at every time. For this purpose, an accumulator of a large capacity, an accurate ammeter and a rheostat were connected with D'' in series. The temperature change was observed by heating with a certain quantity of electricity per unit time and also after shutting off the electric current. The resistance of the platinum wire was measured preliminarily at 37.0°C. by means of Wheatstone's bridge. For the measurements of k and W', the reaction system was employed to know how they changed with the kind and concentration of substances in the reaction system and to compare them with those of the distilled water.

3) In this case an aqueous solution of methylene blue was transferred from E into A to observe the mixing rate and it was mixed almost instantaneously with the contents of A.

The results of the preliminary experiment show that the temperature and the hydrogen ion concentration suitable for the present enzyme preparation are 37.0°C. and $p_H=4.2$ respectively. Accordingly, the experiments were carried out in the following four groups.

1st group. A sucrose solution whose concentration was 15%; $p_H=4.2$; 37.0°C. The amount of the original enzyme solution was changed.

2nd group. 13.5 c.c. of the enzyme solution; $p_H=4.2$; 37.0°C. The concentration of the sucrose solution was changed.

3rd group. A sucrose solution whose concentration was 15.0%; 13.5 c.c. of enzyme solution; 37.0°C. p_H was changed.

4th group. A sucrose solution whose concentration was 15.0%; 13.5 c.c. of the enzyme solution; $p_H=4.2$. The temperature was changed.

For the buffer the following solutions were employed.

For $p_H = 2.9$ 4 c.c. of $\frac{N}{10}$ secondary sodium citrate solution. 6 c.c. of $\frac{N}{10}$ hydrochloric acid.

For $p_H = 4.2$ 8.28 c.c. of $\frac{N}{5}$ secondary sodium phosphate solution. 11.79 c.c. of $\frac{N}{10}$ citric acid.

For $p_H = 5.11$ 9 c.c. of $\frac{N}{10}$ secondary sodium citrate solution. 1 c.c. of $\frac{N}{10}$ caustic soda solution.

For $p_H = 6.2$ 50 c.c. of $\frac{N}{5}$ primary potassium phosphate solution. 8.6 c.c. of $\frac{N}{5}$ caustic soda solution.

For $p_H = 7.0$ 50 c.c. of $\frac{N}{5}$ primary potassium phosphate solution. 29.63 c.c. of $\frac{N}{5}$ caustic soda solution.

Experimental.

(1) The Reaction Heat.

(1) The cooling constant k and the water equivalent W obtained by employing 120.0 c.c. of distilled water are given in Table 1.

Table I.

Water 120.0 c.c. Temp. of Bath 37.0°C.

No. of Exp.	i (amp.)	q (cal.)	W mean	k mean
1	0.736	8.463	165.71	0.0197
2	0.714	7.950	165.72	0.0197
3	0.643	4.543	165.70	0.0197

k and W observed by employing the reaction system are given in the column "Blank Test" of Table 2 and almost no difference can be seen between the values of k and W in the reaction system and those in distilled water.

Table 2.

Quantity of Sucrose (g.)	Quantity of Enzyme Solution (c.c.)	Reaction (pH)	Temperature of Bath (°C)	Measurement of Reaction Heat			Blank Test	
				Inverted Sucrose (%)	Weight of Section Paper occupied by the $t-q$ curve (g.)	Reaction Heat (cal./g.mol.)	k	W (cal.)
15.0	5.0	4.2	37.0	100.0	0.3784	4.026	0.0197	165.71
,,	10.0	,,	,,	100.0	0.3850	4.090	0.0197	165.72
,,	13.5	,,	,,	100.0	0.3826	4.065	0.0196	165.73
,,	16.0	,,	,,	100.0	0.3830	4.069	0.0198	165.72
,,	18.0	,,	,,	100.0	0.3829	4.069	0.0198	165.72
,,	20.0	,,	,,	100.0	0.3710	4.054	0.0195	165.70
2.0	13.5	,,	,,	100.0	0.0503	4.015	0.0197	165.71
4.0	,,	,,	,,	100.0	0.1028	4.103	0.0197	165.68
5.0	,,	,,	,,	100.0	0.1243	3.965	0.0197	165.71
7.5	,,	,,	,,	100.0	0.1918	4.081	0.0196	165.72
10.0	,,	,,	,,	100.0	0.2576	4.111	0.0198	165.71
12.5	,,	,,	,,	100.0	0.3230	4.125	0.0199	165.71
20.0	,,	,,	,,	100.0	0.5132	4.092	0.0198	165.71
25.0	,,	,,	,,	95.8	0.6108	4.068	0.0198	165.70
30.0	,,	,,	,,	90.4	0.7015	4.072	0.0198	165.72
35.0	,,	,,	,,	97.2	0.8490	3.980	0.0197	165.71
15.0	13.5	7.0	37.0	17.07	0.0642	4.001	0.0197	165.73
,,	,,	6.2	,,	82.2	0.3100	4.008	0.0196	165.70
,,	,,	5.11	,,	100.0	0.3809	4.053	0.0195	165.70
,,	,,	2.9	,,	100.0	0.3818	4.062	0.0197	165.72
,,	,,	4.2	42.0	89.4	0.3334	3.965	0.0196	165.71
,,	,,	,,	38.0	100.0	0.3708	4.052	0.0197	165.71
,,	,,	,,	35.0 (I)	100.0	0.3901	4.150	0.0196	165.70
,,	,,	,,	35.0 (II)	97.0	0.3680	4.032	0.0196	165.71
,,	,,	,,	32.0	100.0	0.3919	4.170	0.0199	165.70
Mean						4.058	0.0197	165.71
Mean Square Error						±0.0515	±0.000108	±0.0108

(2) The Determination of the Reaction Heat.

Observing the $JT-t$ curve at various conditions, the relation between $\frac{dT}{dt}$ and t was measured and the value of $\frac{dQ}{dt}$ or q for each t calculated from equation (4), applying $k=0.0197$ and $W=165.71$. The results of this calculation are given in Tables 3, 4 and 5. From the $\frac{dQ}{dt}-t$ curve (this is corresponding to that of Fig. 2 whoseordinate $\frac{dx}{dt}$ was exchanged with $\frac{dQ}{dt}$, the unit of the ordinate

being cal./min. and that of the abscissa min.) Q was graphically obtained. The mean value of Q was 4.1 cal., which was in good agreement in every experiment. When the reaction had not terminated, the decomposition rate was calculated from the rotating power of the reaction system measured immediately after the interruption and then the reaction heat per 1 g. mol of sucrose—342.24 g. calculated. It is apparent from the above table that the determination of the reaction heat by the present method provides a sufficiently reliable result and accordingly that the thermal analysis of the reaction velocity based upon the $\frac{dQ}{dt}-t$ relation is reliable.

(3) Comparison of the Observed Value with that obtained thermochemically.

The inversion of sucrose proceeds according to the following equation:

$$\underset{\text{sucrose}}{C_{12}H_{22}O_{11}}+H_2O=\underset{\text{glucose}}{C_6H_{12}O_6}+\underset{\text{fructose}}{C_6H_{12}O_6}. \qquad (5)$$

Therefore the inversion heat can be calculated as follows:

$$\begin{aligned}
&(C_{12}H_{22}O_{11}\text{Aq.})=[C_{12}H_{22}O_{11}]-c \\
&[C_6H_{12}O_6]=(C_6H_{12}O_6\text{Aq.})+a \qquad \text{(glucose)} \\
&[C_6H_{12}O_6]=(C_6H_{12}O_6\text{Aq.})+b \qquad \text{(fructose)} \\
&24O_2+[C_{12}H_{22}O_{11}]=12O_2+11(H_2O)+x \\
&6CO_2+6(H_2O)=[C_6H_{12}O_6]+12O_2-y \qquad \text{(glucose)} \\
+\quad &6CO_2+6(H_2O)=[C_6H_{12}O_6]+12O_2-z \qquad \text{(fructose)} \\
\hline
&(C_{12}H_{22}O_{11}\text{Aq.})+(H_2O)=2(C_6H_{12}O_6\text{Aq.})+a+b-c+x-y-z. \qquad (6)
\end{aligned}$$

Substituting the following values into equation (6), we can obtain 4.1 cal. per 1 g. mol for Q.

