

Die Kinetik der Invertinwirkung

Von

L. Michaelis and Miss Maud L. Menten

(Received 4 February 1913.)

With 19 Figures in Text.

The Kinetics of Invertase Action

translated by

Roger S. Goody¹ and Kenneth A. Johnson²

The kinetics of enzyme³) action have often been studied using invertase, because the ease of measuring its activity means that this particular enzyme offers especially good prospects of achieving the final aim of kinetic research, namely to obtain knowledge on the nature of the reaction from a study of its progress. The most outstanding work on this subject is from Duclaux⁴), Sullivan and Thompson⁵), A.J. Brown⁶) and in particular V. Henri⁷). Henri's investigations are of particular importance since he succeeded, starting from rational assumptions, in arriving at a mathematical description of the progress of enzymatic action that came quite near to experimental observations in many points. We start from Henri's considerations in the present work. That we have gone to the lengths of reexamination of this work arises from the fact that Henri did not take into account two aspects, which must now be taken so seriously that a new investigation is warranted. The first point to be taken into account is the hydrogen ion concentration, the second the mutarotation of the sugar(s).

The influence of the hydrogen ion concentration has been clearly demonstrated by the work of Sørensen⁸) and of Michaelis and Davidsohn⁹). It would be a coincidence if Henri in all his experiments, in which he did not consider the hydrogen ion concentration, had worked at the same hydrogen ion concentration. This has been conveniently addressed in our present contribution by addition of an acetate mixture that produced an H⁺-concentration of $2 \cdot 10^{-5}$ M¹⁰)

¹ Director of the Dept. of Physical Biochemistry, Max-Planck Institute of Molecular Physiology, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany. Email: roger.goody@mpi-dortmund.de

² Professor of Biochemistry, Institute for Cell and Molecular Biology, 2500 Speedway, University of Texas, Austin, TX 78735 USA. Email: kajohnson@mail.utexas.edu

³ Michaelis and Menten use the word "ferment", but we adopt the word "enzyme" following papers from the same period written in English.

⁴ Duclaux, *Traité de Microbiologie* 0899, Bd. II.

⁵ O. Sullivan and Thompson, *J. Chem. Soc.* (1890) **57**, 834.

⁶ A. J. Brown, *J. Chem. Soc.* (1902), 373.

⁷ Victor Henri, *Lois générales de l'action des diastases*, Paris (1903).

⁸ S. P. L. Sørensen, *Enzymstudien II. Biochemische Zeitschrift* (1909) **21**, 131.

⁹ L. Michaelis and H. Davidsohn, *Biochemische Zeitschrift* (1911) **35**, 386.

¹⁰ As in many places throughout the article, no units are given and we presume M, giving pH 4.7.

in all solutions, which is on the one hand the optimal H^+ -concentration for the activity of the enzyme and on the other hand the H^+ -concentration at which there is the lowest variation of enzyme activity as a result of a small random deviation from this concentration, since in the region of the optimal H^+ -concentration the dependence of the enzyme activity on the H^+ -concentration is extremely small.

At least as important in the work of Henri is the lack of consideration of the fact that on inversion of the sugar, glucose is formed initially in its birotational form and is only slowly converted to its normal rotational form.¹¹⁾ Monitoring the progress of the inversion reaction by direct continuous observation of the polarization angle therefore leads to a falsification of the true rate of inversion, since this is superimposed on the change in polarization of the freshly formed glucose. This could be allowed for by including the rate of glucose equilibration in the calculations. However, this is not realistic, since highly complex functions are generated which can be easily avoided experimentally. A better approach is to take samples of the inversion reaction mixture at known time intervals, to stop the invertase reaction and to wait until the normal rotation of glucose is reached before measuring the polarization angle. Sørensen used sublimate ($HgCl_2$) while we used soda, which inactivates the invertase and removes the mutarotation of the sugar within a few minutes.¹²⁾

Incidentally, it should be noted that Hudson¹³⁾ already adopted the approach of removing mutarotation experimentally using alkali, but came to a quite different conclusion to ours concerning the course of the invertase reaction. Thus, he is of the opinion that after removing the problem of mutarotation, inversion by invertase follows a simple logarithmic function similar to that of inversion by acid, but this result is contrary to all earlier investigations and according to our own work is not even correct to a first approximation. Even if Henri's experiments need to be improved, their faults are not as grave as Hudson believes. (Sørensen also noticed that Hudson's conclusions were incorrect). On the contrary, we are of the opinion that the basic considerations that started with Henri are indeed rational, and we will now attempt to use improved techniques to demonstrate this. It will become apparent that the basic tenets of Henri are, at least in principle, quite correct, and that the observations are now in better accord with them than are Henri's own experiments.

Henri has already shown that the cleavage products of sugar inversion, glucose and fructose, have an inhibitory effect on invertase action. Initially, we will not attempt to allow for this effect, but will choose experimental conditions which avoid this effect. Since the effect is not large, this is, in principle, simple. At varying starting concentrations of sucrose, we only need to follow the inversion reaction in a time range where the influence of the cleavage products is

¹¹ The cleavage of sucrose initially gives the α -anomer (α -D-glucopyranose), which then equilibrates to a mixture of α - and β -anomers (ca. 65% β); the meaning of birotational is not entirely clear.

¹² This is not strictly correct since mutarotation describes the equilibration of the α and β anomers, which is not removed; rather, the treatment with alkali accelerates the equilibration.

¹³ C. S. Hudson, J. Amer. Chem. Soc. (1908) **30**, 1160 and 1564; (1909) **31**, 655; (1910) **32**, 1220 and 1350 (1910).

not noticeable. Thus, we will initially only measure the starting velocity of inversion at varying sucrose concentrations. The influence of the cleavage products can then be easily observed in separate experiments.

1. The initial reaction velocity of inversion at varying sucrose concentrations

The influence of the sucrose concentration on enzymatic inversion was examined by all authors already cited and led to the following general conclusions. At certain intermediate sucrose concentrations the rate is hardly dependent on the starting amount of sugar. The rate is constant at constant enzyme concentration but is reduced at lower and also at higher sugar concentration¹⁴). Our own experiments were performed in the following manner. A varying quantity of a sucrose stock solution was mixed with 20 ccm of a mixture of equal parts of 1/5 M acetic acid, 1/5 M sodium acetate, a certain quantity of enzyme, and water to give a volume of 150 ccm. All solutions were prewarmed in a water bath at $25 \pm <0.05^\circ$ and held at this temperature during the reaction. The first sample was taken as soon as possible after mixing the solution, followed by further samples at appropriate intervals. Every sample of 25 ccm was transferred to a vessel containing 3 ccm of 1/2 M Soda to immediately stop the enzyme activity. The solution was examined polarimetrically after approximately 1/2 hour. The initial polarization angle was extrapolated from the first actual measurements. This extrapolation is certainly valid, since it was only over a few hundredths of a degree. Regular checks that the mutarotation was complete were made by repeated measurements 1/2 hour later. Every measurement recorded in the protocol is the average of 6 individual measurements, which only differed by a few hundredths of a degree. If we now plot the rotation as a function of time for a single experiment, we see that at the beginning of the process the rotation decreases linearly with time over a fairly long stretch. We define the initial velocity of the inversion as the decrease of rotation per unit time in the phase that can be regarded as linear. The experiments led to the following results:

¹⁴ Perhaps the authors were referring to substrate inhibition at high sucrose concentrations, which is evident in Figs. 2a and 4a, and explained on page 14 as possibly due to changes in the solvent at high concentrations (e.g., 34% sucrose).

In Tables I through IV we give the rotation angle relative to the real zero point of the polarimeter, corrected for the (very small) rotation of the enzyme solution.

Table I (Fig. 1)

Time (t) in minutes	Corrected rotation	Change in rotation x	Initial concentration of Sucrose	Time (t) in Minutes	Corrected rotation	Change in rotation x	Initial concentration of Sucrose
1. 0	[14.124]	0	0.333 M	2. 0	[7.123]	0	0.167 M
1	14.081	0.043		1	7.706	0.047	
7	13.819	0.305		8	6.749	0.374	
14	13.537	0.587		16	6.528	0.595	
26	13.144	0.980		28	6.109	1.014	
49	12.411	1.713		52	5.272	1.851	
75	11.502	2.602		82	4.316	2.807	
117	10.156	3.968		103	3.592	3.531	
1052	- 4.129	18.253		24 Std.	- 2.219	9.342	
theor. endpoint		18.57		theor. endpoint		9.35	
3a. 0	[3.485]	0	0.0833 M	3b. 0	3.394	0	0.0833 M
2.5	3.440	0.045		1	3.367	0.027	
12.5	3.262	0.223		6	3.231	0.163	
49.5	1.880	1.605		13	2.941	0.453	
90.0	0.865	2.620		21	2.672	0.722	
125.0	0.340	3.145		22	2.302	1.092	
151.0	0.010	3.496		57	1.626	1.768	
208.0	- 0.617	4.102		90	0.824	2.570	
267.0	- 0.815	4.300		24 Std.	- 1.109	4.503	
24 Std.	- 0.998	4.483		theor. endpoint		4.56	
theor. endpoint		4.560					
4. 0	[1.745]	0	0.0416 M	5. 0	[0.906]	0	0.0208 M
2.25	1.684	0.061		1	0.881	0.025	
10.25	1.487	0.258		6	0.729	0.177	
30.75	0.929	0.816		17	0.512	0.394	
61.75	0.359	1.386		27	0.369	0.537	
90.75	0.061	1.684		38	0.179	0.727	
112.75	- 0.169	1.914		62	0.029	0.877	
132.75	- 0.339	2.084		95	- 0.117	1.023	
154.75	- 0.374	2.119		1372	- 0.230	1.136	
1497.0	- 0.444	2.189		24 Std.	- 0.272	1.178	
theor. endpoint		2.247	theor. endpoint		1.190		
6. 0	[0.480]	0	0.0104 M	7. 0	[0.226]	0	0.0052 M
0.5	0.472	0.012		1	0.219	0.007	
5.5	0.396	0.084		8	0.172	0.054	
11.0	0.329	0.151		16	0.092	0.134	
19.0	0.224	0.251		28	0.056	0.170	
35.0	0.127	0.353		50	- 0.012	0.238	
75.0	0.021	0.459		80	- 0.089	0.315	
117.0	- 0.059	0.539		114	- 0.117	0.343	
149.0	- 0.114	0.594		2960	- 0.104	0.330	
24 Std.	- 0.127	0.607		—	—	—	
theor. endpoint		[0.630]	—	—	—		

