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The kinetics of invertin action

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Enzyme kinetics has been most studied with invertin where the simple reaction may be followed so easily that there are especially good prospects of reaching the goal of kinetics research, namely to understand the essence of a reaction by means of observations on its course. The most important work up to now has been by Duclaux,[1] O'Sullivan and Tompson,[2] A.J. Brown[3] and particularly V. Henri.[4] The investigations of Henri are especially important because starting from reasonable ideas on the nature of enzyme action he arrives at a mathematical formulation of the course of enzyme action which fits the facts quite well, at many points. In this work, we also start out from these ideas of Henri. We undertook to re-investigate the whole work because Henri did not take into account two influences which are of very great importance and the neglect of which in Henri's work now appears so serious that a new investigation is worthwhile. The first is the effect of hydrogen ion concentration ($[H^+]$), the second the effect of mutarotation.

The effect of hydrogen ion concentration is today fully clarified, through the work of Sørensen[5] and Michaelis and Davidsohn.[6] In all his work, Henri took no account of $[H^+]$, so that if his experiments were actually at constant $[H^+]$ this could only have been by chance. In the present work this has been taken into account simply by adding an acetate mixture, which gave the solution the H^+-concentration 2×10^{-5}, which on the one hand is the optimal $[H^+]$ for the action of the enzyme and on the other is the $[H^+]$ where accidental minor deviations of $[H^+]$ are least significant, since in the region of the optimal $[H^+]$ the dependence of enzyme action on $[H^+]$ is minimal.

But hardly less important in Henri's work is neglect of the fact that, during inversion, glucose is first produced in its mutarotating form and eventually spontaneously converts into the normal form. Thus if one observes the course of inversion by observations of the angle of rotation, the true picture of inversion is distorted in that inversion of sucrose and the change of rotation of newly formed glucose are superposed. One could take this into account by calculating the change of rotation due to glucose. But this is not very practical since very complicated functions are involved which can be avoided more easily experimentally. The better way is to take samples of the mixture from time to time during the course of inversion, to interrupt the enzyme action and await arrival of the normal rotation of glucose before taking readings. Sørensen used sublimate; we use a soda solution which simultaneously renders invertin inactive and completes mutarotation within a few minutes.

Hudson[7] had already used the method of eliminating mutarotation by the action of alkali, but arrived at a quite different conclusion from ours on the course of invertin action. He claims, in fact, that if one eliminates mutarotation, inversion by invertin becomes a simple logarithmic function like that of inversion by acid. This result stands in contradiction to all earlier observations and also does not once agree with our experiments, within even coarse approximation. Although the researches of Henri are doubtless capable of improvement, this error is not so great as Hudson claims. (Sørensen has also already found that the results of Hudson are discrepant.) We claim, on the contrary, that the assumptions from which Henri set out are thoroughly reasonable and we shall therefore attempt to set out from the same foundations and to control the methods better. It turns out that in principle at least Henri's ideas are completely correct, and the observations agree with them even better than Henri's own experiments.

Henri had already shown that the lysis products of inversion, glucose and fructose, have an inhibitory influence on invertin action. As a matter of principle we have worked here in such a way that the inhibitory influence of the lysis products — at first minimal — is not corrected for by calculation but eliminated experimentally. As the influence is not very large, it is easy to carry out this principle. At various initial concentrations of sucrose, one follows the inversion only so far as the influence of the lysis products formed is still not detectable experimentally. Thus, at first, in all experiments we follow only the initial velocity of inversion at various initial concentrations of sucrose. The action of the lysis products in special experiments is then easily observed.

1. The initial velocity of inversion at varying sucrose concentration

The influence of sucrose concentration upon enzymatic inversion has already been studied by all the cited preceding authors with results something like the following. At certain intermediate sucrose concentrations, the velocity of inversion is scarcely dependent at all on the amount of sugar, it is constant for a given amount of enzyme, but diminishes at lower and also at higher sugar concentrations. Our own experiments were carried out as follows. A chosen amount of a known sucrose solution was mixed with 20 cm^3 of a mixture of equal parts of 1/5 mol/L acetic acid and 1/

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5 mol/L sodium acetate, with a fixed amount of enzyme, and water to bring to a constant volume of 150 cm^3. All solutions were well warmed in advance in a large water-bath, accurate to 25.0± <0.05°, and held at this temperature during the reaction. The first sample was removed as quickly as possible after mixing the solutions, and then further samples taken at suitable intervals. Each sample, of 25 cm^3, was mixed in a flask with 3 cm^3 of a 1/2n soda solution and the enzyme action thus interrupted. The solution was measured polarimetrically after about half-an-hour. The initial rotation was extrapolated from the first effective reading. Since this extrapolation was at most a few hundredths of a degree, it was permissible in all cases. Most importantly, it was checked that the mutarotation of the sugar was effectively complete by repeating the reading after half-an-hour. The reading given in the results is the mean of 6 individual readings which only differed by a few hundredths of a degree. If we show the decrease in rotation as a function of time in an individual experiment we see that for a reasonable distance after the outset, rotation decreases with time in a linear manner. We take as initial velocity of inversion the decrease in rotation per minute during this initial period, when it can still be regarded as linear. The experiments gave the results shown in Figs. 1–4.

To interpret these experiments we shall make the assumption, with Henri, that invertin forms a compound with sucrose which is very labile and breaks down into free enzyme, glucose and fructose. From our experiments, we shall test whether this assumption is justified. If this assumption is correct, the velocity of inversion must be proportional to the instantaneous concentration of sucrose–enzyme compound. If 1 mol of enzyme plus 1 mol of sucrose yields 1 mol of sugar–enzyme compound, the Law of Mass Action states,

$$[S] \cdot [\Phi - \phi] = k\varphi \tag{1}$$

Here $[S]$ is the concentration of free sucrose, or, since only a vanishingly small part of the sucrose is segregated by the enzyme, also the total concentration of sucrose; Φ is the total molar enzyme concentration, ϕ the concentration of bound enzyme or of enzyme–sugar compound, thus $(\Phi - \phi)$ is the concentration of free enzyme; k is the dissociation constant. Hence it follows that

$$\phi = \Phi \cdot \frac{[S]}{[S] + k} \tag{2}$$

This quantity must thus always be proportional to the initial velocity of inversion, v, or