$a=-0.9$ cal.[4]	$x=1353.5$ cal.[5]
$b=-2.3$ cal.[4]	$y=674.0$ cal.[5]
$c=-2.3$ cal.[4]	$z=671.6$ cal.[5]

But the results of the combustion heat measurement by various investigators do not coincide with respect to the numbers smaller than the first decimals of the large calorie value. Therefore the value of Q fluctuates from 2.0 cal. to 10.0 cal., if it is calculated by employing various values. On the other hand, calculating from the heats of the formation of $C_{12}H_{22}O_{11}$Aq., H_2O, $C_6H_{12}O_6$Aq. glucose and

4) *Table annuelles internat.*, 4 (1922).
5) Landolt, *Physik. Chem. Tab.*, (1923).

$C_6H_{12}O_6$Aq. fructose, namely 534.8 cal., 69.0 cal., 300.4 cal. and 300.4 cal.[6] respectively, a value—3.0 cal. per 1 g. mol for Q—can be obtained. As the above values are all within the limit of the measurement error they are not all accurate, and only indicate a rough standard. The value obtained by the present research lies within the limit of this rough standard indicated by the value calculated from the combustion heat, and so the present value, namely Q for 1 g. mol=4.1 cal., is certainly a reliable one, and consequently it seems to ensure again the applicability of the present analytical method.

(II) Thermal Analysis of the Invertase Action.

Observing $t-\Delta T$ in a number of experiments performed under the above-mentioned conditions to obtain $\frac{dT}{dt}$, q was calculated, and then dividing it by Q per 1 mg. mol=4.1 cal., $\frac{dx}{dt}$ was calculated. Further, in the present research the relation $x-t$ was unknown and the relation $\frac{dx}{dt}-t$ or $q-t$ was known and so from this relation k_m was calculated as follows.

$$\frac{dx}{dt}=k_m(a-x) \tag{7}$$

Integrating this we have

$$k_m t=\ln\frac{a}{a-x}.$$

Then
$$a-x=ae^{-k_m t} \tag{8}$$

Substituting (8) in (7), we have

$$\frac{dx}{dt}=ak_m e^{-k_m t}. \tag{9}$$

Putting the values of $\frac{dx}{dt}$ at t_1 and t_2 as $\left(\frac{dx}{dt}\right)_{t_1}$ and $\left(\frac{dx}{dt}\right)_{t_2}$ respectively, we have

$$\ln\left(\frac{dx}{dt}\right)_{t_1}-\ln\left(\frac{dx}{dt}\right)_{t_2}=k_m(t_2-t_1)$$

$$\therefore\quad k_m=\frac{\log\left\{\left(\frac{dx}{dt}\right)_{t_1}\Big/\left(\frac{dx}{dt}\right)_{t_2}\right\}}{0.4343\,(t_2-t_1)}$$

$$=\frac{\log\,(q_{t_1}/q_{t_2})}{0.4343\,(t_2-t_1)}, \tag{10}$$

6) Berthelot, *Thermochimie*, 2, 696 & 796 (1897).

where q_{t_1} and q_{t_2} are the values of q or $\frac{dQ}{dt}$ at t_1 and t_2 respectively. Using this equation, k_m can be easily calculated from the relation $q-t$.

In this calculation the value of k_m fluctuated when both t_1 and t_2 were freely taken, and so it was calculated, taking only t_1 to be a certain constant value. From the relation between $\frac{dx}{dt}$ and t and that between $\frac{dx}{dt}$ and k_m the reaction was analysed as will be seen later.

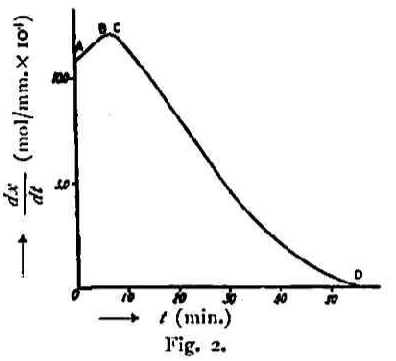

Fig. 2.

As is seen from the following tables and figures the results obtained in the present experiments show that the sucrose inversion by invertase takes a type quite different from that of the first order reaction in its earlier stage and similar to it in its later stage—the reaction consists of three parts, AB, BC and CD, as is shown in Fig. 2.

Since the BC part seems to be the transition part from AB to CD as will be also mentioned later, the velocity formulae of only the two parts, AB and CD, and the influence of various conditions on these parts will be considered.

(1) The AB-Part.

The relation between $\frac{dx}{dt}$ and t is linear as is seen in Figs. 6, 9, 12 and 15, and the velocity equation was derived as follows.

Let a' and b represent AO and $\tan\alpha$ respectively in Fig. 3, and the equation of the straight line AB is expressed by

$$\frac{dx}{dt}=bt+a'. \qquad (11)$$

Integrating this, we have

$$x=\frac{b}{2}t^2+a't+C.$$

where $C=0$, as $x=0$ when $t=0$.

Therefore $$x=\frac{b}{2}t^2+a't.$$

Hence $$t=\frac{-a'+\sqrt{a'^2+2bx}}{b}.$$ (The negative sign is unfit.)

Substituting this in (11),

$$\frac{dx}{dt}=\sqrt{a'^2+2bx}\,.$$

Putting $\sqrt{2b}\equiv k_1$ and $\frac{a'^2}{2b}\equiv a_1$,

$$\frac{dx}{dt}=k_1\sqrt{a_1+x}\,. \qquad (12)$$

From this equation a and b were calculated under various conditions, and then k_1 and a_1 obtained.

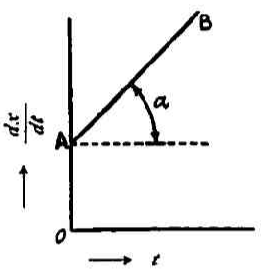

Fig. 3.

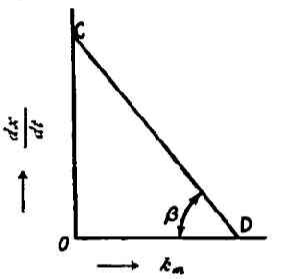

Fig. 4.

(2) The CD-Part.

k_m is almost constant, but, strictly speaking, it is not. The relation between $\frac{dx}{dt}$ and k_m is linear as seen in Figs. 7, 10, 13 and 16, and so the velocity equation was derived as follows.

Let b_1 and c represent OD and cot β respectively in Fig. 4. and the straight line CD is expressed by

$$c\frac{dx}{dt}+k_m=b_1\,. \qquad (13)$$

And $$k_m=\frac{dx}{dt}\cdot\frac{1}{a-x}\,.$$

Therefore, $$\frac{dx}{dt}=b_1\cdot\frac{a-x}{ac+1-cx}=b_1\cdot\frac{a-x}{1+c(a-x)}\,. \qquad (14)$$

If $ac+1$ is replaced by a_2, then

$$\frac{dx}{dt}=b_1\cdot\frac{1}{a_2-cx}\cdot(a-x)\,. \qquad (14)'$$

b_1 and c were obtained and then a_2 was calculated.

(2) Amount of Enzyme and Reaction Velocity.

As for pepsin, Schütz[7] reported that the following relation held between the

7) E. Schütz, *Z. physiol. C.*, 9, 577 (1885).

amount of enzyme and the reaction velocity:

$$x = k \cdot t \cdot \sqrt{F}, \qquad (15)$$

where k is a constant, F the amount of enzyme, x the amount of the split protein for the time t. As for invertase, the results of investigators[8] are in agreement, showing the amount of the split sucrose for a given time is directly proportional to the amount of enzyme:

$$x = k \cdot F \cdot t. \qquad (16)$$

Then, let v be the initial velocity, and $v = k \cdot F$.