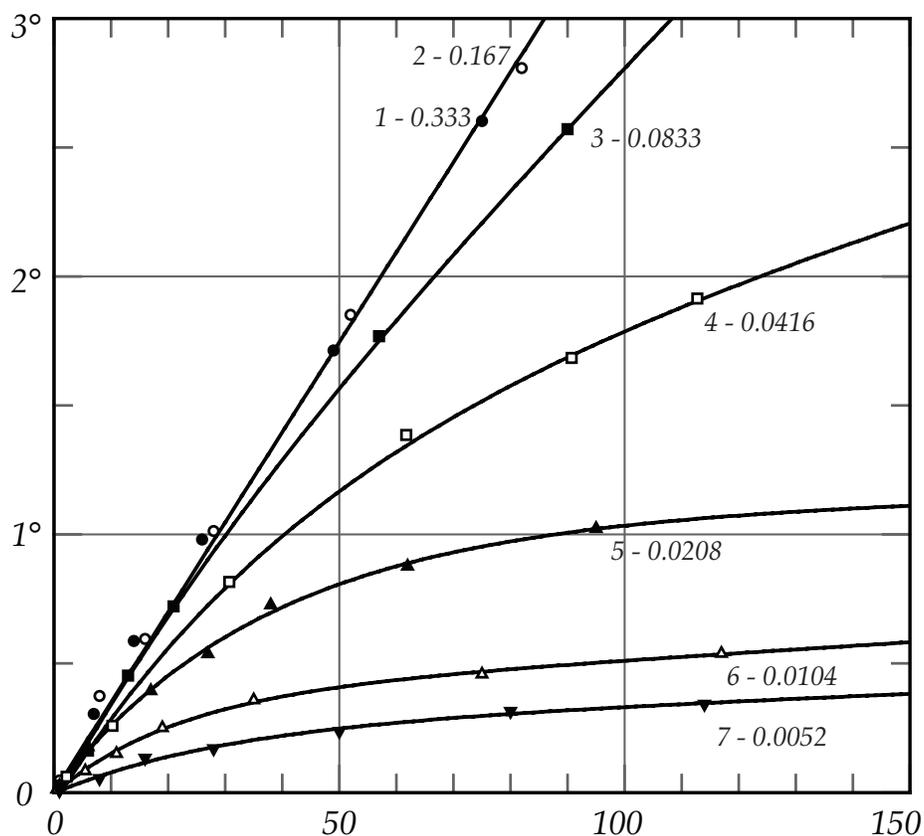


Fig. 1. Abscissa: Time in minutes. Ordinate: Decrease in rotation in degrees. Each curve is for an experiment with the given starting concentration of sucrose. The numbers of the experiments (1 to 7) correspond to those of Table I.¹⁵⁾ Experiment 3 represents the combined results of the parallel experiments 3a and 3b. Amount of enzyme is the same in all experiments.

Results of the experiment in Table I (Fig. 1a)

	Initial velocity	Initial Concentration of Sucrose a	$\log a$
1.	3.636	0.3330	- 0.478
2.	3.636	0.1670	- 0.777
3.	3.236	0.0833	- 1.079
4.	2.666	0.0416	- 1.381
5.	2.114	0.0208	- 1.682
6.	1.466	0.0104	- 1.983
7.	0.866	0.0052	- 2.284

¹⁵⁾ The numbers on the figure define the experiment number and the molar concentration of sucrose.

Fig 1a. Abscissa: Logarithm of initial concentration of sucrose. Ordinate: The initial rate of cleavage, expressed as the decrease of rotation (in degrees) per unit time (minutes), extracted graphically from Fig. 1. Concerning the "rational scale" of the ordinate, see pp. 12-13.

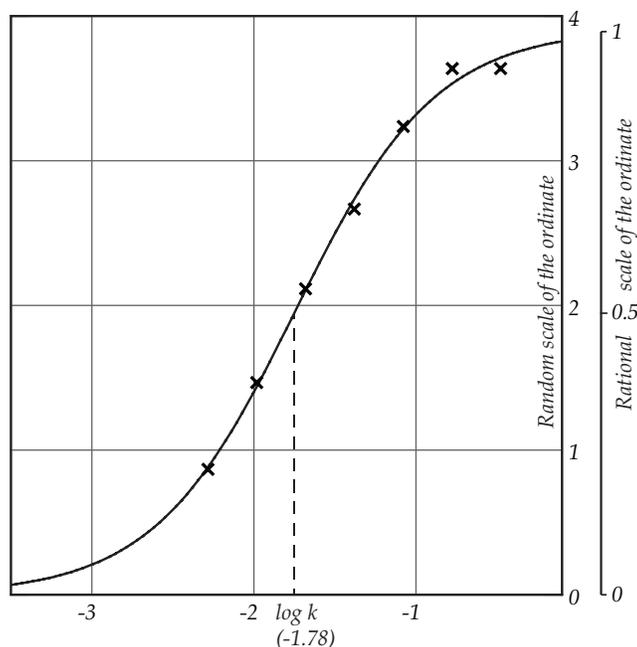


Table II (Fig. 2)

Time (t) in minutes	Rotation	Change in rotation	Initial concentration of Sucrose	Time (t) in minutes	Rotation	Change in rotation	Initial concentration of Sucrose
<i>A</i> 0	[31.427]	0	0.77 M	<i>B</i> 0	[15.684]	0	0.385 M
0.5	31.393	0.034		0.5	15.643	0.041	
7.0	30.951	0.476		7.0	15.148	0.536	
15.0	30.486	0.941		15.0	14.543	1.141	
23.0	30.025	1.402		23.0	13.935	1.749	
38.0	29.185	2.242		38.0	13.183	2.501	
<i>C</i> 0	[7.949]	0	0.192 M	<i>D</i> 0	[3.853]	0	0.096 M
0.5	7.910	0.039		0.5	3.810	0.043	
7.0	7.407	0.542		9.0	3.090	0.763	
15.0	6.790	1.159		17.0	2.741	1.112	
23.0	6.161	1.788		25.0	2.063	1.790	
32.0	5.523	2.426		34.0	1.551	2.302	
<i>E</i> 0	[2.063]	0	0.048 M	<i>F</i> 0	[1.374]	0	0.0308 M
0.5	2.033	0.030		0.5	1.348	0.026	
7.0	1.643	0.420		6.0	1.055	0.319	
15.0	1.197	0.866		13.0	0.706	0.668	
23.0	0.791	1.272		22.0	0.403	0.971	
32.0	0.440	1.623		32.0	0.138	1.236	
<i>G</i> 0	[0.707]	0	0.0154 M	<i>H</i> 0	[0.360]	0	0.0077 M
0.5	0.690	0.017		0.5	0.348	0.012	
6.0	0.505	0.202		6.0	0.220	0.140	
13.0	0.340	0.367		13.0	0.161	0.199	
22.0	0.160	0.547		22.0	0.105	0.255	
32.0	0.050	0.657		32.0	0.046	0.314	

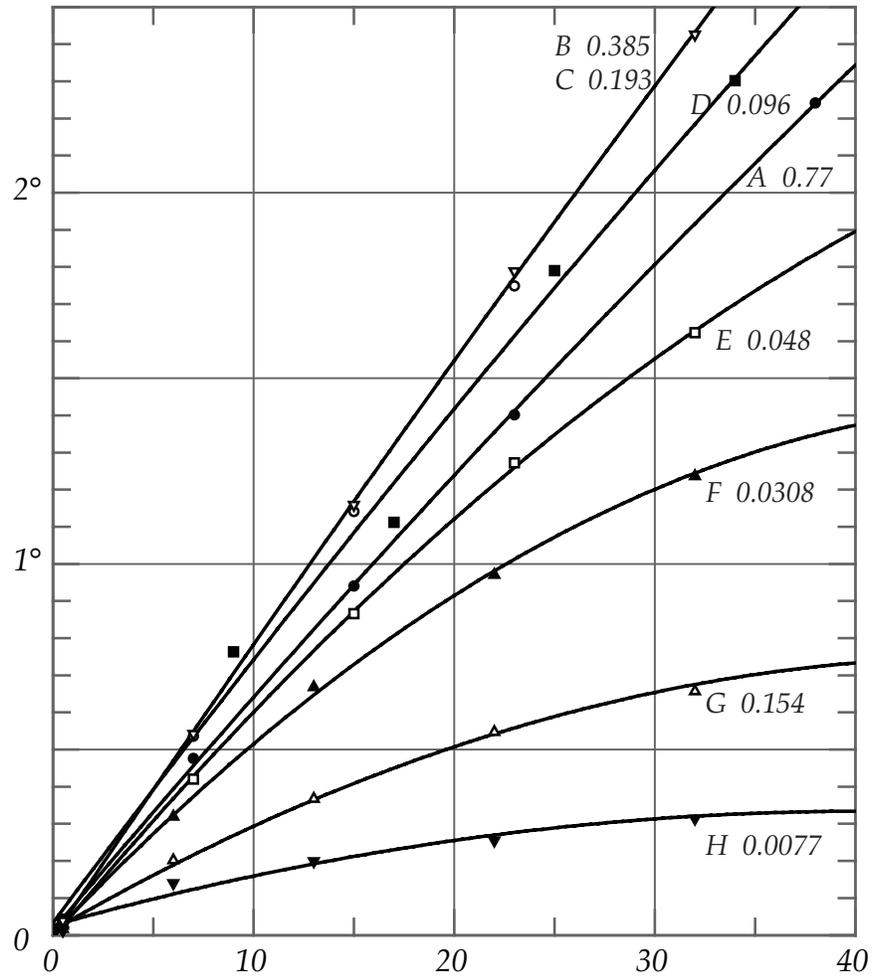


Fig. 2. Terms as in Fig. 1. Graphical representation of the experiment in Table II. Approximately double the enzyme amount as in Fig. 1.¹⁶⁾

Results of the experiment in Table II (Fig. 2a)

	Initial velocity	Initial Concentration of Sucrose <i>a</i>	log <i>a</i>
1.	0.0630	0.7700	- 0.114
2.	0.0750	0.3850	- 0.414
3.	0.0750	0.1920	- 0.716
4.	0.0682	0.0960	- 1.017
5.	0.0583	0.0480	- 1.318
6.	0.0500	0.0308	- 1.517
7.	0.0350	0.0154	- 1.813
8.	0.0267	0.0077	- 2.114

¹⁶⁾ The concentrations of sucrose in M are listed in Fig. 2 for each experiment (A-H) according to Table II.

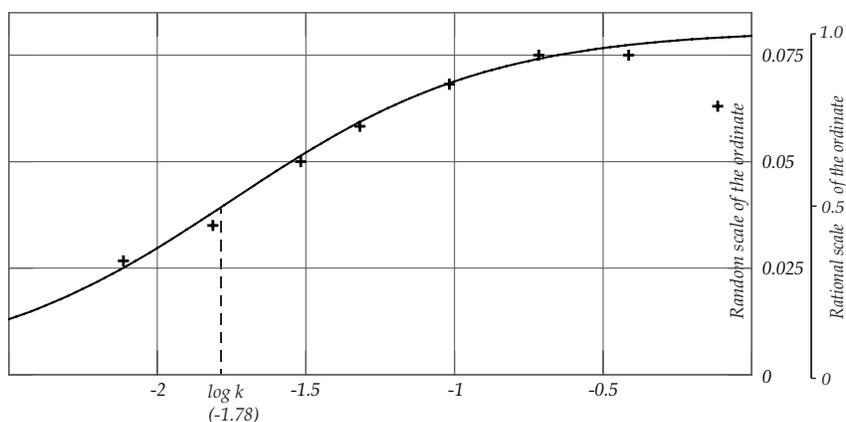


Fig 2a. The presentation corresponds to Fig. 1a; calculated from Fig. 2.