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

where C is the proportionality factor. Since we measure v experimentally in an arbitrary system of units (change of rotation per minute) and since ϕ will be held constant in one series of experiments, we can write $v/C\phi$ simply as V. V is thus an arbitrary function, but one which is proportional to the true initial velocity. Thus

$$V = \frac{[S]}{[S] + k} \tag{4}$$

This function is formally the same as the dissociation curve of an acid[8]

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

and we can best represent it graphically, as earlier, by placing the logarithm of the independent variable on the abscissa. We thus represent V as a function of $\log[S]$, and must obtain the well-known dissociation curve. Here, we still do not know what to select as the unit for the ordinate. We know only that the maximal value of V (estimated asymptotically) must be 1 and that the perpendicular from the ordinate having the value 1/2 must indicate the logarithm of k. To determine this scale we use the following graphical procedure. We first assume that we have obtained a number of experimental points, which give a curve of the form of a dissociation curve. Since the scale in which we measure the ordinates of these points is arbitrary, it is assumed that this scale is different from that of the abscissa. The function which we represent graphically is, if $s = \log[S]$

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

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

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

By differentiation we obtain

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

This differential expression represents the tangent of the slope of the section of the curve concerned. Now, the dissociation curve has a section whose slope is particularly easily determined, since this section displays almost exactly a straight line over a long stretch. This is the middle part of the curve, particularly the point where the ordinate = 1/2. We know (from the just-cited work) that this ordinate corresponds to the point $\log k$ on the abscissa. If we now set V equal to 1/2 and s equal to $\log k$ (or e^{ps} equal to k in our differential expression) then

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

Thus the middle, almost linear, section of the curve has a slope of 0.576 (that is, a slope of almost exactly 30°). This holds, naturally, for the assumption that ordinate and abscissa were measured in the same units. Now we join up the experimental points of the middle part of the curve with a straight line and find, say, that the tangent to its slope = v. Thence we can conclude that the ratio of the ordinate units to abscissa units must be $0.576 : v$, that is, the ordinate unit is $v/0.576$ times the abscissa unit. Now we can mark off the correct scale on the ordinate (cf. Figs. 1b–4b: "rational units"). We first fix the position of the point 0.5 using this new scale. The ordinate corresponding to this point gives, at its perpendicular to the abscissa, the value of $\log k$. Now we know k and can graphically construct the whole dissociation curve point for point. We do this and shall examine whether all the observations fit this curve well and above all, that the value 1 is not significantly exceeded. If we use this procedure for our experiments, we determine for each curve a value of v; we then construct the curves and obtain very good superposition of the observed points with the calculated, with one exception to be mentioned soon.

A second method for graphical determination of the scale of the ordinate is as follows. If at the right-hand end of the curve there are several well-determined points of which one can say that for practical purposes they attain the maximal value of the ordinate, one may simply set the appropriate ordinate = 1. One then again constructs the oblique middle part of the curve by joining together the observed points in this section with a straight line and determines which of these points corresponds to ordinate 0.5 in the new scale. Thus again we obtain all the data for construction of the curve.

The first method is chosen if the middle points of the curve are better defined, the second if the points towards the right-hand end

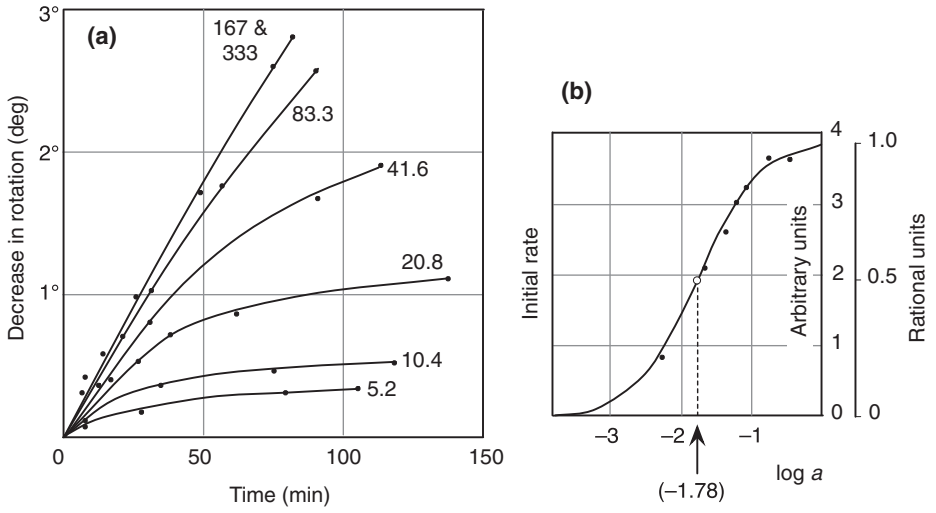

Fig. 1. (a) Abscissa: time in minutes. Ordinate: decrease in rotation in degrees. Each curve is from a single experiment at the initial concentration of sucrose shown (mmol/L), except that for 83.3 mmol/L which is from two parallel experiments. Amount of enzyme the same in each case. (b) Abscissa: logarithm of the initial concentration of sucrose. Ordinate: initial velocity of hydrolysis expressed as decrease in rotation (in degrees) per minute, obtained graphically from Fig. 1a. For "rational units" of the ordinate, see text.