Further, it was admitted that this relation was justified by the assumption that the invertase action was a homogeneous reaction, whose velocity is proportional to the product of the concentrations of enzyme and sucrose. Haldane[9] considers that the enzyme reaction is by no means a reaction in the homogeneous system. Therefore, it is interesting to see the influence of the amount of enzyme on the rate of this reaction which seems to be consecutive.

Experimental Results. Experiments were carried out with 5.0, 10.0, 13.5,

Table 3.

Enzyme 5 c.c.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.01242	2.058	5.0		40.0	0.363	0.00370	1.797	5.4	0.01231
1.0	0.012	0.01242	2.096	5.1	−0.02018	50.0	0.393	0.00206	1.623	3.9	0.01162
2.0	0.025	0.01242	2.139	5.2	−0.02018	60.0	0.406	0.00060	1.423	3.5	0.01206
3.0	0.037	0.01242	2.177	5.3	−0.02018	70.0	0.405	0.00077	1.193	2.9	0.01309
4.0	0.050	0.01242	2.220	5.4	−0.02018	80.0	0.390	0.00195	0.949	2.3	0.01464
5.0	0.062	0.01242	2.260	5.5	−0.02018	90.0	0.367	0.00263	0.760	1.8	0.01572
7.0	0.087	0.01242	2.341	5.7	−0.02039	100.0	0.338	0.00301	0.603	1.4	0.01659
9.0	0.110	0.01212	2.366	5.8	−0.01008	110.0	0.307	0.00312	0.483	1.1	0.01721
12.5	0.151	0.01141	2.382	5.8	−0.00285	120.0	0.275	0.00306	0.389	0.9	0.01765
15.0	0.179	0.01084	2.379	5.8	0.00039	130.0	0.246	0.00417	0.111	0.26	0.01742
17.5	0.207	0.01030	2.381	5.8	−0.00400	140.0	0.219	0.00389	0.069	0.16	0.01748
20.0	0.230	0.00942	2.311	5.6	0.01180	150.0	0.195	0.00375	0.015	0.03	0.01738
25.0	0.274	0.00791	2.203	5.4	0.01024	160.0	0.174	0.00323	0.002	0.005	0.01768
30.0	0.312	0.00658	2.107	5.1	0.00976						

8) C. O'Sullivan & F. W. Tompson, *J. C. Soc. London*, 57, 834 & 848 (1890).
C. S. Hudson, *Am. J. C. S.*, 30, 1564 & 1575 (1908).
H. v. Euler & O. Svanberg, *Z. physiol. C.*, 107, 269 & 275 (1919).
R. Willstätter, J. Graser & R. Kuhn, *Z. physiol. C.*, 123, 72 (1922).

9) J. B. S. Haldane, *Enzymes* (1930).

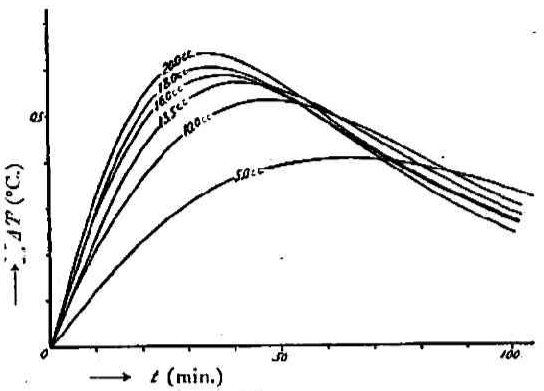

Fig. 5.

18.0, and 20.0 c.c. of the enzyme solutions whose concentration was 15%, $p_H=4.2$, at 37.0°C., and the results obtained are shown in Fig. 5. The observed values are tabulated in Tables 3, 4 and 5. a' was calculated from the value of $\frac{dx}{dt}$ at $t=0$ and b also from the $\frac{dx}{dt}-t$ curve in Fig. 6, and hence k_1 and a_1; b_1, c and a_2 were calculated from the $\frac{dx}{dt}-k_m$ curve in Fig. 7. These values are given in Table 6.

Table 4.

Enzyme 13.5 c.c.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02473	4.098	10.0		40.0	0.568	0.00083	1.990	4.8	0.02548
2.0	0.05	0.02473	4.260	10.4	−0.02039	50.0	0.551	0.00319	1.269	3.1	0.03015
4.0	0.10	0.02473	4.424	10.8	−0.02039	60.0	0.501	0.00584	0.666	1.6	0.03676
6.0	0.15	0.02410	4.482	10.9	−0.00501	70.0	0.448	0.00589	0.485	1.1	0.03593
8.2	0.20	0.02316	4.480	10.9	0	80.0	0.387	0.00603	0.263	0.6	0.03945
10.3	0.25	0.02169	4.409	10.7	0.00471	90.0	0.332	0.00616	0.062	0.13	0.03950
15.3	0.35	0.01838	4.187	10.2	0.00819	100.0	0.284	0.00535	0.040	0.08	0.03962
21.6	0.45	0.01290	3.605	8.8	0.001604	110.0	0.244	0.00451	0.004	0.01	0.03980
30.0	0.532	0.00703	2.901	7.1	0.01979						

Table 5.

Enzyme 20 c.c.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.03551	5.884	14.4		40.0	0.628	0.00422	1.350	3.3	0.04290
2.0	0.070	0.03551	6.111	14.9	−0.02039	44.0	0.607	0.00554	1.062	2.5	0.04461
4.0	0.140	0.03551	6.340	15.5	−0.02039	48.0	0.583	0.00644	0.835	2.0	0.04604
8.0	0.273	0.02728	5.410	13.2	0.03922	52.0	0.558	0.00656	0.734	1.7	0.04698
12.0	0.383	0.02467	5.337	13.0	0.02067	56.0	0.530	0.00712	0.550	1.3	0.05115
16.0	0.470	0.01978	4.810	11.7	0.02249	60.0	0.500	0.00767	0.361	0.8	0.05105
20.0	0.540	0.01495	4.238	10.3	0.02490	64.0	0.470	0.00828	0.165	0.4	0.05201
24.0	0.590	0.00935	3.474	8.5	0.02992	68.0	0.442	0.00818	0.086	0.2	0.05211
28.0	0.620	0.00558	2.947	7.2	0.03188	72.0	0.415	0.00795	0.022	0.05	0.05125
32.0	0.635	0.00196	2.396	5.8	0.03464	76.0	0.392	0.00768	0.003	0.008	0.05150
36.0	0.637	0.00041	2.010	4.9	0.03584						

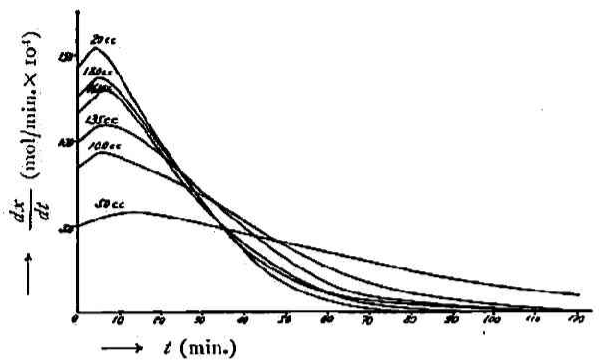

Fig. 6.

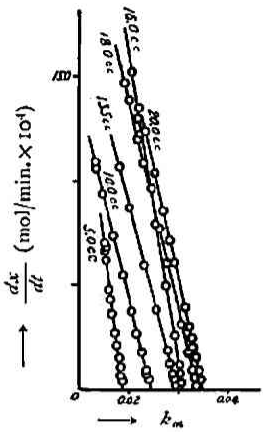

Fig. 7.

Table 6.