Table III (Fig. 3)

Time (t) in minutes	Rotation	Change in rotation	Initial quantity of Sucrose	Time (t) in minutes	Rotation	Change in rotation	Initial quantity of Sucrose
A 0	[30.946]	0	0.77 M	B 0	[15.551]	0	0.385 M
0.5	30.935	0.011		0.5	15.541	0.010	
30.0	30.325	0.621		30.0	14.973	0.578	
60.0	29.715	1.231		60.0	14.353	1.198	
90.0	29.286	1.660		90.0	13.810	1.741	
123.0	28.506	2.440		123.0	13.138	2.413	
C 0	[7.623]	0	0.193 M	D 0	[3.869]	0	0.096 M
0.5	7.613	0.010		0.5	3.860	0.009	
31.0	6.990	0.633		27.0	3.366	0.503	
55.0	6.430	1.193		53.0	2.791	1.078	
74.0	6.040	1.583		74.0	2.533	1.336	
—	—	—		101.0	1.998	1.871	
E 0	[2.004]	0	0.048 M	F 0	[0.967]	0	0.024 M
0.5	1.995	0.009		0.5	0.953	0.004	
27.0	1.485	0.546		27.0	0.711	0.246	
53.0	1.113	0.891		53.0	0.446	0.511	
74.0	0.848	1.156		73.0	0.343	0.614	
101.0	0.555	1.449		100.0	0.195	0.762	

Results of the experiment in Table III (Fig. 3a)¹⁷

	Concentration (x)	log(x)	Initial velocity (v)
1.	0.770	- 0.114	0.3166 (0.02)
2.	0.385	- 0.414	0.3166 (0.02)
3.	0.193	- 0.716	0.2154 (0.0215)
4.	0.096	- 1.017	0.0192
5.	0.048	- 1.318	0.0166
6.	0.024	- 1.619	0.0088 (0.0135)

¹⁷ Numbers in this table were inconsistent with Fig. 3a. To reproduce the figure, we used a micrometer to estimate the values from the graph, as indicated by the numbers in parenthesis in the table, which were used to recreate Fig. 3a.

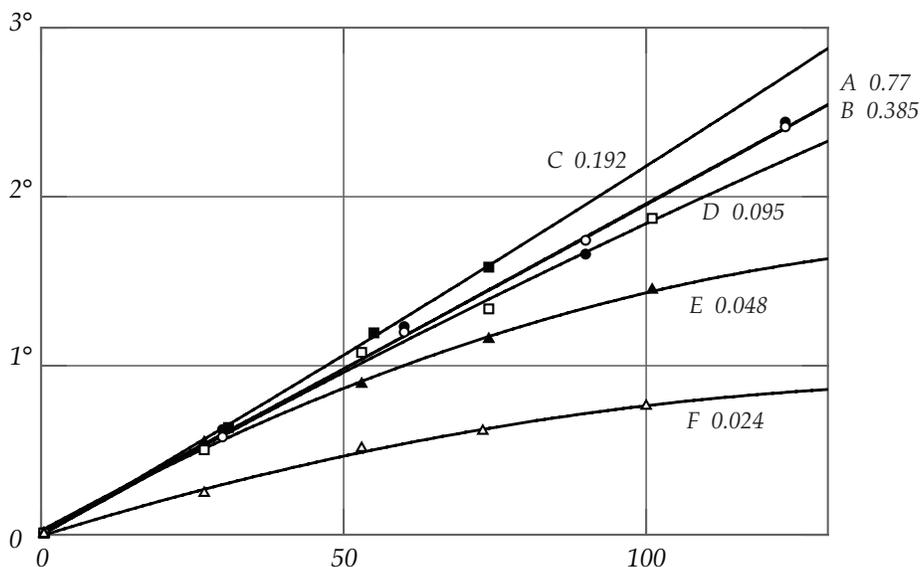


Fig 3. Graphical representation of the experiment in Table III. Amount of enzyme about half as large as in Fig. 1.

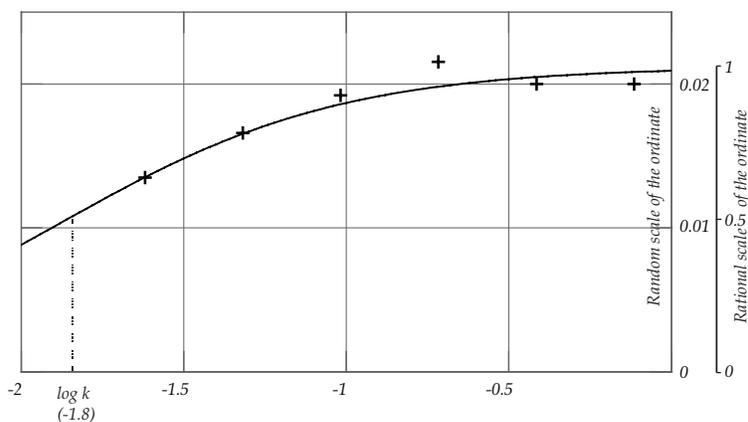


Fig 3a. Representation as Fig. 1a, calculated from Fig. 3.

Table IV (Fig. 4)

Time (t) in minutes	Rotation	Change in rotation	Initial quantity of Sucrose	Time (t) in minutes	Rotation	Change in rotation	Initial quantity of Sucrose
1. 0	[31.205]	0	0.77 M	2. 0	[15.588]	0	0.385 M
0.5	31.190	0.015		0.5	15.570	0.018	
68.0	29.183	2.022		67.0	13.140	2.448	
3. 0	[7.849]	0	0.193 M	4. 0	[3.980]	0	0.096 M
0.5	7.830	0.019		0.5	3.963	0.017	
62.0	5.416	2.433		62.0	1.840	2.140	
5. 0	[1.984]	0	0.048 M	6. 0	[1.031]	0	0.024 M
0.5	1.970	0.014		0.5	1.013	0.018	
30.0	1.133	0.851		10.0	0.665	0.366	
-	-			29.0	0.415	0.616	
-	-			36.0	0.321	0.710	

Results of the experiment in Table IV (Fig. 4a)

	Concentration (x)	$\log x$	Initial Velocity v
1.	0.770	- 0.114	0.0297
2.	0.385	- 0.414	0.0365
3.	0.193	- 0.716	0.0374
4.	0.096	- 1.017	0.0345
5.	0.048	- 1.318	0.0284
6.	0.024	- 1.619	0.0207

To analyze these experiments, we assume with Henri that invertase forms a complex with sucrose that is very labile and decays to free enzyme, glucose and fructose. We will test whether such an assumption is valid on the basis of our experiments. If this assumption is correct, the rate of inversion must be proportional to the prevailing concentration of the sucrose-enzyme complex.¹⁸⁾

If 1 mole of enzyme and 1 mole of sucrose form 1 mole of sugar-enzyme complex, the law of mass action requires that

$$[S] \cdot [\Phi - \phi] = k \cdot \phi \dots \dots (1)$$

where $[S]$ is the concentration of free sucrose, or since only a vanishingly small fraction of it is bound by enzyme, the total concentration of sucrose; Φ is the total molar enzyme concentration, ϕ is the concentration of the complexed enzyme, $[\Phi - \phi]$ is the concentration of free enzyme, and k is the dissociation constant.

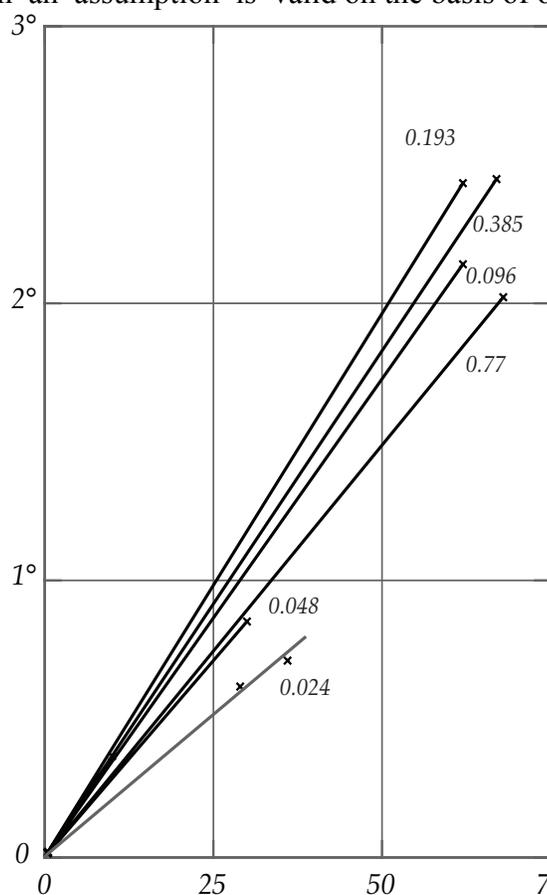


Fig 4. Graphical representation of the experiment in Table IV. Enzyme amount approximately the same as in the experiment of Fig. 1.

¹⁸ The authors use the word "Verbindung", which is normally used these days for compound. English texts of the period use the expression "molecular compound" for the invertase:sucrose complex (A.J. Brown, J. Chem. Soc. Vol. 81, pp. 373-388, 1902).

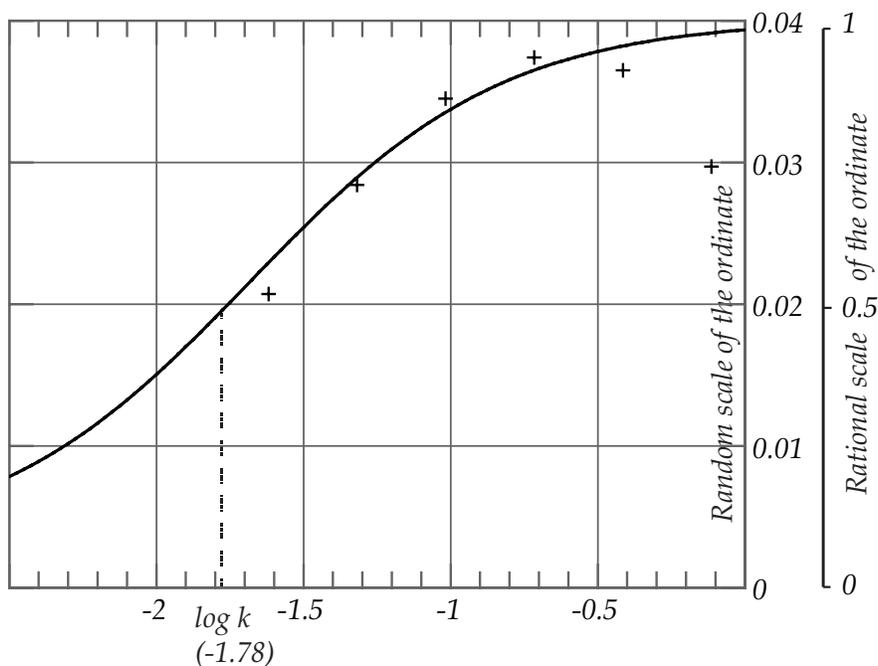


Fig 4a. Representation as Fig. 1a. Calculated from the experiment of Fig. 4.