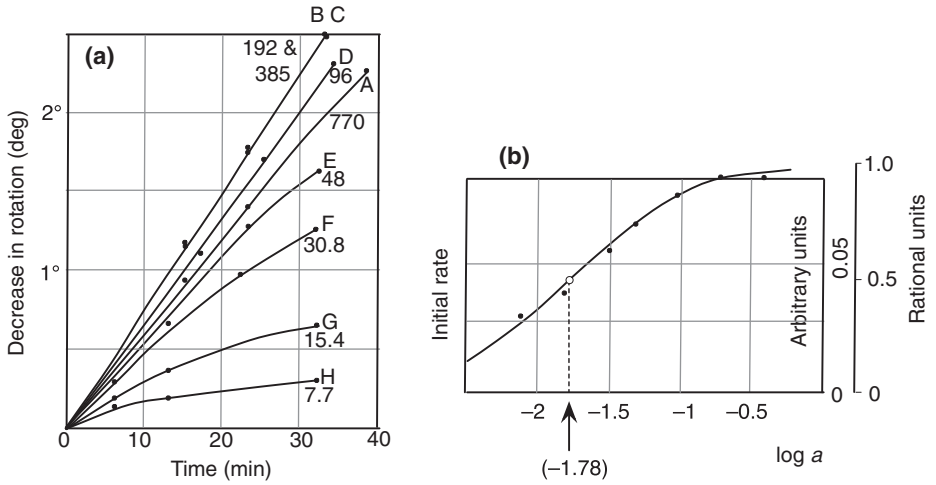

Fig. 2. (a) Notation as Fig. 1. Experimental series 2. Amount of enzyme about double that of the experiments of Fig. 1. (b) Representation as Fig. 1b; calculated from the experiments of Fig. 2a.

are experimentally better established. Where possible, both methods are used. Equality of the results is then convincing; failing this, in the case of a small difference in the measured dissociation constants, the mean of the two graphical results is chosen as the most probable value. All the curves shown were obtained by these two methods. In each of the 4 cases (the curves in Figs. 1b–4b) a family of dissociation curves was thus constructed, for all possible values of the scale units of the ordinate which fell within the range of probability on the basis of graphical estimations by the two methods described, and the best fit sought for each by shifting it to right and left, up and down, until all the observed points fitted it as well as possible. It is possible to find thus a curve in each of the 4 cases, such that the observed points agree with it within the range of experimental error, even though the 4 series of experiments were carried out with quite different amounts of enzyme.

The dissociation constants of the invertin-sucrose compound found by these graphical methods were, in the individual experiments:

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

Agreement is good, even though the individual series of experiments were carried out with different amounts of enzyme.[9] We have here for the first time a picture of the magnitude of the affinity of an enzyme for its substrate, and we have measured here for the first time "specific" affinity following van't Hoff's definition of chemical affinity value.

The meaning of this affinity constant is as follows. If we could prepare the enzyme-sucrose compound pure and dissolve it in water in such concentration that the undissociated part of it was at 1 mol/L, there would also be in the solution $\sqrt{0.0167}$ or 0.133 mol of free enzyme and an equal amount of free sucrose. The accuracy with which it is possible to estimate k is of different degree in the 4 different experiments (Figs. 1b–4b). To the inexperienced observer, the inevitably arbitrary fitting of experimental points will probably appear doubtful. And yet that amounts to very little. For example, the worst of our 4 curves is perhaps that of Fig. 3b. Graphically, we here find $\log k = -1.8$. We could possibly calculate the curve so that $\log k$ was −1.9 or −1.7, but, already, the assumption that $\log k$ was −2.0 would no longer be compatible with the shape of a dissociation curve and the same holds for the assumption $\log k = -1.5$. The latitude for the chosen value of k is

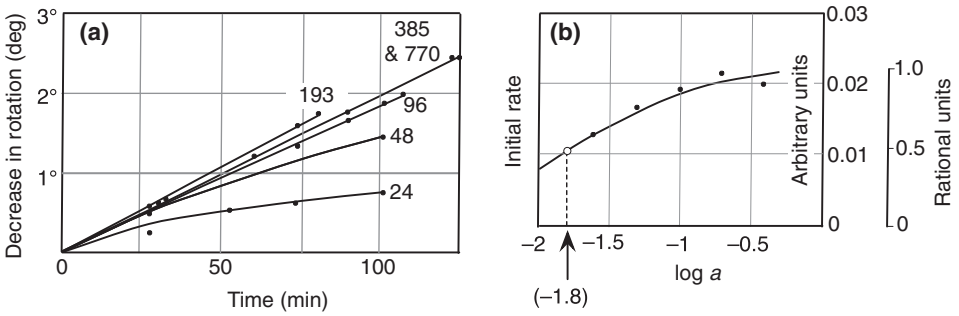

Fig. 3. (a) Experimental series 3. Amount of enzyme about half as much as in Fig. 1. (b). Representation as Fig. 1, calculated from the experiments of Fig. 3(a).

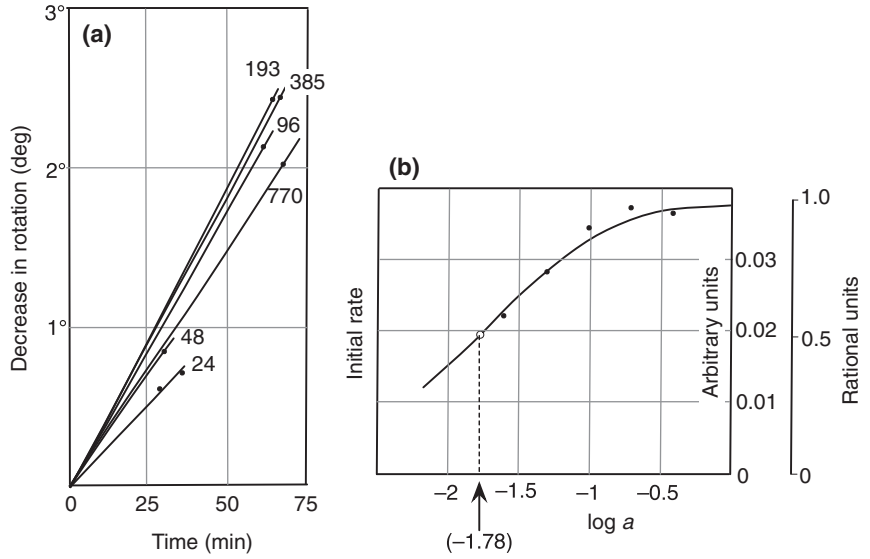

Fig. 4. (a) Experimental series 4. Amount of enzyme approximately equal to that in the experiments of Fig. 1. (b) Representation as Fig. 1a. Calculated from the experiments of Fig. 4a.

thus not large, even for so bad a curve as that of Fig. 3b, as soon as it is accepted, on the basis of evidence from other, better, experiments that we are permitted to interpret the curves in general as dissociation curves.