Enzyme	$a'(\times 10^4)$	$k(\times 10^6)$	$k_1(\times 10^3)$	$a_1(\times 10^2)$	b_1	c	a_2
5.0 c.c.	5.0	1.00	1.41	1.24	0.018	1.12	1.0448
10.0 „	8.5	1.87	1.93	1.92	0.029	2.00	1.0800
13.5 „	10.0	2.03	2.01	2.44	0.040	2.08	1.0832
16.0 „	11.7	2.63	2.29	2.60	0.042	1.36	1.0544
18.0 „	12.6	2.63	2.29	3.00	0.048	2.00	1.0872
20.0 „	14.4	2.80	2.36	3.68	0.050	1.02	1.0768

As for the AB part, k_1 could be regarded as almost constant, and had a tendency to increase in proportion to the increase of the enzyme amount ; a_1 had a similar tendency.

As for the CD part, b_1 increased almost in proportion to the amount of enzyme ; c showed a remarkable fluctuation ; a_2 was always constant regardless of the amount of enzyme.

Again it was found that b_1 and k_1 could be represented by $b_1'F$ and $k_1'F$ respectively in the case where b_1' and k_1' were constants indifferent to the amount of enzyme and F was the amount of enzyme. The fact that k_1 and b_1 corresponding to the velocity constants of the reaction can be represented by $k_1'F$ and $b_1'F$ is in agreement with the results obtained by various investigators though not discussed in the same breath.

The duration of the AB part is about 4–5 minutes though a little longer in the case where the amount of enzyme is small. The larger the $\frac{dx}{dt}$ value of the AB part, the shorter the duration of the BC part is, and at last the BC part seems to be a transition point from the AB part to the CD.

(3) Concentration of Sucrose and Reaction Velocity.

Brown[10] reported that in the range between 5–40% the reaction velocity increased in proportion to the concentration of sucrose, namely, the velocity constant of the first order reaction could be always given, and accordingly the sucrose inversion by succharase satisfied what the first order reaction required. The results obtained by other investigators[11], however, proved that below 5% the reaction velocity increased till k_m=const., but not above 5% and also that in the range between 5–20% the velocity reached the maximum with 5% according to the expression $k_m \times c$=const. (c is the concentration denoted by %.) The latter fact can not be satisfactorily interpreted by the theory of the first order reaction already admitted. In order to know which of the two, sucrose or water, had the main influence upon the reaction velocity, Nelson and Ingersoll[12] changed the concentrations of sucrose and water in the reaction system by replacing part of them with alcohol which would not take part in the reaction and ascertained that the concentration of water higher than 5% mainly affected the reaction velocity. Here Nelson[13] suggests that the deviation of this enzyme reaction from the first order reaction, namely the existence of the maximum value with respect to the concentration of sucrose, may easily be explained by the assumption that it is a reaction of the heterogeneous system based upon the adsorption phenomenon, taking the concentration of water into consideration.

Experimental Results.

The results of the experiments in which the concentrations of the sucrose used were 2.0, 4.0, 5.0, 7.5, 10.0, 12.5, 15.0, 20.0, 25.0, 30.0 and 35.0%, are shown in Fig. 8 and Tables 7, 8 and 9. The $\frac{dx}{dt}-t$ curve and $\frac{dx}{dt}-k_m$ curve obtained from the values tabulated in these

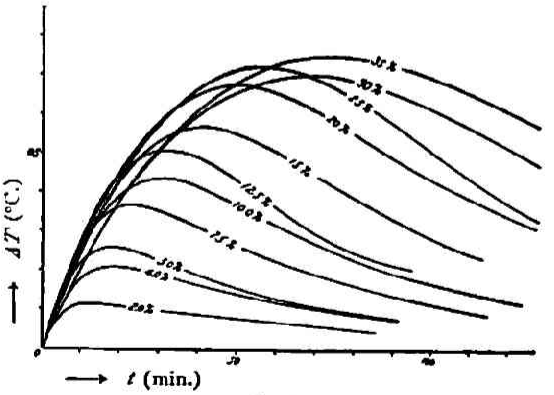

Fig. 8.

10) A. S. Brown, *J. C. Soc. London*, 81, 373 (1902).
11) L. Michaelis & M. L. Menten, *Bioch. Z.*, 49, 333 (1913).
J. M. Nelson & W. C. Vosburgh, *J. A. C. S.*, 39, 790 (1917).
L. Michaelis, *Biochem. Z.*, 115, 269 (1921).
12) J. M. Nelson & M. D. Schubert, *J. A. C. S.*, 50, 2188 (1925).
C. D. Ingersoll, *Bull. soc. chim. biol.*, 8, 264 & 276 (1926).
13) J. M. Nelson, *Chem. Rev.*, 12, 1 (1933).

tables are shown in Figs. 9 and 10, and the velocity constants k_1, a_1, b_1, c and a_1 are given in Table 10.

Table 7.

Sucrose 2%.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02055	3.405	8.3		10.0	0.115	0.00205	0.714	1.7	0.17538
0.5	0.01	0.02055	3.436	8.4	−0.04037	12.5	0.117	0.00011	0.362	0.8	0.19642
1.0	0.02	0.02055	3.469	8.5	−0.04037	15.0	0.115	0.00100	0.208	0.5	0.20091
2.0	0.04	0.01758	3.042	7.4	0.13098	20.0	0.110	0.00167	0.080	0.15	0.16185
3.0	0.057	0.01593	2.825	6.9	0.09940	30.0	0.101	0.00187	0.019	0.04	0.09974
5.0	0.083	0.01088	2.073	5.1	0.12817	40.0	0.093	0.00183	0	0	0.07776
7.5	0.105	0.00640	1.401	3.4	0.13906						

Tabla 8.

Sucrose 25%.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02420	4.010	9.8		32.0	0.60	0.01277	3.711	9.0	0.00410
2.2	0.05	0.02420	4.172	10.2	−0.01853	37.3	0.65	0.00798	3.443	8.4	0.00449
4.0	0.10	0.02420	4.336	10.6	−0.02267	45.0	0.70	0.00470	3.063	7.4	0.00508
6.1	0.15	0.02420	4.499	11.0	−0.01942	55.0	0.725	0.00075	2.490	6.0	0.00597
8.1	0.20	0.02363	4.568	11.1	−0.00501	65.0	0.715	0.00280	1.869	4.5	0.00723
10.3	0.25	0.02188	4.441	10.8	0.00897	75.0	0.675	0.00450	1.456	3.5	0.00781
12.7	0.30	0.02110	4.475	10.9	−0.00416	85.0	0.623	0.00626	0.995	2.4	0.00902
15.1	0.35	0.01946	4.366	10.6	0.00358	95.0	0.535	0.00706	0.641	1.5	0.01025
17.8	0.40	0.01754	4.212	10.3	0.00496	105.0	0.482	0.00726	0.379	0.9	0.01161
20.8	0.45	0.01578	4.083	9.9	0.00462	115.0	0.412	0.00667	0.238	0.5	0.01245
24.0	0.50	0.01428	3.998	9.7	0.00401	125.0	0.347	0.00621	0.102	0.2	0.01462
27.0	0.55	0.01277	3.910	9.5	0.00379						

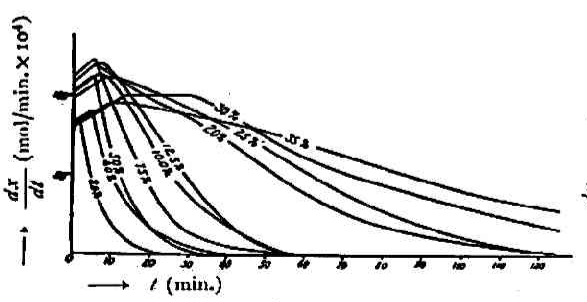

Fig. 9.