From this it follows that

$$\varphi = \Phi \cdot \frac{[S]}{[S] + k} \dots\dots\dots (2)$$

This quantity must be proportional to the starting velocity, v , of the inversion reaction, therefore

$$v = C \cdot \Phi \cdot \frac{[S]}{[S] + k} \dots\dots\dots (3)$$

where C is the proportionality constant.¹⁹⁾ Since we measure v in arbitrary units (change of rotation angle per minute), and since Φ is held constant in an experimental series, we can refer to $\frac{v}{C \cdot \Phi}$ as V . Thus, V is a function that is proportional to the true starting velocity, so that²⁰⁾

$$V = \frac{[S]}{[S] + k} \dots\dots\dots (4)$$

¹⁹⁾ Equation 3 is the closest they come to the Michaelis-Menten equation. The constant C contains k_{cat} and a factor to convert the change of optical rotation to concentration so that $C \cdot \Phi$ is V_{max} in units of optical rotation degrees per minute.

²⁰⁾ In equation 4, V is actually a dimensionless number giving the fraction of maximum velocity, v/V_{max} as we know it.

This function is formally the same as the association curve²¹⁾ of an acid²²⁾

$$\rho = \frac{[H^+]}{[H^+] + k}$$

and in order to achieve a better graphical representation we will plot the logarithm of the independent variable on the abscissa. We can therefore plot V as a function of $\log[S]$ and should obtain the well known association curve. At this point, we do not know the true scale of the ordinate. We only know that the maximal value $V = 1$ should be reached asymptotically and that the foot of the ordinate of value $\frac{1}{2}$ should give the value of k . In order to find the scale, we use the following graphical procedure.

Let us assume that we have a number of points from the experiment that we assume should give an association curve. Since the scale of the points on the ordinate is arbitrary, we have to assume that it will be different from that of the abscissa. Setting $s = \log[S]$, the function that we wish to display graphically is

$$V = \frac{10^s}{10^s + k}$$

or, if we substitute $10 = e^p$, where $p (= 2.303)$ is the modulus of the decadic logarithm system,

$$V = \frac{e^{ps}}{e^{ps} + k}$$

Differentiating, we obtain

$$\frac{dV}{ds} = \frac{p \cdot k \cdot e^{ps}}{(e^{ps} + k)^2}$$

This differential quotient defines the tangent of the slope of the specified part of the curve. The association curve has a region whose slope is especially easy to determine, since it is practically linear over an extended stretch. This is the middle of the curve, in particular around the region where the ordinate has a value of $\frac{1}{2}$. We know (cf. the work just referenced) that this ordinate corresponds to the point $\log k$ on the abscissa. If we now substitute the value $\frac{1}{2}$ for V and $\log(k)$ for s , i.e. k for e^{ps} , in the differential equation, we obtain

$$\frac{dV}{ds} \text{ for } V = 1/2 = \frac{p}{4} = \frac{2.3026}{4} = 0.576$$

²¹ The term used was "Restdissoziationskurve", which we translate according to the meaning implied by the equation defining the fractional association of an acid versus pH.

²² L. Michaelis, *Biochemische Zeitschrift* **33**, 182 (1911); see also, by the same author, *The General Significance of the Hydrogen-Ion Concentration etc.*, in Oppenheimer's *Handbook of Biochemistry*, supplementary volume, 1913.

This means that the middle, almost linear part of the curve has a slope relative to the abscissa whose tangent is 0.576 (i.e. a slope of almost exactly 30°). This obviously only applies if the ordinate and the abscissa have the same scales. We now join the experimental points of the middle part of the curve by a straight line and find that the tangent of its slope has the value v .²³⁾ From this we can conclude that the units of the abscissa are related to those of the ordinate in the ratio of 0.576: v , i.e. that the units of the ordinate are the $v/0.576$ of those of the abscissa. We can now calculate the proper scale of the ordinate. (cf. Fig. 1a, 2a, 3a, 4a; "rational scale"). We now determine the position of the point 0.5 on this new scale. The ordinate of the curve, which corresponds to this point, gives the value of $\log k$ at its foot on the abscissa. We now know the value of k and can construct the whole association curve point for point. We will do this to test whether all the observed points fit well to this curve, and in particular that the value of 1 is not exceeded. Doing this for our experiments, we determine a value for v for each curve; we then construct the curve according to this and find, with one exception to be discussed, a good agreement of the observed and calculated points.

A second method to determine the scale of the ordinate is the following. If several points at the right hand end of the curve are well determined, and if it is clear that the maximal value has been reached, we can rescale the ordinate to make this value equal to 1. Then we again construct the sloping middle part of the curve by joining the points with a straight line and determine which point corresponds to the ordinate 0.5 on the new scale. We now have all data to construct the curve.

The first method will be chosen if the middle part of the curve is well determined, the second if the points at the right hand end of the curve are determined more reliably. If possible, both methods are used to confirm the agreement of the values obtained; in case of slight disagreements, the average value is taken. Using a combination of these methods we were able to obtain all of the curves shown. In all 4 cases (curve 1a, 2a, 3a, 4a), a family of dissociation curves was constructed for all possible combinations of likely scales for the ordinate and the best fitting curve was selected by shifting to the right or the left until the observed experimental points gave the best fit. It is indeed possible to find curves in all cases that fit within the limits of the allowed tolerances, even though the 4 experimental series were performed with quite different amounts of enzyme.

The dissociation constant for the invertase-sucrose complex found in the individual experiments were:²⁴⁾

	1	2	3	4
$\log k =$	-1.78	-1.78	-1.80	-1.78
$k =$	0.0167	0.0167	0.0160	0.0167

²³⁾ This is the Greek letter ν , not be confused with the velocity, v .

²⁴⁾ The dissociation constant is given in units of M.

in good agreement, although experiments were carried out with different amounts of enzyme. We have here, for the first time, a picture of the magnitude of the affinity of an enzyme for its substrate and we measure the size of a “specific” affinity according to the van’t Hoff definition of chemical affinity.

The meaning of this affinity constant is the following. If we could prepare the enzyme-sucrose complex in a pure form and were to dissolve it in water at a concentration such that the undissociated fraction was present at a concentration of 1 mol in 1 liter, there would be $\sqrt{0.0167}$ mol or 0.133 mol of free enzyme and the same amount of free sucrose in the solution.

The accuracy with which k can be determined is different in the 4 different experiments (Fig. 1a, 2a, 3a, 4a). To an inexperienced observer, the unavoidable arbitrariness in plotting the observed points will appear questionable. But in fact this has little influence. For example, the worst of our curves is arguably Fig. 3a. Here we find $\log k = 1.8$. Perhaps we could draw an acceptable curve for $\log k = -1.7$ or -1.9 . But assuming $\log k = -2.0$ would not be compatible with the shape of a dissociation curve, and the same applies for $\log k = -1.5$.²⁵) Thus, the variance of the true value of k is not large, even for a curve as poor as in Fig. 3a, as long as we have shown in a number of better experiments that the curve can be regarded as an “association curve”.

The agreement of the theoretical curve with the observed points is satisfactory from the lowest useable sucrose concentrations up to ca. 0.4 M (corresponding to a logarithmic value of ca. -0.4). However, at higher concentrations there is a deviation such that the rate becomes slower rather than remaining constant.²⁶) However, we are not concerned with this deviation, since in this situation we are not confronted with the pure properties of a dilute solution. It is to be expected that the developed quantitative relationships are only valid over a limited range. The reasons for the failure of the law at high sugar concentrations can be attributed to factors whose influence we cannot express quantitatively. The most important influence can be summarized as “change of the nature of the solvent”. We cannot regard a 1 molar solution of sucrose, containing 34% sugar, simply as an aqueous solution, since the sugar itself changes the character of the solvent. This could lead to a change in the affinity constant between enzyme and sugar as well as the rate constant for the decay of the complex. As an example of the manner in which an affinity constant can change when the nature of the solvent changes on addition of an organic solvent, we can consider the investigation of Löwenherz²⁷) on the change in the dissociation constant of water on addition of alcohol. There is no change in the affinity up to 7% alcohol, but there is a progressive decrease as the concentration is increased further.

²⁵ Theoretical dissociation curves can obviously be generated with $\log k = -2.0$ or -1.5 ; they mean the points are not well explained assuming these values of k .

²⁶ The quantities of enzyme in the experimental series I, II, III, IV are calculated from the initial velocities to be almost exactly 1:2:0.5:1.

²⁷ R. Löwenherz, *Zeitschr. f. physikal. Chem.* **20**, 283 (1896) *Biochemische Zeitschrift* Band 42.

2. The influence of the cleavage products and other substances.

The cited authors, especially Henri, have already shown that the cleavage products glucose and fructose have an influence on the hydrolysis of sucrose. Henri found that the influence of fructose is greater than that of glucose. We now have the task of determining this influence in a quantitative manner. Like Henri, we assume that invertase has affinity not only for sucrose, but also for fructose and glucose, and we attempt to determine the values of the affinity constants. We did this in the following manner:

As before, the initial rate of hydrolysis of sucrose at a certain enzyme concentration is determined. In a second experiment, a known concentration of fructose or glucose is added and the initial rate of hydrolysis of sucrose is determined and compared. It is found that this is reduced. We can conclude from this that the concentration of the sucrose-enzyme complex is reduced in the second case, under the assumption that the initial rate is always an indicator of the complex. If v_0 and v are the initial velocities and φ_0 and φ the corresponding sucrose-enzyme complex concentrations, then

$$v_0 : v = \varphi_0 : \varphi$$

If the concentration of enzyme, Φ , partitions between the sucrose concentration S and the fructose concentration F , and if φ is the concentration of the sucrose-enzyme complex and ψ that of the fructose-enzyme complex, it follows from the law of mass action that

$$S \cdot (\Phi - \varphi - \psi) = k \cdot \varphi,$$

$$F \cdot (\Phi - \varphi - \psi) = k_1 \cdot \psi,$$

where k and k_1 are the respective affinity constants.