The agreement of the theoretical curves with the observed points is satisfactory for observations from the lowest usable concentrations of sucrose up to about 0.4 mol/L (corresponding to the logarithm −0.4). At higher concentrations there was a deviation, in that the velocities became smaller again, instead of remaining constant.

We have no doubt, looking back over these deviations, that we are here no longer dealing with the properties of a dilute solution. It is to be expected that the above law has only a certain range of validity. The failure of the law at higher sugar concentrations can be attributed to various conditions, whose precise influence we cannot state quantitatively. The most important influence is that which one can summarize as "alteration of the nature of the solvent". In molar, that is 34%, sucrose solution we no longer have to consider simply water as the solvent, in that the sugar itself is already changing the nature of the solvent. Connected with this, the affinity constant of the enzyme for sugar would also change, and probably also the velocity constant of the breakdown of this compound. As an example of the change of an affinity constant when the character of the solvent was changed by addition of an organic solvent, the researches of Löwenherz[10] on the change of dissociation constant of water on addition of alcohol may be quoted. Only up to an alcohol content of 7% is no definite alteration detectable; at higher concentrations it diminishes progressively.

2. The effect of lysis products and other substances

The above-cited authors, especially Henri, have already demonstrated that the lysis products glucose and fructose have an inhibitory effect on the lysis of sucrose. The latter also found that the effect of fructose is greater than that of glucose. Our task is now to estimate this influence quantitatively. We assumed with Henri that invertin has affinity not only for sucrose, but also for fructose and glucose, and undertook to estimate the three affinity constants quantitatively; we worked to this end as now described.

The velocity of hydrolysis was first determined, as before, for a certain quantity of enzyme. Then in a second experiment a certain amount of fructose (or glucose) was added, and the initial velocity of sucrose lysis measured. It was found that this velocity was diminished. We can thus conclude that the concentration of sucrose–enzyme compound is less in the second case than in the first, always assuming that the initial velocity remains an accurate measure of the concentration of sucrose–enzyme compound. Thus, writing v_0 and v for the two initial velocities, ϕ_0 and ϕ for the corresponding concentrations of sucrose–enzyme compound, we have $v_0 : v = \phi_0 : \phi$.

If the amount of enzyme, Φ, is divided between the amount of sucrose S and the amount of fructose F, and if ϕ represents the

concentration of sucrose–enzyme compound, ψ that of fructose-enzyme compound, we have from the Law of Mass Action

$$S(\Phi - \phi - \psi) = k \cdot \phi$$
$$F(\Phi - \phi - \psi) = k_1 \psi$$

where k and k_1 are the two affinity constants.

It follows, by elimination of ψ from these two equations.

$$k_1 = \frac{F \cdot k}{S\left(\frac{\Phi}{\phi} - 1\right) - k} \tag{5}$$

Φ/ϕ is obtained in the following manner: A parallel experiment without fructose gives the initial velocity v_0 and the concentration of sucrose–enzyme compound ϕ_0: in the corresponding main experiment with fructose let these values be v and ϕ, respectively, then $v : v_0 = \phi : \phi_0$ and $\phi = v \cdot \phi_0 / v_0$. In the fructose-free control experiment we have from Eq. 2,

$$\phi_0 = \Phi \cdot \frac{S}{S + k}$$

and thus

$$\phi = \frac{v}{v_0} \Phi \cdot \frac{S}{S + k} \tag{6}$$

or

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

and finally, by substitution of this value in Eq. (5)

$$k_1 = \frac{F \cdot k}{(S + k)\left(\frac{v_0}{v} - 1\right)} \tag{7}$$

2.1. Precise description of experiments on the inhibitory influence of other substances (fructose and glucose)

An overall protocol shows how experiments are arranged. In each case the progress of hydrolysis is compared in solutions having the same concentrations of sucrose and enzyme, run at optimal acidity and the same temperature, and differing only in the content of glucose or fructose or the lack of these substances. There are limits to the choice of experimental conditions. Firstly one must avoid too high carbohydrate concentrations, in order not to change the nature of the solvent. In general, it is not advisable to go above a total concentration of 0.3 mol/L in respect of carbohydrates. Thus it becomes necessary to work with relatively low concentrations of sucrose. But it follows, unfortunately, that the velocity of the reaction does not remain constant for long but instead the curve begins to deviate from a straight line rather quickly, so that determination of the initial velocity is based on only a small change in rotation or on a graphical extrapolation, in which a certain arbitrariness cannot be avoided. This deviation from linearity is often more serious with pure sucrose (for example, Fig. 8, I) than in experiments with mixtures (Fig. 8, II), since the concentration of inhibitory lysis products changes relatively more rapidly in experiments with pure sucrose than in those experiments where a certain amount of inhibitory substances was already present at the outset. Initial velocities usable for calculations could only be obtained by this graphical extrapolation: the observed points are employed to construct a curve by eye and the initial velocity is considered to be the tangent to this curve judged by eye, at the zero time point. No claim can be made to great accuracy for this, the only possible mode of procedure, but it certainly gives us a correct idea of the order of magnitude of the numbers concerned. These (geometric) tangents are given as broken lines in Fig. 5. The ratio of the corresponding trigonometric tangents, $\text{tangent}_I/\text{tangent}_{II}$, obtained by measurement from Fig. 5, is 1.18; the ratio $\text{tangent}_I/\text{tangent}_{III} = 1.29$. Thus from this experiment v_0/v for glucose is 1.18, for fructose it equals 1.29. Using equation 7 we calculate $k_{glucose}/k_{sucrose} = 4.8$ and $k_{fructose}/k_{sucrose} = 3.0$. Applying the same procedure to the experiment of Fig. 7, we obtain $\text{tangent}_I/\text{tangent}_{II} = 1.18$ and $\text{tangent}_I/\text{tangent}_{III} = 1.26$ and thus $k_{glucose}/k_{sucrose} = 4.6$ and $k_{fructose}/k_{sucrose} = 3.2$. In the experiment of Fig. 9, there was no deviation from a linear course. We obtain $\text{tangent}_I/\text{tangent}_{II} = 1.27$ and $\text{tangent}_I/\text{tangent}_{III} = 1.43$, whence $k_{glucose}/k_{sucrose} = 5.3$ and $k_{fructose}/k_{sucrose} = 3.3$. From the experiments of Fig. 6, it appears $\text{tangent}_I/\text{tangent}_{II} = 1.133$ and thus $k_{glucose}/k_{sucrose} = 6.7$. From the experiments of Fig. 8, it appears $\text{tangent}_I/\text{tangent}_{II} = 1.3$ and thus $k_{fructose}/k_{sucrose} = 4.3$.