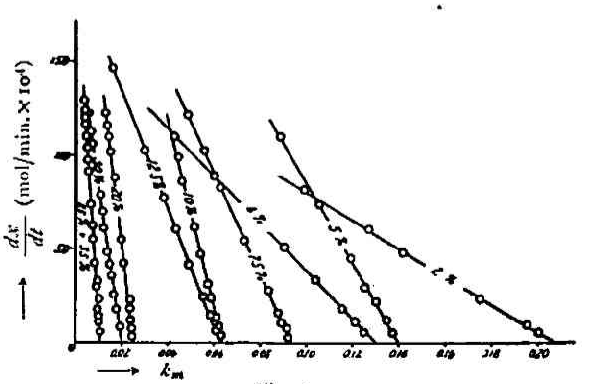

Fig. 10.

Table 9.

Sucrose 35%.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02000	3.314	8.1		85.0	0.737	0.00170	2.132	5.2	0.00833
1.25	0.025	0.02000	3.395	8.3	−0.02435	95.0	0.712	0.00311	1.807	4.4	0.00932
2.50	0.050	0.02000	3.476	8.5	−0.01217	105.0	0.675	0.00387	1.560	3.8	0.00994
3.75	0.075	0.02000	3.557	8.7	−0.02435	115.0	0.634	0.00424	1.365	3.3	0.01026
5.00	0.100	0.02000	3.640	8.9	−0.02435	125.0	0.589	0.00452	1.173	2.8	0.01070
6.25	0.125	0.02000	3.721	9.1	−0.02435	135.0	0.542	0.00490	0.956	2.3	0.01148
7.50	0.150	0.02000	3.803	9.3	−0.01217	145.0	0.594	0.00468	0.836	2.0	0.01162
10.0	0.200	0.01971	3.919	9.6	−0.01632	155.0	0.450	0.00434	0.749	1.8	0.01160
12.5	0.250	0.01825	3.839	9.4	0.00706	165.0	0.407	0.00421	0.629	1.5	0.01197
15.3	0.300	0.01694	3.786	9.3	0.00393	175.0	0.365	0.00395	0.536	1.3	0.01222
20.0	0.375	0.01486	3.685	9.0	0.00465	185.0	0.328	0.00347	0.495	1.2	0.01197
25.0	0.444	0.01302	3.605	8.8	0.00437	195.0	0.295	0.00312	0.445	1.0	0.01190
30.0	0.506	0.01146	3.549	8.7	0.00421	205.0	0.265	0.00292	0.381	0.9	0.01208
35.0	0.560	0.01013	3.506	8.6	0.00377	215.0	0.237	0.00284	0.301	0.7	0.01266
45.0	0.646	0.00708	3.281	8.0	0.00534	225.0	0.210	0.00268	0.240	0.5	0.01312
55.0	0.705	0.00452	3.049	7.5	0.00580	235.0	0.183	0.00257	0.170	0.4	0.01408
65.0	0.735	0.00194	2.719	6.7	0.00692	250.0	0.146	0.00244	0.071	0.2	0.01687
75.0	0.745	0.00008	2.444	6.0	0.00750						

Table 10.

Sucrose	$a'(\times 10^4)$	$k(\times 10^6)$	$k_1(\times 10^2)$	$a_1(\times 10^2)$	b_1	c	a_2
2.0%	8.3	1.60	1.79	2.16	0.207	13.20	1.0792
4.0 ,,	8.3	1.60	1.79	2.16	0.130	7.76	1.0222
5.0 ,,	10.3	2.03	2.01	2.60	0.140	4.64	1.0713
7.5 ,,	11.3	2.03	2.01	3.12	0.094	3.68	1.0832
10.0 ,,	10.2	2.03	2.01	2.56	0.064	2.00	1.0600
12.5 ,,	10.9	2.03	2.01	3.48	0.063	3.12	1.1168
15.0 ,,	10.0	2.03	2.01	2.44	0.040	2.08	1.0832
20.0 ,,	10.8	2.03	2.01	3.44	0.025	1.04	1.0624
25.0 ,,	9.8	1.77	1.88	2.72	0.011	0.32	1.0240
30.0 ,,	8.0	1.60	1.79	2.00	0.020	1.20	1.1080
35.0 ,,	8.1	1.60	1.79	2.04	0.011	0.32	1.0316

As for the AB part, k_1 increased a little below 5%, took quite a constant value for 5–20%, and decreased slightly for more than 20%. Generally it seems to be indifferent to the concentration of sucrose. a_1 increased a little in proportion to the concentration of sucrose below 7.5%, and as a whole it tended to decrease though fluctuating below 20.0%. Above 20%, it showed a tendency similar to k_1. k_1 had almost no relation to the concentration of sucrose, and a_1

was influenced by the amount of sucrose, decreasing as the concentration of sucrose was increased except in the case of low concentration. Therefore, as for this part it was found that the results obtained was not in agreement with those of other investigators.

As for the *CD* part, both b_1 and c remarkably decreased with the increase of the amount of sucrose within the limit of 7.5%, and became constant beyond the limit. a_2 was also constant. In short, b_1 and a_2 are the amounts indifferent to the concentration of sucrose; a_1 decreased with the increase of the concentration except in the range between 2.0–7.5% ; b_1 and c can be regarded as constants indifferent to the concentration of sucrose except in the case of low concentration.

The duration of the *AB* part was about 2–12 minutes and the lower the initial concentration of sucrose, the shorter it was. The lower the concentration of sucrose, the shorter the duration of the *BC* part, and for lower concentrations the *BC* part was not observed.

(4) Hydrogen Ion Concentration and Reaction Velocity.

The influence of the hydrogen ion concentration upon the invertase action has been investigated at 52.5°C. by Sørensen[14] and at 18.0°C. by Michaelis[15]. The results obtained by the former can be unreliable, because invertase must have been destroyed at such a high temperature as 52.5°C. According to the latter, the relation between the hydrogen ion concentration and the invertase activity can be denoted by a curve just like the dissociation curve having its maximum value at $p_H=4.5$, and so invertase is an amphoteric electrolyte, whose isoelectric point is $p_H=4.5$, and the non-dissociated part acts as a catalyser for the inversion of sucrose. Further, judging from the fact that invertase would be destroyed in the medium whose p_H value is smaller that 4.0. Nelson and Bloomfield[16] concluded that the isoelectric point is not necessarily the optimum point ofthe invertase action as has been stated by Michaelis. There is, however, no satisfactory theory to take the place of that of Michaelis.

Experimental Results.

The results of experiments at which p_H was 2.9, 4.2, 5.11, 6.2, and 7.0 are shown in Fig. 11 and Tables 11 and 12. The $\frac{dx}{dt}-t$ curve and $\frac{dx}{dt}-k_m$ curve

14) S. P. L. Sørensen, *Bioch. Z.*, 21, 268 (1909).
15) L. Michaelis & H. Davidson, *Bioch. Z.*, 35, 386 (1911).
16) J. M. Nelson & G. Bloomfield, *J. A. C. S.*, 46, 1025 (1924).

obtained are shown in Figs. 12 and 13. The constants, k_1, a_1, b_1, c, and a_2, calculated similarly are tabulated in Table 13.

As for the AB part, though k_1 fluctuated a little at $p_H=2.9-5.11$, reaching the maximum at $p_H=4.2$, yet in general it seemed to be almost constant in this range. At the p_H value larger than 5.11, however, it markedly decreased. a_1 gradually increased below $p_H=6.2$, and suddenly decreased at $p_H=7.0$.