From these 2 equations, elimination of ψ leads to

$$k_1 = \frac{F \cdot k}{S \cdot \left(\frac{\Phi}{\varphi} - 1 \right) - k} \dots \dots \dots (1)$$

$\frac{\Phi}{\varphi}$ can be determined as follows: In a parallel experiment without fructose, the initial rate is v_0 and the concentration of the sucrose-enzyme complex is φ_0 ; in the main experiment, these two are equal to v and φ , respectively; therefore

$$v : v_0 = \varphi : \varphi_0$$

$$\text{and } \varphi = \frac{v}{v_0} \cdot \varphi_0$$

In the fructose-free experiment, according to equation (2) on p. 11

$$\varphi_0 = \Phi \cdot \frac{S}{S + k}$$

And therefore

$$\varphi = \frac{v}{v_0} \cdot \Phi \cdot \frac{S}{S + k} \dots \dots \dots (2)$$

or

$$\frac{\Phi}{\varphi} = \frac{v_0}{v} \cdot \frac{S+k}{S}$$

and finally by substitution in (1)

$$k_1 = \frac{F \cdot k}{(S+k) \left(\frac{v_0}{v} - 1 \right)} \dots \dots \dots (3)$$

Accurate description of experiments on the inhibition
by other substances (Fructose and Glucose)

Table 5 (Fig. 5)

Time in minutes		Rotation	Change in rotation	Concentration
I	0.0	[3.905]	0.000	Sucrose 0.1 M
	0.5	3.896	0.009	
	15.0	3.640	0.365	
	30.0	3.183	0.722	
I (repeats)	0.0	[3.926]	0.000	Sucrose 0.1 M
	0.5	3.915	0.011	
	30.0	3.223	0.703	
	46.0	2.971	0.935	
II	0.0	[5.643]	0.000	Sucrose 0.1 M Glucose 0.1 M
	0.5	5.633	0.010	
	30.0	5.033	0.610	
	46.0	4.788	0.855	
III	0.0	[1.022]	0.000	Sucrose 0.1 M Fructose 0.1 M
	0.5	1.013	0.009	
	30.0	0.468	0.554	
	46.0	0.237	0.785	

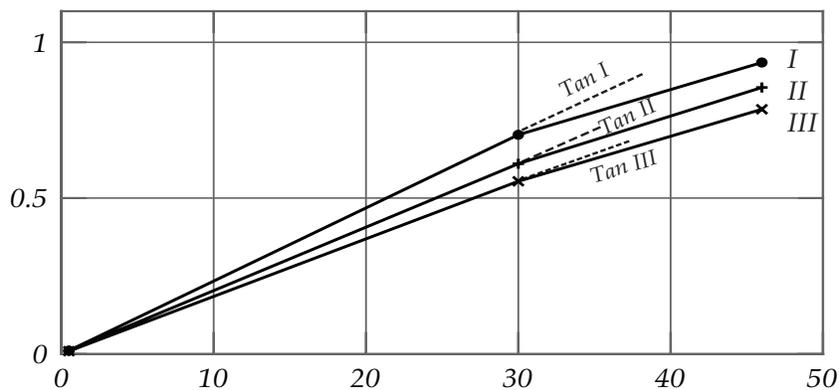


Fig 5. Graphical representation of the experiment in Table 5.
Influence of glucose and fructose.

Table 6 (Fig. 6)

	Time in minutes	Rotation	Change in rotation	Concentration
I	0.0	[5.579]	0.000	Sucrose 0.133 M
	0.5	5.568	0.011	
	30.0	4.891	0.688	
	0.0	[5.361]	0.000	Sucrose 0.133 M
	0.5	5.350	0.011	
	30.0	4.691	0.670	
	45.0	4.373	0.988	
II	0.0	[7.678]	0.000	Sucrose 0.133 M + Glucose 0.133 M
	0.5	7.665	0.013	
	30.0	7.080	0.598	
	0.0	[7.595]	0.000	Sucrose 0.133 M + Glucose 0.133 M
	0.5	7.585	0.010	
	30.0	6.971	0.624	
	45.0	6.735	0.860	

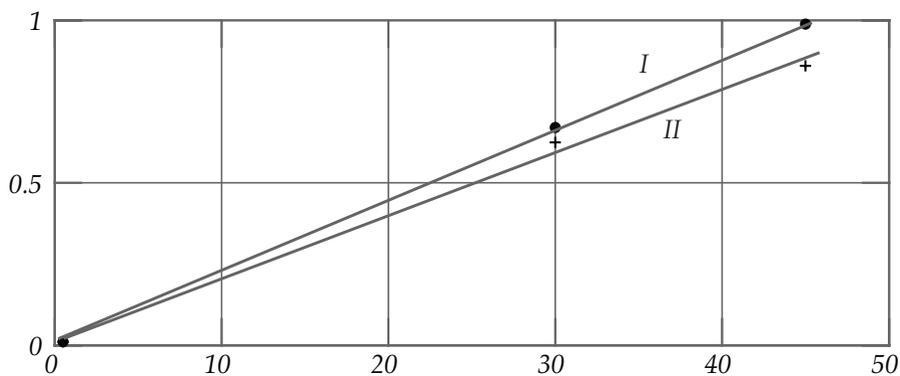


Fig. 6. Graphical representation of the experiment in Table 6. Influence of glucose.

Table 7 (Fig. 7)

	Time in minutes	Rotation	Change in rotation	Concentration
	0.0	[3.384]	0.000	Sucrose 0.0833 M
	0.5	3.358	0.026	
	10.0	3.021	0.363	
	20.0	2.691	0.693	
	30.0	2.365	1.019	
	0.0	[4.758]	0.000	Sucrose 0.0833 M Glucose 0.0833 M
	5.0	4.736	0.022	
	10.0	4.453	0.305	
	20.0	4.190	0.568	
	30.0	3.950	0.808	
	0.0	[0.885]	0.000	Sucrose 0.0833 M Fructose 0.0833 M
	5.0	0.863	0.022	
	10.0	0.570	0.315	
	20.0	0.305	0.580	
	30.0	0.083	0.802	

The protocol given describes the design of the experiment. As seen, the progress of cleavage is compared at optimal acidity and identical temperature in mixtures that are identical in terms of sucrose and enzyme but which differ in their content of fructose or glucose or in the absence of these substances. The nature of such experiments leads to certain limitations. The total concentration of sugars should not be so high that the character of the solvent is changed. In general, it is not advisable to use total concentrations of more than 0.3 M. This necessitates the use of relatively low concentrations of sucrose. This means that the rate of conversion does not stay constant for long periods, so that the progress curve deviates from linearity after small changes in optical rotation, which leads to difficulties in estimating the initial rate unless graphical extrapolation procedures are used that are not free of arbitrariness. These deviations from linearity are often more pronounced with pure sucrose

(e.g. Fig. 8, I) than in experiments with mixed sugars (Fig. 8, II), since the concentration of the inhibitory cleavage products changes relatively more strongly in the pure sucrose experiments than in experiments in which a certain amount of the inhibitory substance is present from the beginning of the experiment. The initial velocities needed for the calculations can only be obtained by graphical extrapolation: the actual curve is constructed by eye from the observed points and a tangent is estimated by eye to give the initial rate. This procedure cannot be regarded as highly accurate, but will suffice to give us a good idea of the size of the value we are interested in. The (geometrical) tangents are shown as dotted

lines in Fig. 5. The value of the ratio of the trigonometrical tangents $\frac{\text{Tan I}}{\text{Tan II}}$ is

calculated from Fig. 5 to be 1.18; the value of $\frac{\text{Tan I}}{\text{Tan III}} = 1.29$.

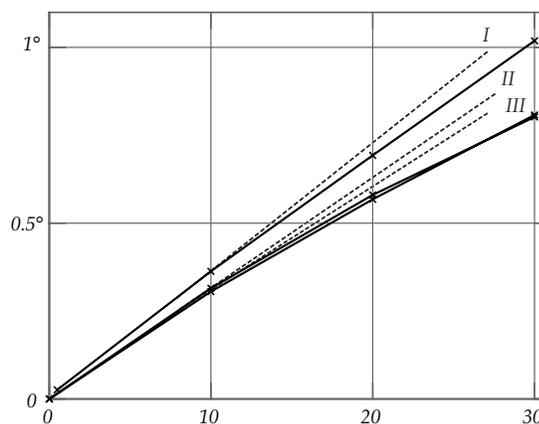


Fig. 7. Graphical representation of the experiment in Table 7.

I = Experiment with 0.0833 M sucrose

II = Experiment with 0.0833 M sucrose + 0.0833 M glucose

III = Experiment with 0.0833 M sucrose + 0.0833 M fructose

Initial tangent is shown as a dashed line.

From this experiment we now know that $\frac{v_0}{v} = 1.18$ for glucose and 1.29 for fructose. Using formula (3) from p. 16 we can calculate that

$$\frac{k_{\text{glucose}}}{k_{\text{sucrose}}} = 4.8 \quad \text{and} \quad \frac{k_{\text{fructose}}}{k_{\text{sucrose}}} = 3.0$$

Table 8 (Fig. 8)

Time in minutes		Rotation	Change in rotation	Concentration
I	0.0	[1.728]	0.000	Sucrose 0.0416 M
	0.5	1.715	0.013	
	7.0	1.552	0.176	
	14.0	1.360	0.368	
	21.0	1.168	0.560	
	28.0	0.982	0.746	
	36.0	0.862	0.866	
	44.0	0.403	1.325	
II	0.0	[-0.809]	0.000	Sucrose 0.0416 M Fructose 0.0833 M
	1.0	-0.831	0.022	
	7.0	-0.961	0.152	
	15.0	-1.116	0.307	
	22.0	-1.238	0.429	
	32.0	-1.471	0.662	

Applying the same procedure to experiment (Fig. 7), we obtain

$$\frac{\text{Tang I}}{\text{Tang II}} = 1.18 \quad \text{and} \quad \frac{\text{Tang I}}{\text{Tang III}} = 1.26$$

and therefore

$$\frac{k_{\text{glucose}}}{k_{\text{sucrose}}} = 4.6 \quad \text{and} \quad \frac{k_{\text{fructose}}}{k_{\text{sucrose}}} = 3.2$$

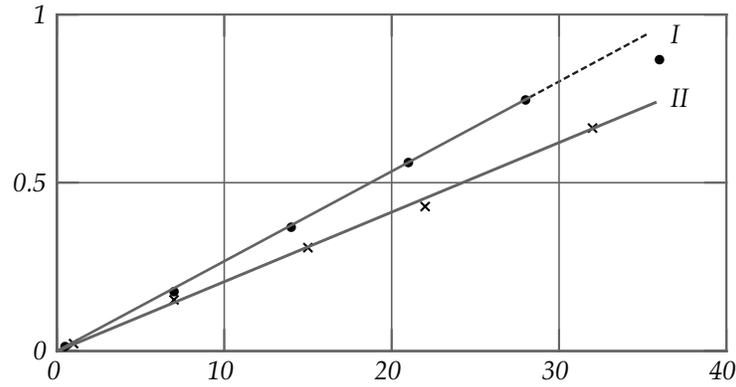


Fig. 8. Graphical representation of the experiment in Table 8.
Influence of fructose.