Bringing all these results together yields

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

and thus from 7, the dissociation constants are: of the glucose–invertin compound 0.088, of the fructose–invertin compound 0.058.

The inhibitory influence of several other substances was measured in the same way. But first we checked the validity of the calculations by showing that foreign substances which certainly cannot have any chemical affinity to invertin also exert no

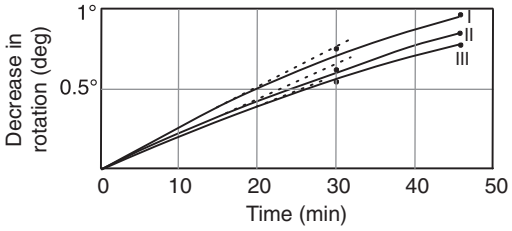

Fig. 5. Experimental series 5. Influence of glucose and of fructose, sucrose 0.1 mol/L. Curve (I) no addition; (II) glucose 0.1 mol/L; (III) fructose 0.1 mol/L.

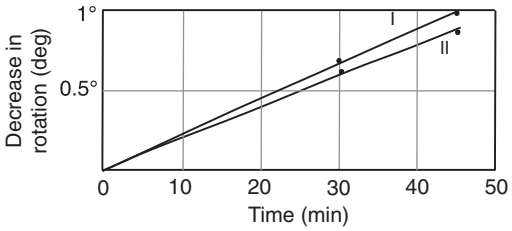

Fig. 6. Experimental series 6. Influence of glucose. Sucrose 0.133 mol/L. (I) No addition; (II) glucose 0.133 mol/L.

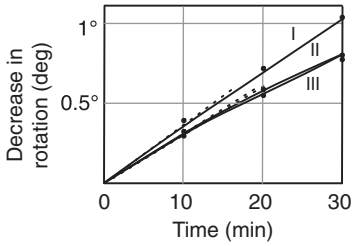

Fig. 7. Experimental series 7. Initial slopes shown by broken lines. Concentrations in mol/L. Sucrose 0.0833. (I) No addition; (II) glucose 0.0833; (III) fructose 0.0833.

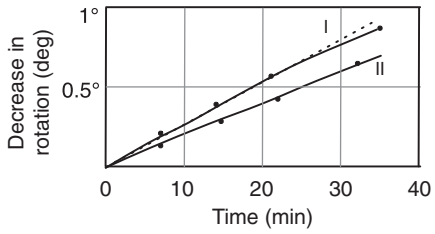

Fig. 8. Experimental series 8. Influence of fructose. Concentrations in mol/L. Sucrose 0.0416. (I) No addition; (II) fructose 0.0833.

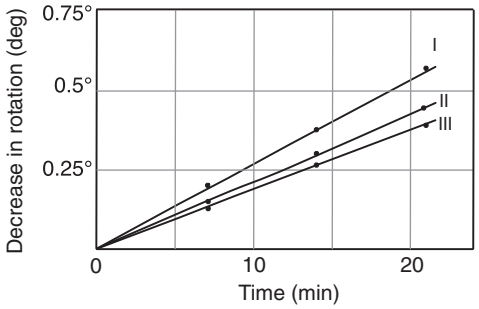

Fig. 9. Experimental series 9. Influences of glucose and fructose. Concentrations in mol/L. Sucrose 0.0416. (I) no addition; (II) glucose 0.0832; (III) fructose 0.0832.

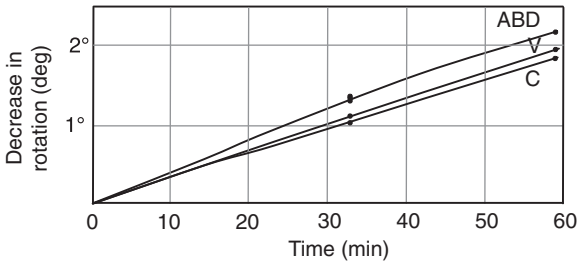

Fig. 10. Experimental series 10. Concentrations in mol/L. Sucrose 0.1. (A) no addition; (B) KCl 0.1; (C) ethanol 1.0; (D) ethanol 0.2; (V) glycerol 0.1.

inhibitory influence on sucrose hydrolysis as long as their concentration is not sufficient to change the nature of the solvent. Thus we showed that potassium chloride at a concentration of 0.1 mol/L had not the slightest inhibitory influence (Fig. 10B) nor even any definite inhibitory influence in molar concentration (Fig. 13f). Ethyl alcohol gave no trace of inhibition at a concentration of 0.2 mol/L (Fig. 10D). However, a slight inhibition was present at molar concentration (Fig. 10C), which is without doubt attributable to the change in the nature of the solvent and not to an affinity of the enzyme for the alcohol. If one regards this inhibition in the sense of an affinity and calculates graphically the ratio $k_{\text{alcohol}}/k_{\text{sucrose}}$ the result is 36. Such a weak affinity can be put equal to zero within experimental error (that is, k_{alcohol} is put = ∞) especially when we consider that another inhibitory influence, namely the alteration of the nature of the solvent, certainly exists.

It was now of particular interest to investigate the influence of other carbohydrates or higher polyfunctional alcohols, and especially lactose (Fig. 11). Its inhibitory action turns out to be so weak that we could scarcely distinguish it from experimental error. If the very small deviations are actually used for calculation, we obtain

	Experiment 1	*Experiment 2*
$\frac{k_{\text{lactose}}}{k_{\text{sucrose}}} =$	at least 30	36

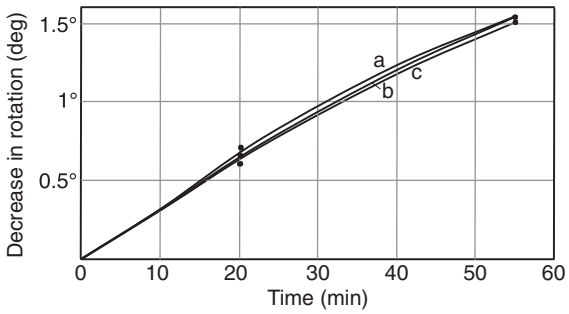

Fig. 11. Experimental series 11. Effect of lactose. Concentrations in mol/L. Sucrose 0.05. (a) no addition; (b) lactose 0.1; (c) lactose 0.2.