Table 11.

pH 7.0.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.00110	0.182	0.4		55.0	0.057	0.00072	0.304	0.7	0.00197
5.0	0.005	0.00110	0.197	0.4	−0.01666	65.0	0.064	0.00065	0.316	0.7	−0.00407
10.0	0.010	0.00110	0.213	0.5	−0.04461	75.0	0.070	0.00051	0.311	0.7	0.00098
15.0	0.017	0.00110	0.236	0.5	−0.02106	85.0	0.075	0.00028	0.289	0.7	0.00430
25.0	0.028	0.00110	0.273	0.6	−0.01862	100.0	0.076	0.00010	0.263	0.6	0.00520
35.0	0.039	0.00110	0.313	0.7	−0.10853	115.0	0.077	0.00008	0.263	0.6	0.00363
45.0	0.050	0.00091	0.313	0.7	0	130.0	0.078	0.00008	0.266	0.6	0.00253

Table 12.

pH 2.29.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02148	3.559	8.7		25.0	0.430	0.01028	3.171	7.8	0.01899
1.25	0.025	0.02148	3.640	8.9	−0.02435	30.0	0.493	0.00675	2.727	6.7	0.02189
2.30	0.050	0.02148	3.721	9.1	−0.02900	35.0	0.517	0.00329	2.232	5.5	0.02543
3.50	0.075	0.02148	3.803	9.3	−0.02536	40.0	0.527	0.00061	1.821	4.4	0.02803
5.0	0.108	0.02148	3.910	9.6	−0.02720	50.0	0.510	0.00360	1.036	2.5	0.03519
7.5	0.161	0.02148	4.084	10.0	−0.01632	60.0	0.465	0.00500	0.689	1.6	0.03630
10.0	0.215	0.02136	4.240	10.4	−0.01632	70.0	0.413	0.00546	0.442	1.0	0.03766
12.5	0.267	0.01926	4.061	10.0	0.01567	80.0	0.357	0.00634	0.114	0.25	0.04173
15.0	0.312	0.01735	3.892	9.5	0.01537	90.0	0.302	0.00588	0.010	0.02	0.04656
17.5	0.355	0.01560	3.743	9.2	0.01627	100.0	0.251	0.00494	0	0	0.04782
20.0	0.391	0.01323	3.468	8.5	0.01588						

Table 13.

pH	$a'(\times 10^4)$	$k(\times 10^6)$	$k_1(\times 10^3)$	$a_1(\times 10^2)$	b_1	c	a_2
7.0	0.4	0.03	0.21	0.32	0.007	2.08	1.0832
6.2	1.6	0.03	0.21	5.12	0.007	2.08	1.0832
5.11	9.5	1.65	1.82	2.72	0.040	2.08	1.0832
4.2	10.0	2.03	2.01	2.44	0.040	2.08	1.0832
2.9	8.7	1.87	1.93	2.00	0.040	2.08	1.0832

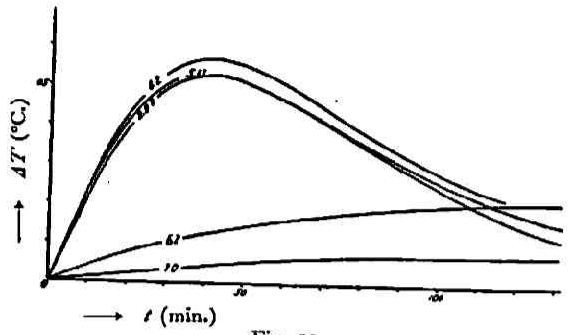

Fig. 11.

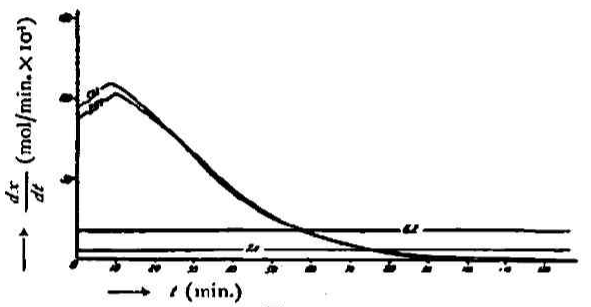

Fig. 12.

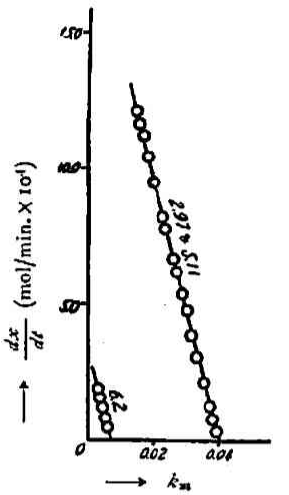

Fig. 13.

As for the *CD* part, b_1 showed a tendency similar to k_1: it was quite constant below $p_H=5.11$ and decreased remarkably. Both c and a_2 were indifferent to the p_H value of the medium. In other words, k_1 and b_1, which are to be regarded as the velocity constant of each part, showed a similar tendency: they were constant below $p_H=5.11$, being indifferent to the hydrogen ion concentration and decreased markedly at larger p_H values. This fact is a little different from the dissociation curve of Michaelis and others. It is evident from many reports concerning the preparation of invertase that the adsorption or the elusion of invertase on or from kaolin and other adsorbing substances is influenced by the p_H value of the medium and this influence is similar to that upon k_1 and b_1. Accordingly, the influence of p_H upon k_1 and b_1 may be easily explained if the inversion of sucrose due to invertase is assumed to be a reaction in the heterogeneous system based upon the adsorption phenomenon. a_2 and c were indifferent to the p_H value. a_1 fluctuated in the neighbourhood of a constant value.

The duration of the *AB* part was almost constant at $p_H=6.2-7.0$, where k_1 and b_1 were very small. The duration of the *BC* part was not observed at $p_H=2.9-5.11$; it was considerably long at $p_H=6.2-7.0$; and the *CD* part could not be found at $p_H=7.0$.

(5) Temperature and Reaction Velocity.

The influence of temperature upon the invertase action has been studied for a long time by various investigators. According to them, the equilibrium constant is slightly influenced by temperature on account of the smallness of the inversion heat as in the case of an acid catalysis. As for the reaction velocity, it has been admitted[17] that the reaction velocity has an optimum point with respect to temperature, because it increases with the rise of temperature according to van't Hoff's law and, on the other hand, it decreases with the rise of temperature on account of the heat inactivation of enzyme. As to the optimum point, however, any accurate value has not been obtained and yet it is considered to be about 55.0°C.[18] As the temperature coefficient, the value of 1.58[19] is given in the temperature range between 30.0°–40.0°C. It is reported that the value of the activation heat, E, calculated from Arrhenius' equation[20] is 8,800 cals. and is far less than that of the acid catalysis—25500 cals.

Experimental Results.

A number of experiments were carried out at 32.0°, 35.0°, 37.0, 38.0° and 42.0°C. Moreover, in order to see the change of the reaction velocity due to temperature under the same influence by the heat inactivation an experiment was carried out at 35.0°C., employing the material preliminarily kept at 42.0°C. for one hour, and the result of this experiment will be shown as "35.0°C. (II)". The results obtained are shown in Fig. 14, and some of the observed values are tabulated in Tables 14 and 15. The $\frac{dx}{dt}-t$ curve and $\frac{dx}{dt}-k_m$ curve are shown Figs. 15 and 16, and the velocity constants k_1, a_1, b_1, c and a_2 in Table 16.

As for the AB part, k_1 increased with the rise of temperature (till 37.0°C.), and decreased suddenly in the temperature range between 37.0° and 38.0°C. and then gradually decreased till 42.0°C. The heat inactivation began to come forth at 35.0°C. and it was affected most in the range between 37.0° and 38.0°C. and and then gradually increased. a_1 increased till 35.0°C. and decreased a little at 35.0°–37.0°C. and then gradually at 37.0°–42.0°C. On the whole it may be regarded as constant.

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17) W. M. Bayliss, *Nature of Enzyme Action*, 93 (1914).
18) J. B. S. Haldane, *Enzymes*, 65 (1930).
19) H. v. Euler & J. Laurin, *Z. Physiol. C.*, 108, 64 (1919).
20) Sv. Arrhenius, *Z. physik. C.*, 4, 226 (1889).

Table 14.

32.0°C.

