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XXXVI.—*Enzyme Action.*

By Adrian J. Brown.

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Introduction.

In a paper on the fermentative functions of yeast (Trans., 1892, **61**, 380), the author described some experiments which showed that the character of the action of fermentation differed in a very marked manner from the character of the action usually attributed to enzyme change.

The author's experiments indicated that during fermentative change a constant amount of yeast decomposes an approximately *constant weight* of sugar in unit time in solutions of varying concentration, and that the velocity of fermentative action is therefore represented graphically by a *straight line*. On the other hand, the character of the action usually attributed to an enzyme is that a constant amount of the ferment changes in unit time a *constant fraction* of the reacting substance present, and that the velocity of its action is represented by the *logarithmic curve* of mass action.

At the time the author's work (*loc. cit.*) was published, the fermentative power of the yeast cell was considered to be a life function inseparable from the cell, and there appeared to be nothing specially remarkable in the observation that fermentation, a life function, differed in the velocity of its action from enzyme action. But since the more recent work of Buchner has demonstrated that the phenomenon of fermentation is caused by enzyme action, the question assumed another aspect. If fermentation is now regarded as an enzyme action, then, either the velocity of its action must be regarded as differing essentially from that which is usually attributed to other enzymes, or the experimental evidence on which the assumed difference rests must be regarded as misleading.

It was with the intention of investigating this question that the author commenced the work described in the following paper.

Velocity of the Action of Fermentation.

If the view is adopted as a working hypothesis that the supposed difference in velocity of the actions of fermentation and ordinary enzyme change does not exist, but that it is due to some misconception, it is evident misconception may have arisen concerning either the velocity of fermentation or that of ordinary enzyme change, and consequently a re-examination of the experiments by which both velocities have been determined appeared desirable.

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As the author is responsible for the experiments by which the velocity of fermentation has been determined, he commenced his investigations by repeating them. It does not appear necessary, however, to give the results of this work, for the experiments were similar to those described in the earlier paper (*loc. cit.*), and the results fully confirmed the conclusion that fermentative action does not proceed in accordance with the law of mass action.

As the general character of the action of fermentation appeared to be thus established, the author proceeded to examine the experimental evidence from which the conclusion is drawn that the velocity of enzyme action accords with the law of mass action.

Velocity of Enzyme Action.

The generally accepted view regarding the velocity of enzyme action is based on the researches of Cornelius O'Sullivan and F. W. Tompson on the action of invertase on cane sugar (Trans., 1890, **57**, 865). These authors demonstrated the velocity of the action of invertase in the following manner.

Invertase was caused to act in solutions of cane sugar, and during the progress of the actions the quantities of sugar inverted during a succession of time intervals were determined. By this means, observations were obtained from which time curves were constructed which represented graphically the velocity of the action of inversion. When these curves were compared with the curve representing simple mass action, a very close agreement in shape was observed, which appeared to indicate that they were of the same order, and from this close agreement in shape, C. O'Sullivan and Tompson concluded that the action of invertase instanced the operation of the law of mass action.

This conclusion has also received confirmation from the researches of James O'Sullivan on the power of inversion of living yeast cells (Trans., 1892, **61**, 926), the experiments of this author indicating that the velocity of action of the living cell is the same as that of the extracted invertase used by C. O'Sullivan and Tompson in their experiments.

The evidence referred to is, so far as the author is aware, all that has been brought forward to support the conclusion that the velocity of enzyme action indicates the operation of a simple mass action.

Hitherto, no doubt, the want of additional evidence has not been felt, owing to C. O'Sullivan and Tompson's experiments appearing conclusive so far as invertase is concerned, and also to the fact that the conclusion these authors arrived at with regard to the character of enzyme action is one which there is every reason to anticipate.

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But when the character of the action of fermentation, now very generally recognised as an enzyme action, was found to differ essentially from that attributed to invertase both in the free state and within the living yeast cell,* it raised doubt in the author's mind regarding the accuracy of C. O'Sullivan and Tompson's conclusion. Moreover, the author found that he was not alone in his distrust, for it has already been pointed out by Duclaux, in a criticism on C. O'Sullivan and Tompson's work (*Ann. Inst. Pasteur*, 1898, **12**, 96), that the logarithmic curve representing the action of invertase, on which C. O'Sullivan and Tompson founded their conclusion, may be shaped by other causes than the supposed action of mass. For Duclaux maintains that such a curve represents, not only a decrease in a changing substance, but also, and equally well, an increase in the products of change, and it is possible these products of change may act as the influence shaping the curve and not the influence of mass action. No experimental evidence sustaining this point is, however, brought forward by Duclaux.

As C. O'Sullivan and Tompson's conclusion rests entirely on the shape of the curve representing the action of invertase, the author considered it advisable first to repeat the experiments from which the curve was derived. Conditions of experiment similar to those used by C. O'Sullivan and Tompson were employed, but the invertase used was prepared in a different manner from the enzyme with which these authors experimented.

C. O'Sullivan and Tompson worked with invertase obtained from an extract of auto-digested yeast by precipitation with alcohol, and in so doing encountered the difficulty that the action of invertase prepared in this manner was very irregular unless it was associated with a small quantity of sulphuric acid. Moreover, the amount of acid required to reach the point described by these authors as "the most favourable condition of acidity," at which point it was necessary to work, varied in every experiment in a most remarkable manner.