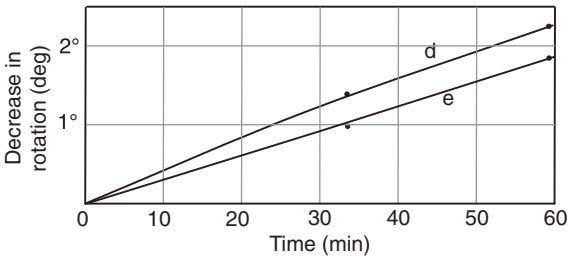

Fig. 12. Experimental series 12. Influence of mannose. Concentrations in mol/L. Sucrose 0.1. d, no addition; e, mannose 0.2.

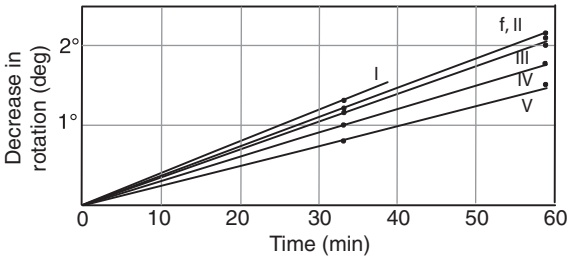

Fig. 13. Series 13. Influence of mannitol. Concentrations in mol/L. Sucrose 0.1. (I) no addition; (II) mannitol 0.1; (III) mannitol 0.25; (IV) mannitol 0.5; (V) mannitol 0.75; (f) KCl 1.0.

We cannot be sure whether calculations based on such small deviations are valid and must be content with establishing that the affinity of lactose for invertin is not measurable with certainty. This corresponds with expectation in that we might anticipate that binding of a disaccharide to invertin would lead to hydrolysis, whereas lactose in fact is not split by invertin.

2.1.1. Mannose

Fig. 12 shows $k_{\text{mannose}}/k_{\text{sucrose}} = 5.0$. Repetitions are necessary for more accurate estimation; however it may already be seen that the affinity of mannose is about equal to that of glucose.

2.1.2. Mannitol

The inhibitory influence was very slight. This example was used, for once, to estimate a weak affinity quantitatively, by appropriate variations of the experimental conditions. From Figs. 13 and 14 we see the following: the influence of 0.1 mol/L mannitol upon the lysis of 0.1 mol/L sucrose is not measurable with certainty. By raising the amount of mannitol while the amount of sucrose remains unchanged the influence gradually becomes distinct. Following the above procedure we can calculate

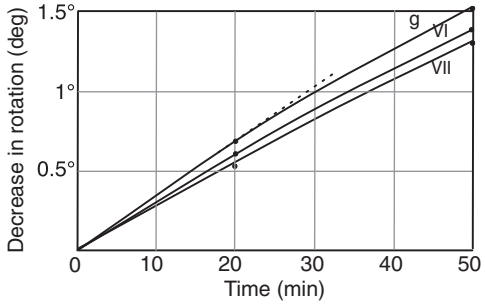

Fig. 14. Series 14. Influence of mannitol. Concentrations in mol/L. Sucrose 0.05. g, no addition; (VI) mannitol 0.1; (VII) mannitol 0.2.

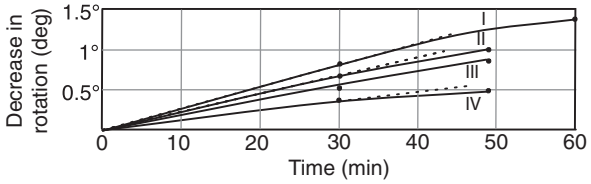

Fig. 15. Experimental series 15. Influence of glycerol. Concentrations in mol/L. Sucrose 0.166. I, no addition; II, glycerol, 0.111; III, glycerol 0.453. IV, glycerol 0.906. Experiment V (glycerol 0.1 and sucrose 0.1) is shown in Fig. 10.

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

In view of the very small differences in velocity the agreement is not bad, and thus it is reasonable to give the mean value, $k_{\text{mannitol}}/k_{\text{sucrose}} = 13$, as giving a satisfactory picture of the situation.

2.1.3. Glycerol

We consider the experimental series of Fig. 15 and one other individual experiment (Fig. 10, V). We find

Experiment	II	III	IV	V	
$\frac{k_{\text{glycerol}}}{k_{\text{sucrose}}}$	3.4	5.6	3.9	5.1	(mean 4.5)

Thus, contrary to expectation, glycerol has a high affinity for invertin. If we gather the dissociation constants together, we have

Sucrose	$k = 0.0167$	or	1/60
Fructose	$k = 0.058$	or	1/17
Glucose	$k = 0.089$	or	1/11
Mannose	$k \approx 0.083$	or	1/12
Glycerol	$k \approx 0.075$	or	1/13
Mannitol	$k = 0.22$	or	1/4.5
Lactose	$k =$ at least0.5 (probably close to ∞)	or	1/2
Sucrose	60		
Fructose	17		
Glucose	11		
Mannose	about 12		
Glycerol	13		
Mannitol	4.5		
Lactose	0		(that is, unmeasurably small)
Ethyl alcohol	0		ditto

To understand these numbers, recall that a rise of dissociation constant corresponds to a fall of affinity of the enzyme for the substance concerned. The affinity for sucrose is thus by far the greatest. If the dissociation constant of the invertin–sugar compound is defined by the magnitude [Enzyme] × [Sugar]/[Enzyme – Sugar compound], we can designate the reciprocal value [Enzyme – Sugar compound]/[Enzyme] × [Sugar] as the affinity constant of the enzyme for the sugar. This is, thus, for

3. The reaction equation for enzymatic lysis of cane sugar

On the basis of these data, we are now in a position to solve the old problem of the reaction equation of invertin in a rational manner and without the help of more than one arbitrary constant.