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.02137	3.541	8.7		35.0	0.542	0.00550	2.679	6.6	0.01800
1.3	0.025	0.02137	3.622	8.9	−0.02341	40.0	0.561	0.00236	2.222	5.4	0.02138
2.6	0.050	0.02137	3.704	9.1	−0.02341	45.0	0.567	0.00004	1.856	4.5	0.02352
4.0	0.083	0.02137	3.811	9.3	−0.02175	50.0	0.563	0.00130	1.622	3.9	0.02387
5.0	0.110	0.02137	3.899	9.6	−0.04081	55.0	0.554	0.00230	1.426	3.5	0.02410
7.5	0.158	0.02137	4.057	10.0	−0.01632	65.0	0.517	0.00480	1.891	2.1	0.02826
10.0	0.212	0.02137	4.232	10.4	−0.01632	75.0	0.465	0.00540	0.623	1.5	0.03156
12.5	0.266	0.01992	4.169	10.2	0.00395	85.0	0.409	0.00554	0.415	1.0	0.03093
15.0	0.312	0.01746	3.911	9.6	0.01537	95.0	0.355	0.00521	0.286	0.7	0.03167
20.0	0.392	0.01434	3.656	9.0	0.01397	105.0	0.305	0.00537	0.104	0.25	0.03169
25.0	0.458	0.01135	3.375	8.3	0.01487	115.0	0.261	0.00495	0.032	0.06	0.03146
30.0	0.508	0.00786	2.959	7.2	0.01786	125.0	0.221	0.00429	0.009	0.02	0.03280

Table 15.

35.0°C. (II)

t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m	t (min.)	ΔT (°C.)	$\frac{dT}{dt}$ (°C./min.)	q (cal./min.)	$\frac{dx}{dt}$ (mol/min.)	k_m
0	0	0.00937	1.552	3.8		35.0	0.264	0.00477	1.652	4.0	0.00488
1.0	0.008	0.00937	1.577	3.8	−0.02018	40.0	0.287	0.00426	1.642	4.0	0.00386
2.0	0.017	0.00937	1.607	3.9	−0.03045	45.0	0.307	0.00366	1.607	3.9	0.00384
3.0	0.028	0.00937	1.643	4.0	−0.03045	50.0	0.324	0.00287	1.532	3.7	0.00522
4.0	0.037	0.00937	1.672	4.1	−0.03045	55.0	0.337	0.00215	1.454	3.5	0.00605
5.0	0.047	0.00937	1.705	4.2	−0.03045	60.0	0.346	0.00171	1.411	3.4	0.00589
7.5	0.070	0.00937	1.779	4.3	−0.01217	70.0	0.360	0.00092	1.327	3.2	0.00598
10.0	0.094	0.00906	1.807	4.4	−0.01217	80.0	0.365	0.00016	1.217	2.9	0.00649
12.5	0.116	0.00846	1.779	4.3	0.00791	90.0	0.363	0.00026	1.141	2.8	0.00653
15.0	0.136	0.00803	1.773	4.3	0.00119	100.0	0.360	0.00060	1.075	2.9	0.00642
20.0	0.174	0.00733	1.781	4.3	0.00200	110.0	0.348	0.00152	0.883	2.1	0.00796
25.0	0.209	0.00644	1.748	4.3	0.00198	120.0	0.328	0.00198	0.742	1.8	0.00897
30.0	0.238	0.00548	1.683	4.1	0.00589						

Table 16.

Temperature (°C.)	$a'(\times 10^4)$	$b(\times 10^6)$	$k_1(\times 10^3)$	$a_1(10\times^2)$	b_1	c	a_2
32.0	8.7	1.87	1.93	2.00	0.032	1.60	1.0640
35.0 (I)	9.7	1.87	1.93	2.48	0.039	1.28	1.0512
37.0	10.0	2.03	2.01	2.44	0.040	2.08	1.0832
38.0	7.8	1.50	1.72	2.04	0.035	1.68	1.0672
42.0	5.8	1.12	1.49	1.48	0.019	1.28	1.0512
35.0 (II)	3.8	0.80	1.26	0.80	0.011	1.28	1.0512

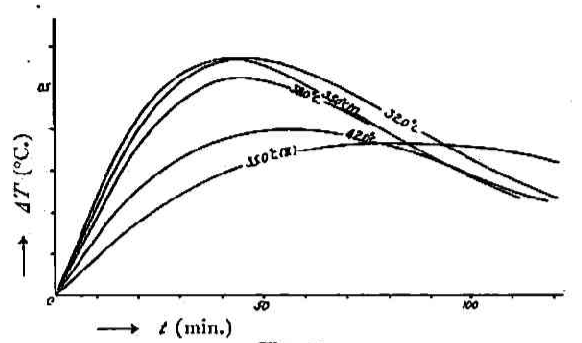

Fig. 14.

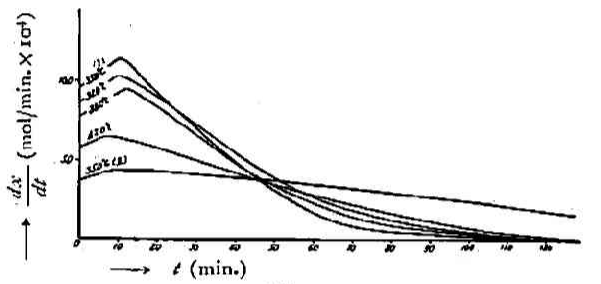

Fig. 15.

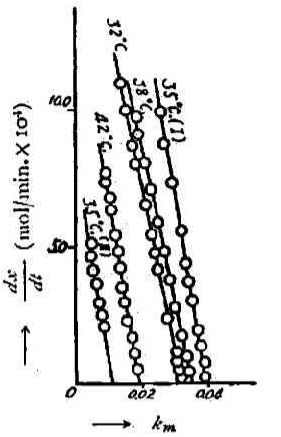

Fig. 16.

As for the *CD* part, b_1 gradually increased till 37.0°C. and then suddenly decreased. The heat inactivation seems to have come forth at 35.0°C. c was almost indifferent to temperature and almost constant though with slight fluctuation. a_2 was also constant.

In short, k_1, a_1 and b_1 have a similar tendency and the result is in agreement with those already published by others. In the present research, however, the optimum point is 37.0°C., being different from that hitherto obtained. c and a_2 have no relation to the temperature.

The duration of the *AB* part was short at 42.0°C. and 35.0°C. (II), and at 32.0°–38.0°C. it was indifferent to the temperature. The *BC* part was very short except at 35.0°C. (II).

From the data obtained, calculating the temperature coefficients k_1 and b_1 which correspond to the velocity constants of the *AB* and *CD* parts respectively, the activation heat E was calculated from Arrhenius' equation, and the results were obtained thus:

Temperature coefficient in the range between 32.0° and 42.0°C.

AB part	(k_1)	1.26
CD part	(b_1)	2.03.

Both are different a little from each other, but they are approximate to the

values hitherto obtained.

Activation heat.

AB part	4013 cals.
CD part	15175 cals.

The discrepancy between these can be considered as a matter of course, for the reaction velocity of each part quite differs and its velocity constant does not contain the same factor. These values are far smaller than 25,5000 cals. obtained in the inversion by acid catalysis. Thus, as far as the influence of temperature upon the invertase action is concerned, the results obtained were almost similar to those of other investigators.

Theoretical Consideration of the Invertase.

By the thermal analysis of the invertase action it was ascertained that the sucrose inversion by invertase is a reaction consisting of two stages, and not of one stage. This fact, therefore, can never be explained by the velocity equations already proposed, but it may be explained if the invertase action is considered to be a reaction in the heterogeneous system. The enzyme solution is in fact a colloidal system and not a homogeneous one, so the reaction should not be treated as a reaction in the homogeneous system. Therefore, assuming that the reaction is due to the contact catalysis of the colloidal system, theoretical formulae will be derived and compared with the above-mentioned empirical ones.