From the experiment (Fig. 9) we obtain the following. Note, there is no deviation from a straight line in these experiments.

$$\frac{\text{Tang I}}{\text{Tang II}} = 1.27 \quad \text{and} \quad \frac{\text{Tang I}}{\text{Tang III}} = 1.43$$

and therefore

$$\frac{k_{\text{glucose}}}{k_{\text{sucrose}}} = 5.3 \quad \text{and} \quad \frac{k_{\text{fructose}}}{k_{\text{sucrose}}} = 3.3$$

Table 9 (Fig. 9)

	Time in minutes	Rotation	Change in rotation	Concentration
I	0.0	[1.703]	0.000	Sucrose 0.0416 M
	0.5	1.698	0.015	
	7.0	1.501	0.212	
	14.0	1.335	0.378	
	21.0	1.153	0.560	
II	0.0	[3.039]	0.000	Sucrose 0.0416 M Glucose 0.0832 M
	0.5	3.031	0.008	
	7.0	2.923	0.116	
	14.0	2.745	0.294	
	21.0	2.608	0.431	
III	0.0	[-0.834]	0.000	Sucrose 0.0416 M Fructose 0.0832 M
	0.5	-0.845	0.011	
	7.0	-0.985	0.151	
	14.0	-1.096	0.262	
	21.0	-1.221	0.387	

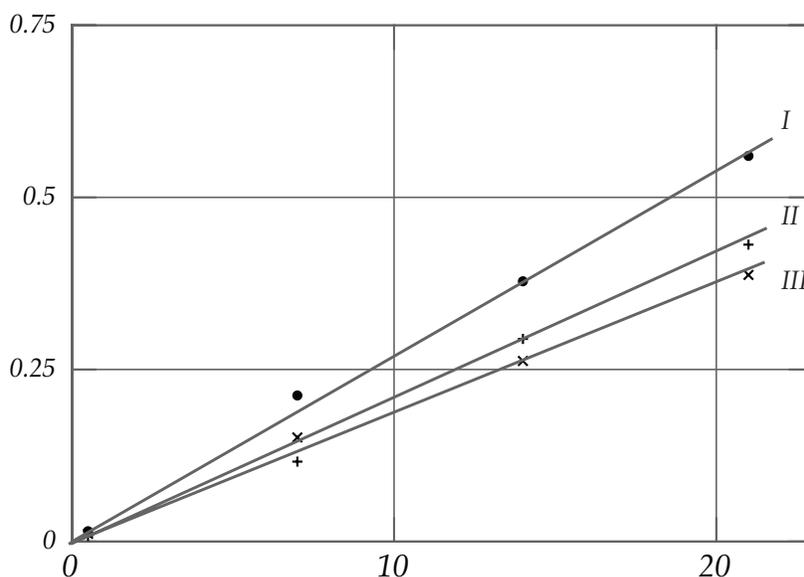


Fig. 9. Graphical representation of the experiment in Table 9. Influence of glucose and fructose.

For experiment (Fig. 6) we obtain

$$\frac{\text{Tang I}}{\text{Tang II}} = 1.133 \quad \text{so that} \quad \frac{k_{\text{glucose}}}{k_{\text{sucrose}}} = 6.7$$

For experiment (Fig. 8) we obtain

$$\frac{\text{Tang I}}{\text{Tang II}} = 1.33 \quad \text{so that} \quad \frac{k_{\text{fructose}}}{k_{\text{sucrose}}} = 4.3$$

Summarizing these data, we have

				Average
$\frac{k_{\text{glucose}}}{k_{\text{sucrose}}} = 4.7$	4.6	5.3	6.7	5.3
$\frac{k_{\text{fructose}}}{k_{\text{sucrose}}} = 3.0$	3.2	3.3	4.3	3.45

Using (3), p. 16, this leads to the following values for the dissociation constants:

$$\text{Glucose-invertase complex} = 0.088 \text{ M}$$

$$\text{Fructose-invertase complex} = 0.058 \text{ M}$$

The inhibitory influence of other substances was measured in the same manner. Before doing this, as a test for the correctness of the procedure described above, we had to show that foreign substances that were expected to have no affinity to invertase did not inhibit the cleavage of cane sugar as long as their concentration did not change the character of the solvent. We therefore convinced ourselves again that a 0.1 normal concentration of potassium chloride had

absolutely no inhibitory effect and that even a normal concentration had no significant effect (Tables 10 and 13).

Table 10²⁸⁾

	Time in minutes	Rotation	Change in rotation	Concentration
A	0.0	[3.901]	0.000	Sucrose 0.1 M
	0.5	3.881	0.020	
	33.0	2.540	1.361	
	59.0	1.716	2.185	
B	0.0	[3.878]	0.000	Sucrose 0.1 M Calcium chloride 0.1 M
	0.5	3.858	0.020	
	33.0	2.561	1.317	
	59.0	1.693	2.185	
V	0.0	[3.907]	0.000	Sucrose 0.1 M Mannitol 0.1 M (cf Table 14)
	0.5	3.885	0.020	
	33.0	2.573	1.334 (1.23)	
	59.0	1.761	2.146 (1.95)	
C	0.0	[4.001]	0.000	Sucrose 0.1 M + 1 M-Alcohol
	0.5	3.985	0.016	
	33.0	2.935	1.006 (1.07)	
	59.0	2.141	1.860	
D	0.0	[3.971]	0.000	Sucrose 0.1 M + Alcohol 0.2 M
	0.5	3.951	0.020	
	33.0	2.601	1.370	
	59.0	1.868	2.103	

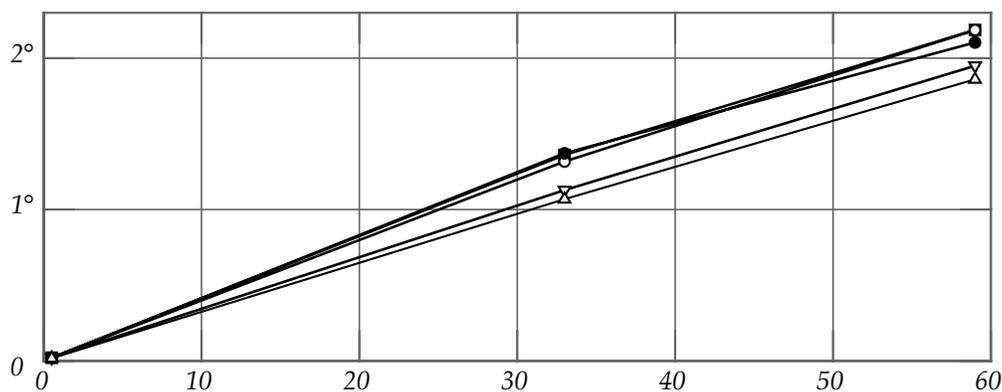


Fig. 10. Graphical representation of the experiment in Table 10. ■○● Trial A, B, D. ▽ Trial V (Glycerin 0.1 M). △ Trial C (Alcohol 1 M).

At a concentration of 0.2 M, ethanol does not show the slightest inhibitory effect (Table 10). In contrast, there is a slight inhibition at normal concentration, which is without doubt due to a change in the character of the solvent and does not

²⁸⁾ There was a discrepancy between the numbers in Table 10 and Fig. 10. In order to reproduce Fig. 10, we measured values from the figure using a micrometer to get the numbers shown in parentheses and used these values to create Fig 10.

arise from an affinity of the enzyme to alcohol. If one wished to calculate the effect in terms of an affinity as done previously, graphical estimation of the ratio

$$\frac{k_{\text{alcohol}}}{k_{\text{sucrose}}}$$

would give a value of 36. Such a weak affinity can be equated to 0 within error limits (i.e. $k_{\text{alcohol}} = \infty$), especially when we bear in mind that another inhibitory factor, namely the change in character of the solvent, certainly plays a role.

The investigation of other carbohydrates or of poly-alcoholic substances was now of particular interest.

Table 11.

Time in minutes	Rotation	Change in rotation	Concentration
0.0	[2.081]	0.000	Sucrose 0.05 M
0.5	2.065	0.016	
20.0	1.386	0.695	
50.0	0.548	1.533	
0.0	[5.373]	0.000	Sucrose 0.05 M + 0.1 M-Lactose (Milk sugar)
0.5	5.358	0.015	
20.0	4.750	0.628	
50.0	3.815	1.558	
0.0	[8.805]	0.000	Sucrose 0.05 M + 0.2 M-Lactose
0.5	8.790	0.015	
20.0	8.168	0.637	
50.0	7.315	1.490	

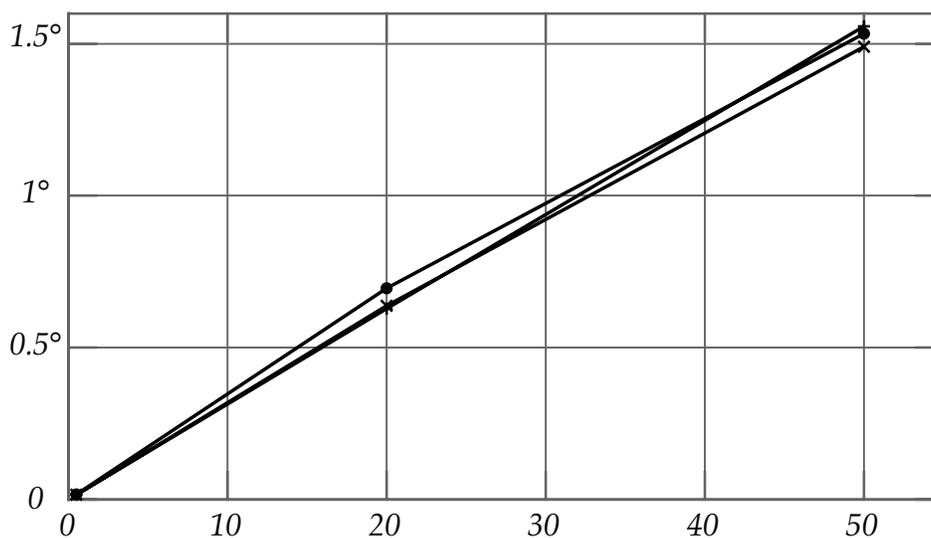


Fig. 11. Graphical representation of the experiment in Table 11.
Effect of lactose.

The behavior of milk sugar was of special interest (Tables 11 and Fig. 11). Its inhibitory influence was so slight, that it was hardly detectable inside the error limits. If we evaluated the very slight signal changes, we would find

$$\frac{k_{\text{lactose}}}{k_{\text{sucrose}}} = \begin{array}{cc} \text{Experiment 1} & \text{Experiment 2} \\ \text{at least 30} & 36 \end{array}$$

Since we cannot say whether the small effects can be used reliably, we have to be satisfied with the statement that an affinity of milk sugar to invertase is not measurable with certainty. This is in agreement with our expectations, since binding of a disaccharide such as lactose to invertase would lead to hydrolysis, as is the case for sucrose, whereas lactose is not cleaved.