V. Henri made the closest approach to this solution of all authors to date and we can consider our derivation as an expanded modification of Henri's, on the basis of newly acquired knowledge.

The basic assumption of this derivation is that the rate of decomposition is at every instant proportional to the concentration of the sucrose–invertin compound and that at every instant the concentration of this compound is determined by the concentrations of enzyme, of sucrose and of those lysis products which bind to the enzyme. But while Henri employed an "affinity constant of the lysis products", we work separately with the dissociation constants of the sucrose–enzyme compound, $k = 1/60$, the fructose-enzyme compound, $k_1 = 1/17$, and the glucose–enzyme compound, $k_2 = 1/11$. We employ, further, the following symbols

Φ = total enzyme concentration
ϕ = concentration of sucrose–enzyme compound
ψ_1 = concentration of fructose–enzyme compound
ψ_2 = concentration of glucose–enzyme compound
S = concentration of sucrose *
F = concentration of fructose *
G = concentration of glucose *

*That is, the concentration with respect to sugars in the free state, which is, however, practically the same as the total concentration of the sugars.

Since the lysis always yields equal quantities of fructose and glucose, G is always equal to F. By the Law of Mass Action we have, at every instant

$$S(\Phi - \phi - \psi_1 - \psi_2) = k\phi \tag{8}$$

$$F(\Phi - \phi - \psi_1 - \psi_2) = k_1\psi_1 \tag{9}$$

$$G(\Phi - \phi - \psi_1 - \psi_2) = k_2\psi_2 \tag{10}$$

From Eq. 8 it follows that

$$\phi = \frac{S(\Phi - \psi_1 - \psi_2)}{S + k} \tag{11}$$

We can eliminate ψ_1 and ψ_2, because we first obtain, by division of Eqs. 9 and 10:

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

and by division of Eqs. 8 and 10

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

so that

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

Now let us abbreviate by writing

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

so

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

Substituting this in Eq. 11 and solving for ϕ,

$$\phi=\Phi\cdot\frac{S}{S+k(1+qF)} \tag{12}$$

Now we can obtain a differential equation. Let a be the initial amount of sucrose. t the time. x the amount of fructose or glucose existing at time t, and therefore. $a-x$ is the amount of sucrose still existing at time t, so that the rate of decomposition at time t is defined as $v_t=dx/dt$.

From the initial assumptions this is proportional to ϕ, so that using Eq. 12 our differential equation becomes

$$\frac{dx}{dt}=C\cdot\frac{a-x}{a+k-x(1-kq)} \tag{13}$$

where C represents the single arbitrary constant, which is proportional to amount of enzyme. The general integral of the equation is calculated without difficulty

$$C\cdot t=(1-kq)x-k(1+aq)\ln(a-x)+\text{const.}$$

To eliminate the constant of integration, we similarly integrate the corresponding equation for the initial state of the process, for which $x=0$ and $t=0$:

$$0=k(1+aq)\cdot\ln a+\text{const.}$$

and by subtraction of the last two equations we finally obtain the definite integral

$$C\cdot t=k(1+aq)\cdot\ln\frac{a}{a-x}+(1-kq)x \tag{14}$$

or, substituting the value of q:

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

Now one can incorporate k into the constant on the right-hand side of the equation, giving

$$\frac{1}{t}\left(\frac{1}{a}+\frac{1}{k_1}+\frac{1}{k_2}\right)\cdot a\cdot\ln\frac{a}{a-x}+\frac{1}{t}\left(\frac{1}{k}-\frac{1}{k_1}-\frac{1}{k_2}\right)x=\text{const.} \tag{15}$$

It is characteristic of this function, as of Henri's, that it is a combination of logarithmic and linear functions, of the form

$$m\cdot\ln\frac{a}{a-x}=n\cdot x=t\cdot\text{const.} \tag{16}$$

where the significance of m and n is obvious from the last equation: they are factors whose magnitude can be determined from the individual dissociation constants and the initial amounts of the sugar. Substituting the values ascertained by us for k, k_1 and k_2 at the experimental temperature of 25°, we obtain

$$\frac{1}{t}(1+28a)\cdot 2.303\log\frac{a}{a-x}+\frac{1}{t}\cdot 32\cdot x=\text{const.} \tag{17}$$

For ease in calculation, we replace $\log[a/(a-x)]$ by $-\log(1-x/a)$.

This constant must be proportional to enzyme concentration. That it is, is shown by all previous researches and in particular it was shown by L. Michaelis and H. Davidsohn (l.c. pp. 398-400) that an equation of the following form is correct:

$$\text{amount of enzyme}\times\text{time}=f(a,x) \tag{18}$$

The undetermined function of the right-hand side of the last equation finds a definite form through our present Eq. 16. Besides this, nothing else is changed. One can see from Eqs. 16 and 18 that the constant of Eq. 16 must be proportional to enzyme concentration.

It is thus superfluous to test the validity of Eq. 17 again, for varying amounts of enzyme, yet it must still be tested whether this constant remains the same with equal amounts of enzyme but various amounts of sugar, and whether it is in general independent of time within a single experiment.

For this purpose we use experimental series I and we must first calculate the value of x in mol. Until now we have expressed it in arbitrary polarimetric units. We make use of the assumption that if a sucrose solution gives a rotation of m°, the theoretical final angle of rotation of the inverted solution is −0.313 m°. (c.f. Sørensen, l.c., p. 262.)

Time (t)	x/a	K	Mean
	I. Sucrose 0.333 mol/L		
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 mol/L		
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 mol/L		
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 mol/L		
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	[0.058]	0.0445
	V. Sucrose 0.0208 mol/L		
17	0.331	0.0510	
27	0.452	0.0464	
38	0.611	0.0500	
62	0.736	0.0419	
95	0.860	[0.0388]	
1372	0.990	[0.058]	0.0474
	Overall average 0.0454		

The values of the constant agree well in all experiments, and apart from slight fluctuations show scarcely any trend with time or with sugar concentration, so that we may consider it as satisfactorily invariant.