Theoretical Derivation of the Velocity Equations.

First let us consider the mechanism of the reaction. The surface of enzyme is a homogeneous adsorptive surface[21] as is stated by Langmuir[22], on which the molecules of sucrose and water can be adsorbed in a monomolecular layer. These adsorbed molecules are activated by the adsorption and among them reactions take place. The adsorption coefficient of sucrose is far larger than that of water, so that at the instant of the mixing with the sucrose solution the surface of enzyme which has been occupied by the water molecules comes to be occupied by the sucrose molecules and almost all the water molecules are driven from the

21) As far as a metal catalyst is concerned, it has been found by further research that the surface of a metol is not homogeneous, and the active centre should be taken into account. Langmuir's simple theory has been adopted here for simplicity, and it explains the results of the present experiment well.

22) J. Langmuir, *J. A. C. S.*, 40, 1361 (1918).
J. Langmuir, *Trans. Farad. Soc.*, 17, 621 (1922).

surface. The area occupied by the sucrose molecules becomes smaller as the concentration of sucrose decreases during the progress of the reaction. Accordingly, at the earlier stage, in which the area occupied by sucrose is very large, the reaction velocity depends mainly upon the adsorbed water, while at the later stage, in which the area occupied by sucrose is very small, it depends upon the adsorbed sucrose. Further, water is adsorbed on the surface of enzyme, not in a molecular state, but in a dissociated state, such as H^+ and OH^-.

From this assumption let us derive velocity formulae. Let S represent the whole surface of enzyme, which is a quantity related to the dispersity and the concentration of the enzyme solution. Let θ_S and θ_{H_2O} be the fractions of the enzyme surface occupied by the sucrose and water molecules respectively (water was first considered to be adsorbed in its molecular state for simplicity.). Then the reaction velocity is proportional to $S\cdot\theta_{H_2O}^{n}$ for the earlier stage where it depends upon water, and to $S\cdot\theta_S$ for the later stage where it depends upon sucrose. According to Langmuir's adsorption equilibrium, θ_S and θ_{H_2O} will be as follows:

$$\theta_S=\frac{b_SC_S}{1+b_SC_S+b_{H_2O}C_{H_2O}}, \tag{17}$$

and

$$\theta_{H_2O}=\frac{b_{H_2O}C_{H_2O}}{1+b_SC_S+b_{H_2O}C_{H_2O}}. \tag{18}$$

where b_S and b_{H_2O} are the adsorption coefficients of sucrose and water, and C_S and C_{H_2O} are the concentrations of sucrose and water respectively.

In equations (17) and (18), $b_S\ll b_{H_2O}$ and C_{H_2O} can be regarded as a constant, so

$$1+b_SC_S+b_{H_2O}C_{H_2O}\fallingdotseq 1+b_SC_S.$$

Therefore, equations (17) and (18) can be rewritten as follows:

$$\theta_S=\frac{b_SC_S}{1+b_SC_S}$$

$$\theta_{H_2O}=\frac{b_{H_2O}C_{H_2O}}{1+b_SC_S}$$

$$\fallingdotseq\frac{b_{H_2O}C_{H_2O}}{b_S}\left(\frac{1}{b_S}-C_S\right)$$

$$=\frac{b_{H_2O}C_{H_2O}}{b_S}\left\{\left(\frac{1}{b_S}-a\right)+x\right\}.$$

Hence the velocity equation for the earlier stage can be obtained thus,

$$\frac{dx}{dt}=k\cdot S\sqrt{\frac{b_{H_2O}C_{H_2O}}{b_S}}\cdot\sqrt{\left\{\left(\frac{1}{b_S}-a\right)+x\right\}}. \qquad (19)$$

Let k_1 and a_1 represent $k\cdot S\sqrt{\frac{b_{H_2O}C_{H_2O}}{b_S}}$ and $\frac{1}{b_S}-a$ respectively, then we have a theoretical formula of the same type as the empirical formula:

$$\frac{dx}{dt}=k_1\sqrt{a_1+x}\ .$$

Similarly, the velocity equation for the later stage is obtained as follows:

$$\frac{dx}{dt}=k'\cdot S\cdot\frac{b_SC_S}{1+b_SC_S}$$

$$=\frac{k'\cdot S\cdot b_S(a-x)}{1+b_S(a-x)}. \qquad (20)$$

Let b_1 and c represent $k'\cdot S\cdot b_S$ and b_S respectively, then we have a theoretical formula of the same type as the empirical formula:

$$\frac{dx}{dt}=\frac{b_1(a-x)}{1+c(a-x)}.$$

Next, let us examine whether the velocity constants experimentally obtained can satisfy the theoretical values or not and then consider the physical meaning of these constants.

Comparison of the Theoretical Formulae with the Empirical Formulae.

(1) The AB part. First, let us discuss $k_1=k\cdot S\sqrt{\frac{b_{H_2O}\cdot C_{H_2O}}{b_S}}$ which is considered to correspond to the velocity constant of the earlier stage. $\frac{b_{H_2O}\cdot C_{H_2O}}{b_S}$ is a constant and S is relative to the amount of enzyme. It follows, therefore, that k_1 should be increased at a certain rate with the increase of the amount of enzyme and in fact this was experimentally proved.

As to the concentration of sucrose, k_1 should be indifferent, because it contains no factor concerning the concentration of sucrose and this was also experimentally confirmed. How can the fact be explained that k_1 is very small at $p_H=5.11-7.0$? This seems to be related to S, and it is perhaps due to the fact that the area to adsorb has considerably been limited before the beginning of the reaction at such p_H values. Whether this limitation is ascribed to the change of dispersity or to the change of the surface owing to unknown substances though dispersity is un-

changed can not be easily determined, but it is probable from the general nature of the colloidal solution that the change of dispersity is the cause.

The duration of the AB part should be the function of the initial area of the surface of enzyme occupied by the sucrose molecules, for the part lasts until the area occupied by sucrose becomes smaller. However, in $\theta_S=\frac{b_S a}{1+b_S a}=\frac{1}{\frac{1}{b_S a}+1}$ the larger the value of a, the larger is the value of θ_S. Therefore, the duration of the AB part should be proportional to the increase of the initial sucrose concentration. This was, in fact, justified by the experimental results. The influences of the hydrogen ion concentration and temperature upon the duration of this part, which were experimentally ascertained, can be well explained by the consideration that the adsorption surface is remarkably limitted as above said.

(2) The CD part. b_1, which can be regarded as the velocity constant of the later stage, contains S, so it must be proportional to the increase of the amount of enzyme, and in fact this was experimentally confirmed. Theoretically b_1 contains no factor concerning the sucrose cencentration, and the experimental results show that it is considered to be constant except at very low concentration of sucrose. To the hydrogen ion concentration b_S is not related, but S is. Therefore, b_1 must be markedly small at $p_H=5.11-7.0$ due to the limitation of the adsorption surface as in the case of k_1, and this was experimentally confirmed, too. The influence of temperature can be similarly explained.

Summary.

(1) A relatively pure invertase which has such a small time value as 0.25 has been prepared from yeast cells by a simple method.

(2) The sucrose inversion has been investigated by thermal analysis, employing the invertase thus obtained.

(3) The observed value of the inversion heat has been 4.1 cal. per g. mol.

(4) The influences of the enzyme amount, the sucrose concentration, the hydrogen ion concentration and the temperature upon the invertase action have been examined by thermal analysis.

(5) The results obtained by thermal analysis have shown that the inversion by invertase proceeds according to the equations: $\frac{dx}{dt}=k_1\sqrt{a_1+x}$, at its earlier stage and $\frac{dx}{dt}=\frac{b_1(a-x)}{1-c(a-x)}$ at its later stage.

(6) The theoretical formulae derived from the assumption that the catalytic

action of invertase is due to the adsorptive phenomenon of a colloidal solution have been well satisfied by the experimental results—(4) and (5).

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