Mannose.

An experiment gave (Tables 12 and Fig. 12)

$$\frac{k_{\text{mannose}}}{k_{\text{sucrose}}} = 5.0$$

Table 12.

Time in minutes	Rotation	Change in rotation	Concentration
0.0	[3.901]	0.000	Sucrose 0.1 M
0.5	3.881	0.020	
33.0	2.540	1.361	
59.0	1.716	2.185	
0.0	[4.717]	0.000	Sucrose 0.1 M + Mannose 0.2 M
0.5	4.703	0.014	
33.0	3.778	0.939	
59.0	2.887	1.830	

For a more accurate determination, multiple repeated experiments would be needed. However, it can be seen that the affinity of mannose and glucose are similar.

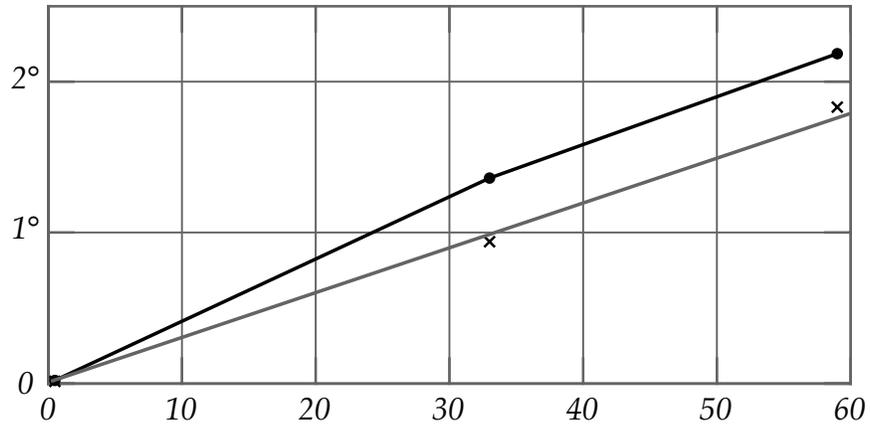


Fig. 12. Graphical representation of the experiment in Table 12. Effect of mannose.

Mannitol

The inhibitory effect was low. This example was used to determine a weak affinity quantitatively by adequate variation of experimental conditions.

Table 13

	Time in minutes	Rotation	Change in rotation	Concentration
<i>I</i>	0.0	[3.928]	0.000	Sucrose 0.1 M
	0.5	3.908	0.020	
	33.0	2.610	1.318	
	59.0	1.751	2.177	
<i>IIa</i>	0.0	[3.971]	0.000	Sucrose 0.1 M + Mannitol 0.1 M
	0.5	3.953	0.018	
	33.0	2.760	1.211	
	59.0	1.747	2.224	
<i>IIb</i>	0.0	[3.907]	0.000	Sucrose 0.1 M + Mannitol 0.1 M
	0.5	3.885	0.020	
	33.0	2.573	1.334	
	59.0	1.761	2.146	
<i>III</i>	0.0	[3.948]	0.000	Sucrose 0.1 M + Mannitol 0.25 M
	0.5	3.930	0.018	
	33.0	2.711	1.237	
	59.0	1.938	2.010	
<i>IV</i>	0.0	[3.953]	0.000	Sucrose 0.1 M + Mannitol 0.5 M
	0.5	3.938	0.015	
	33.0	2.917	1.036	
	59.0	2.205	1.748	
<i>V</i>	0.0	[3.921]	0.000	Sucrose 0.1 M + Mannitol 0.75 M
	0.5	3.910	0.011	
	33.0	3.163	0.758	
	59.0	2.348	1.573	
	0.0	[3.952]	0.000	Sucrose 0.1 M Calcium chloride 1 M
	0.5	3.933	0.019	
	33.0	2.700	1.252	
	59.0	1.744	2.208	

Table 14.

Time in minutes	Rotation	Change in rotation	Concentration
0.0	[2.081]	0.000	Sucrose 0.05 M
0.5	2.065	0.016	
20.0	1.386	0.695	
50.0	0.548	1.533	
<i>VII</i> 0.0	[1.993]	0.000	
0.5	1.980	0.013	
20.0	1.447	0.546	
50.0	0.685	1.308	
<i>VI</i> 0.0	[2.004]	0.000	Sucrose 0.05 M + Mannitol 0.1 M
0.5	1.990	0.014	
20.0	1.403	0.601	
50.0	0.627	1.377	

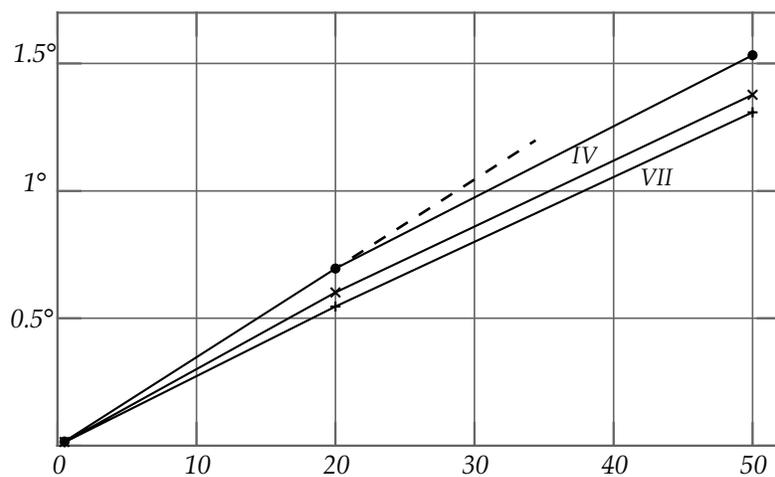
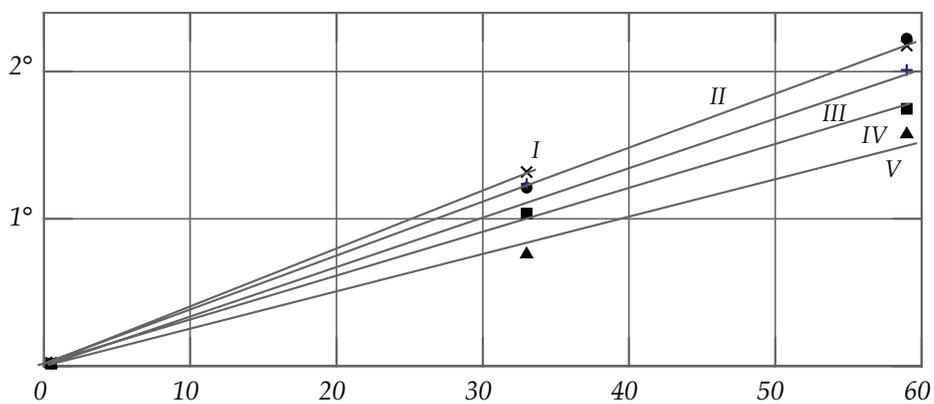


Fig. 13. (corresponding to Table 13) and Fig. 14 (corresponding to Table 14).
Effect of mannose.

The following can be concluded from Table 13 and Fig. 13: The influence of 0.1 M mannitol on the cleavage of 0.1 M sucrose cannot be measured reliably. On increasing the amount of mannitol while keeping the amount of sucrose constant, the influence becomes gradually more obvious. From the procedure described above we obtain

Experiment	III	IV	V	VI	VII
$\frac{k_{\text{mannitol}}}{k_{\text{sucrose}}} =$	17	13.4	10.5	11.4	11.4

Considering the small signals, the agreement is not bad, and the average value of

$$\frac{k_{\text{mannitol}}}{k_{\text{sucrose}}} = 13$$

should give a reasonable impression of the relative affinities.

Glycerin.

We have obtained the experimental series Fig. 15, Table 15 and an individual experiment (Fig. 10). We find

Experiment	II	III	IV	V
$\frac{k_{\text{glycerin}}}{k_{\text{sucrose}}} =$	3.4	5.6	3.9	5.1, with an average of 4.5.

Thus, glycerin has, against expectations, a high affinity to invertase.

Summarizing the dissociation constants, we have:²⁹⁾

Sucrose	k = 0.0167	or	1/60
Fructose	k = 0.058	"	1/17
Glucose	k = 0.089	"	1/11
Mannose	k = ca. 0.083	"	1/12
Glycerin	k = ca. 0.075	"	1/13
Mannitol	k = 0.22	"	1/4.5
Lactose	at least k = 0.5	"	1/2
	(probably approaching ∞)		

To help understand these values, it should be noted that an increase in the dissociation constant corresponds to a decrease of the affinity of the enzyme to the respective substance. Thus, the affinity of sucrose is by far the largest.

²⁹⁾ In units of M.

Table 15.

Time in minutes	Rotation	Change in rotation	Concentration
<i>I</i> 0.0	[6.783]	0.000	Sucrose 0.166 M
0.5	6.770	0.013	
30.0	5.975	0.808	
0.0	[6.652]	0.000	Sucrose 0.166 M
0.5	6.646	0.006	
60.0	5.470	1.182	
<i>II</i> 0.0	[6.672]	0.000	Sucrose 0.166 M + Glycerin 0.453 M
1.0	6.650	0.022	
30.5	6.008	0.664	
49.0	5.690	0.982	
<i>III</i> 0.0	[6.826]	0.000	Sucrose 0.166 M + Glycerin 0.453 M
0.5	6.813	0.013	
30.0	6.013	0.813	
49.0	5.961	0.865	
<i>IV</i> 0.0	[6.789]	0.000	Sucrose 0.166 M + Glycerin 0.906 M
0.5	6.781	0.006	
30.0	6.433	0.354	
49.0	6.321	0.466	

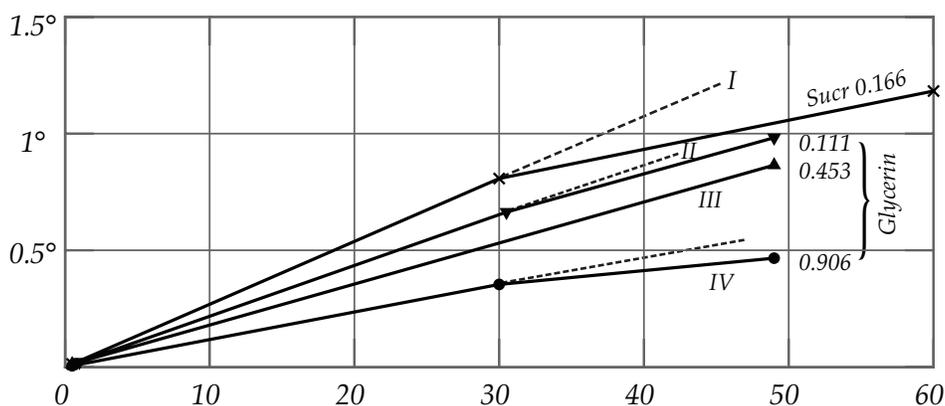


Fig. 15. Graphical representation of the experiment in Table 15. Effect of glycerin. Experiment V is listed in Table 10.