Summary

The course of invertin action on sucrose may be understood on the basis of the following assumptions: Sucrose binds with invertin to form a compound of which the dissociation constant is 0.0167. This compound is labile, decomposing according to the equation

1 mol sucrose–invertin compound → 1 mol fructose + 1 mol glucose + 1 mol invertin

Invertin also has detectable affinity for the lysis products, fructose and glucose, and for several other higher alcohols (mannitol, glycerol) and carbohydrates but, remarkably, not for lactose. This affinity is considerably less than for sucrose. These compounds are not labile, thus they do not lead to a chemical lysis of fructose, etc., but are manifested only in an inhibitory effect of fructose, etc. on the sucrose–invertin process. The concentrations of all these compounds may be calculated from the Law of Mass Action and for each of them the dissociation constant has been obtained fairly accurately, most accurately for the sucrose–invertin compound. Since the breakdown of the sucrose–invertin compound must be a monomolecular reaction, the velocity of decomposition of sucrose is simply proportional to the concentration of sucrose–invertin compound. From all these assumptions, a differential equation may be derived for the course of sucrose lysis, whose integral fits the observations well.

Notes on the translation for FEBS Letters

The original word is used for the principal subject, *invertin* rather than *invertase*; otherwise modern forms are preferred in most places, including cm^3 for ccm and mol for mole. Equations are set as convenient, sometimes inserting the point between symbols to indicate multiplication (e.g. $x \cdot y$ for xy) and sometimes not, as did the original authors. The proportionality operator, :, appears where it does in the original, instead of a simple slash, /. [H^+] is used throughout instead of direct transliteration of the original "H^+-Konzentration". The original authors do this in equations and similarly in most places use square brackets for concentrations of other species. Sørensen is used in place of the German spelling, Sörensen, of the original.

As in an earlier version,[1] the *numbered* Tables (which contain the same information as the corresponding Figures) are omitted to save space, ease the reader's task and avoid the need for explanations where discrepancies occur. Text and Legends to Figures are amended accordingly. The Figures have been re-drawn, copying the original except where labels on the graphs or axes have been added to, modified or removed to improve clarity. In a more recently-published translation,[2] a different treatment was adopted. There the Tables are published in translation (with several errors), and Figures re-drawn from recalculated results, introducing differences from the originals. If the need is felt again to examine Michaelis and Menten's results in detail, the original should be consulted, though taking into account the work of Goody and Johnson.

Footnotes are the authors' own, though with one factual correction, and they have been numbered in one series through the paper instead of page by page. "This journal" means *Biochemische Zeitschrift*, the original name of FEBS Journal. Only a minority of equations are numbered in the original and some numbers are repeated (even once on the same page). Here, a single sequence is used for the originally numbered equations only and internal cross-references by page number are thus eliminated. The original also has Figs. 1a–4a in addition to and in parallel with Figs. 1–4. Here these are numbered and referenced as Figs. 1–4, with Fig. 1a referring to the original Fig. 1, and Fig. 1b referring to the original Fig. 1a, and so on.

There are several other minor difficulties or errors in the original paper, discussed now and not identified in the translated text:

1. First page, footnote 2: The authors of this paper were C. O'Sullivan and F.W. Tompson, not Thompson as given in the original. The same incorrect citation has been made by many other writers, beginning perhaps with Sørensen (cited here in footnote 5).
2. For concentrations Michaelis and Menten use the word "normal", or "n". No problem arises when referring to sugars or buffer, where the meaning can only be mol/L. In these cases it is preferred to use present-day terminology. However a solution of "soda" (meaning Na_2CO_3) was added to terminate the enzymic reaction and accelerate mutarotation. This was "1/2 n", potentially ambiguous, and the original form is preferred in this translation.
3. Fig. 2b (Fig. 2a in the original). Though the points are correctly placed with respect to the ordinate scale, the horizontal line for 0.05 arbitrary units is too low, a feature retained in this translation. The other horizontals are, respectively, 0.075, 0.025 and 0 arbitrary units.
4. Table 10, p. 357 (which does not appear in this translation): Experiment V was with glycerol, not mannitol, and a cross-reference in the original should be to Table 15 not XIV. (One source of confusion is that a different experiment, with mannitol, described in Fig. 13 and Table 13, is also identified by the symbol V.)
5. Fig. 11: Though ignored in this translation, there is a discrepancy between the Figure and the corresponding Table, where the final observations are shown as at 50 min.
6. Page 362: Reference is made to Fig. 13 (and Table 13) for the weak inhibition by mannitol, but the discussion includes results in Fig. 14. In the original, these two Figures share a single Legend.
7. Page 365: Eq. (1), k_1 is printed instead of k. Also two equations are labelled (4).
8. Page 366: A minus sign is omitted in one equation [b].

Endnotes

[1] Duclaux (1899) Traité Microbiol., vol. II.

[2] O'Sullivan, C., Tompson, F.W. (1890) J. Chem. Soc. 57, 834.

[3] Brown, A.J. (1902) J. Chem. Soc., 373.

[4] Victor Henri (1903) Lois générales de l'action des diastases, Paris.

[5] Sørensen, S.P.L. (1909) "Enzymstudien II", this journal 21, 131.

[6] Michaelis, L., Davidsohn, H. (1911) this journal 35, 386.

[7] Hudson, C.S. (1910) J. Am. Chem. Soc. 30, 1160,1564, 1908; 31, 655, 1909; 32, 1220, 1350.

[8] Michaelis, L. (1911) this journal 33, 182 (see also: by the same author, Die allgemeine Bedeutung der Wasserstoffionenkonzentration, etc. in Oppenheimer's *Handbuch der Biochemie*, supplementary volume, 1913).

[9] The amounts of enzyme used in the experimental series I, II, III and IV can be calculated from the initial velocities as almost exactly 1:2:0.5:1.

[10] Löwenherz, R. (1896) Z. Phys. Chem. 20, 283.

[1] Foundation Stones of Biochemistry (1980) (pages 287–316), published by Voile et Aviron, Hong Kong and London (now an imprint of Mount Davis Press), and the book is also available at http://www.trcboyde.net.

[2] K.A. Johnson and R.S. Goody, *Biochemistry* 50 (2011) 8264–8269. The translation is an online publication linked to the paper.