The dissociation constant for the invertase-sugar complex is defined as

$$\frac{[\text{enzyme}] \cdot x[\text{sugar}]}{[\text{enzyme-sugar-complex}]}$$

so we can define the reciprocal value

$$\frac{[\text{enzyme-sugar-complex}]}{[\text{enzyme}] \cdot x[\text{sugar}]}$$

as the affinity constant of the enzyme to the sugar. Thus we have:

Sucrose	60
Fructose	17
Glucose	11
Mannose ca.	12
Glycerin	13
Mannitol	4.5
Lactose	} (i.e. immeasurably small)	0
Ethyl alcohol		

3. The reaction equation of the fermentative splitting of cane sugar.

On the basis of these data, we are now able to solve the old problem of the reaction equation of invertase in a real manner without resorting to the use of more than one arbitrary constant. Of all authors, V. Henri was closest to this solution, and we can regard our derivation as an extended modification of Henri's derivation on the basis of the newly gained knowledge.

The basic assumption in this derivation is that the decay rate at any instant is proportional to the concentration of the sucrose-invertase complex and that the concentration of this complex at any instant is determined by the concentration of enzyme, of sucrose and of reaction products that are able to bind to the enzyme. Whereas Henri introduced an "affinity constant for the cleavage products", we operate with the dissociation constant of the sucrose-enzyme complex, $k = 1/60$, with that of the fructose-enzyme complex, $k = 1/17$, and with that of the glucose-enzyme complex, $k = 1/11$.

We also use the following designations:

Φ = the total enzyme concentration

φ = the concentration of the enzyme-sucrose complex

Ψ_1 = the concentration of the enzyme-fructose complex

Ψ_2 = the concentration of the enzyme-glucose complex

S = the concentration of sucrose

F = the concentration of fructose

G = the concentration of glucose

} i.e. the concentration of the respective sugar in the free state, which is practically equal to the total concentration.

Since the cleavage yields equal amounts of fructose and glucose, G is always equal to F .

According to the law of mass action, at any instant

$$S \cdot (\Phi - \varphi - \psi_1 - \psi_2) = k \cdot \varphi \quad \dots\dots\dots (1)$$

$$F \cdot (\Phi - \varphi - \psi_1 - \psi_2) = k_1 \cdot \psi_1 \quad \dots\dots\dots (2)$$

$$G \cdot (\Phi - \varphi - \psi_1 - \psi_2) = k_2 \cdot \psi_2 \quad \dots\dots\dots (3)$$

From (1) it follows that

$$\varphi = \frac{S \cdot (\Phi - \psi_1 - \psi_2)}{S + k} \quad \dots\dots\dots (4)$$

We can eliminate ψ_1 and ψ_2 by first dividing (2) by (3) to give

$$\psi_2 = \frac{k_1}{k_2} \cdot \psi_1,$$

and further by dividing (1) by (3) to give

$$\psi_1 = \frac{k}{k_1} \cdot \varphi \cdot \frac{F}{S},$$

so that

$$\psi_1 + \psi_2 = k \cdot \varphi \cdot \frac{F}{S} \left(\frac{1}{k_1} + \frac{1}{k_2} \right).$$

For abbreviation we substitute

$$\frac{1}{k_1} + \frac{1}{k_2} = q$$

so that

$$\psi_1 + \psi_2 = k \cdot q \cdot \varphi \cdot \frac{F}{S}.$$

Substituting in (4), this gives

$$\varphi = \Phi \cdot \frac{S}{S + k \cdot (1 + q \cdot F)} \quad \dots\dots\dots (4)^{30}$$

We can now proceed to the differential equation. If

a is the starting amount of sucrose

t is the time

x is the amount of fructose or glucose, so that

$a-x$ is the remaining amount of sucrose at time t , the decay velocity at time t is defined by

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

³⁰ Note the duplicate use of equation number (4).

According to our assumptions, this is proportional to φ , so that the differential equation derived using equation (4) is:

$$\frac{dx}{dt} = C \cdot \frac{a-x}{a+k-x \cdot (1-k \cdot q)} \dots \dots \dots (5)$$

where C is the only arbitrary constant, which is proportional to the amount of enzyme.³¹⁾

The general integral of the equation can be calculated without difficulty:

$$C \cdot t = (1-k \cdot q) \cdot x - k \cdot (1+a \cdot q) \cdot \ln(a-x) + const$$

To eliminate the integration constant, we substitute the values of $x=0$ and $t=0$ for the start of the process to give ³²⁾

$$0 = -k \cdot (1+a \cdot q) \cdot \ln a + const$$

and find by subtraction of the last two equations the definite integral

$$C \cdot t = k \cdot (1+a \cdot q) \cdot \ln \frac{a}{a-x} + (1-k \cdot q) \cdot x \dots \dots \dots (6)$$

or on substituting the value for q:

$$\frac{k}{t} \cdot \left(\frac{1}{a} + \frac{1}{k_1} + \frac{1}{k_2} \right) \cdot a \cdot \ln \frac{a}{a-x} + \frac{k}{t} \cdot \left(\frac{1}{k} - \frac{1}{k_1} - \frac{1}{k_2} \right) \cdot x = C$$

We can now incorporate k into the constant on the right hand side of the equation and obtain

$$\frac{1}{t} \cdot \left(\frac{1}{a} + \frac{1}{k_1} + \frac{1}{k_2} \right) \cdot a \cdot \ln \frac{a}{a-x} + \frac{1}{t} \cdot \left(\frac{1}{k} - \frac{1}{k_1} - \frac{1}{k_2} \right) \cdot x = const \dots (7)$$

Like the Henri function, this is characterized by a superposition of a linear and a logarithmic function of the type

$$m \cdot \ln \frac{a}{a-x} + n \cdot x = t \cdot const \dots \dots \dots (8)$$

where the meaning m and n can be seen by inspection of the previous equation: they are factors whose magnitude is dependent on the respective dissociation constants and starting quantity of the sugar.

³¹ This is not the C used in the earlier equations; rather, it includes the enzyme concentration and, as described below, a conversion from degrees of optical rotation to fractional conversion of substrate to product (x/a), so $C = k_{cat} \cdot E_0$.

³² We corrected a sign error here that was not propagated to the next equation.

Substituting the determined values of k , k_1 and k_2 at 25° we obtain

$$\frac{1}{t} \cdot (1 + 28 \cdot a) \cdot 2.303 \cdot \log_{10} \frac{a}{a-x} + \frac{1}{t} \cdot 32 \cdot x = \text{const} \dots \dots \dots (9)$$

Instead of $\log \frac{a}{a-x}$ we use the simpler expression $-\log \left(1 - \frac{x}{a} \right)$

This constant must be proportional to the quantity of enzyme. That this is the case was shown by L. Michaelis and H. Davidsohn (l.c. p. 398-400), who demonstrated that an equation of the form

$$\text{enzyme quantity } x \text{ time} = f(a, x) \dots \dots \dots (10)$$

is strictly followed. The hitherto unknown function of the right hand side of the equation finds its definitive form in our equation (8). Otherwise nothing is changed and it can be easily seen that the constant in equation (8) must be proportional to the enzyme concentration.

While it is not necessary to test the correctness of equation (9) for varying amounts of enzyme, it still has to be tested whether the constant has the same value if the amount of enzyme is kept constant and the amount of sugar is varied, and whether the constant in a single experiment is independent of the time.

For these calculations, we use the data from experimental series I, and must first convert the values for x , for which we have so far used arbitrary polarimetric units, into concentration units. To do this we use the observation that the theoretical rotation of a sucrose solution which originally shows a rotation of m° is $-0.313 \times m^\circ$ after complete cleavage of the sugar (cf. Sørensen, l.c., p. 262).

<i>Time (t)</i>	<i>x/a</i>	<i>const</i> ³³⁾	<i>Average</i>
I. Sucrose 0.333 M			
7	0.0164	0.0496	
14	0.0316	0.0479	
26	0.0528	0.0432	
49	0.0923	0.0412	
75	0.1404	0.0408	
117	0.2137	0.0407	
1052	0.9834	[0.0498]	0.0439
II. Sucrose 0.1667 M			
8	0.0350	0.0444	
16	0.0636	0.0446	
28	0.1080	0.0437	
52	0.1980	0.0444	
82	0.3000	0.0445	
103	0.3780	0.0454	0.0445
III. Sucrose 0.0833 M			
49.5	0.352	0.0482	
90.0	0.575	0.0447	
125.0	0.690	0.0460	
151.0	0.766	0.0456	
208.0	0.900	0.0486	0.0465
IV. Sucrose 0.0416 M			
10.25	0.1147	0.0406	
30.75	0.3722	0.0489	
61.75	0.615	0.0467	
90.75	0.747	0.0438	
112.70	0.850	0.0465	
132.70	0.925	0.0443	
154.70	0.940	0.0405	
1497.00	0.972	[0.0514]	0.0445
V. Sucrose 0.0208 M			
17	0.331	0.0510	
27	0.452	0.0464	
88	0.611	0.0500	
62	0.736	0.0419	
95	0.860	[0.0388]	
1372	0.990	[0.058]	0.0474

Average of all average values: 0.0454

The value of the constant is very similar in all experiments and despite small variation shows no tendency for systematic deviation neither with time nor with sugar concentration, so that we can conclude that the value is reliably constant.

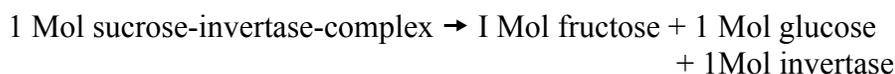
³³ The term, $const = E_0 \cdot k_{cat} / K_m$, which would define the specificity constant if the enzyme concentration were known. In this table, Michaelis and Menten to calculate an average value, representing a global fit to their full time course data including product inhibition.

Summary

The progress of invertase action is understandable based on the following assumptions:

Sucrose binds to invertase to give a complex with a dissociation constant of 0.0167.

This complex is unstable as a consequence of the equation



Invertase has an affinity to the cleavage products, fructose and glucose, as well as to several other higher alcohols (mannitol, glycerin) and carbohydrates (remarkably not to milk sugar), but this affinity is much lower than to sucrose. Since these complexes are not labile,³⁴) they do not lead to a chemical cleavage reaction, but manifest themselves only in the inhibitory action of fructose etc. on the sucrose-invertase-process.

The concentration of all these complexes can be calculated according to the law of mass action and the dissociation constant for each complex can be given fairly accurately, most accurately for the sucrose-invertase-complex.

Since the decay of the sucrose-invertase-complex must be a monomolecular reaction, the respective decay rate of the sucrose is directly proportional to the concentration of the sucrose-invertase-complex.

Based on all these assumptions, a differential equation for the progress of the sucrose cleavage can be derived, whose integral is in good agreement with observations.

³⁴ The authors mean the complexes of invertase formed with other sugars are not labile in terms of cleavage of chemical bonds.