It appeared very desirable to avoid this complication when repeating C. O'Sullivan and Tompson's experiments, so the author employed in his experiments an extract of invertase prepared from dried yeast by digestion with water. An extract of invertase prepared in this manner was quite suitable for the purpose of the experiments, and the risk of modifying the activity of the invertase by precipitation was avoided. That this method of obtaining a preparation of invertase suitable for experiment was preferable to that employed by C. O'Sullivan and Tompson was evidenced by the invertase being free from the irregularities of action associated with the precipitated in-

* Presumably, invertase within the wall of the living cell is in the same position as zymase with regard to its action as an enzyme.

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vertase used by these authors, and, in consequence, it could be employed without the complicating addition of sulphuric acid.

The author's experiments, like those of C. O'Sullivan and Tompson, consisted in the addition of a suitable amount of invertase solution to a solution of cane sugar, and in determining, by means of a polarimeter, the fractions of the sugar inverted during successive intervals of time.

Instead, however, of expressing the velocity of the inversion change by means of a curve, the author preferred to make use of the value k, derived from the expression $\frac{1}{\theta}\log\frac{1}{1-x}$. This well-recognised means of expression, usually adopted now to demonstrate such changes as those of a mass action, has the advantage of avoiding certain difficulties which attend the comparison of calculated and experimental curves.

The results of two series of experiments determining the velocity of the action of invertase are given in Tables I and II:

TABLE I.—*Velocity of inversion change in* 9·48 *per cent. solution of cane sugar.* 500 *c.c. of solution of sugar and* 25 *c.c. of invertase solution used. Temp.* 30°.

Duration of time interval in minutes. θ.	Fraction of sugar inverted in θ. x.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
30	0·265	0·00445
64	0·509	0·00483
120	0·794	0·00571
180	0·945	0·00698
240	0·983	0·00737
300	1·003	

During the course of a change proceeding as a simple mass action, it is well known that the value k, determined for any point of the action, is a constant. But in the experiments described in the above tables it will be noticed that the value k increases in both experiments as inversion proceeds, until the value at the termination of the experiments is about 70 per cent. higher than at the beginning.*

Now, these results do not support the view that the action of inversion instances a mass action, as C. O'Sullivan and Tompson believed, for they differ very materially from the results these authors obtained. But in order to emphasise more distinctly the difference between the

* It will be noticed that there is no indication of "reversion" in these inversion experiment- [illegible] denotes an increasing velocity; re-

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TABLE II.—*Velocity of inversion change in* 19·28 *per cent. solution of cane sugar.* 500 *c.c. of sugar solution and* 25 *c.c. of invertase solution used. Temp.* 30°.

Duration of time interval in minutes. θ.	Fraction of sugar inverted in θ. x.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
30	0·130	0·00201
64	0·256	0·00201
120	0·454	0·00219
180	0·619	0·00232
240	0·738	0·00242
300	0·831	0·00257
360	0·890	0·00265
420	0·935	0·00283
480	0·961	0·00293
540	0·983	0·00327
581	0·990	0·00344

character of the action of inversion and that of a mass action, the results of an experiment involving mass action are given in Table III for the purpose of comparison. In this experiment, the author employed sulphuric acid to invert cane sugar, and thus obtained results from a typical mass action, which are directly comparable with those effected by invertase.

TABLE III.—*Velocity of inversion change of cane sugar by acid.* 600 *c.c. of a* 20 *per cent. solution of cane sugar, and* 35 *c.c. of normal* H_2SO_4 *used. Temp.* 48°.

Duration of time interval in minutes. θ.	Fraction of sugar inverted in θ. x.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
30	0·165	0·00261
61	0·317	0·00271
90	0·433	0·00274
120	0·532	0·00275
150	0·617	0·00278
180	0·688	0·00281
243	0·785	0·00275
302	0·856	0·00279
362	0·902	0·00278
		Mean result 0·00275

The above experiments show very clearly, when a true mass action is followed under experimental conditions similar to those used when determining the character of the action of invertase, that a very differ-

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ent result is obtained. The small and irregular variation in the value of k* is very different from the regular and well marked increase in the value of k observed in the experiments with invertase given in Tables I and II, and from this there can remain but little doubt that the order of progression of inversion differs essentially from that of a mass action. At present, the author has not attempted to determine any expression for the order of progression of inversion under the conditions of his experiment, because, for the immediate purpose of his investigation, it is only necessary to show that inversion does not proceed as a mass action.†

Although the author's experiments throw the greatest doubt on the accuracy of the conclusion that inversion evidences mass action, they cast very little light on the true character of the action of invertase, and in order to obtain more knowledge, it became necessary to adopt some method of experiment different from that which has already been described.

* The variations in value of k are no doubt due to experimental error. Very slight changes in temperature have a marked influence on the velocity of inversion change by acid.

† Since writing the above, a communication from Victor Henri has been published (*Compt. rend.*, 1901, **133**, 891) on the velocity of inversion change. This author arrives at the conclusion that the action proceeds in accordance with the expression $2k_1=\frac{1}{\theta}\log\frac{1+x}{1-x}$.

On applying this purely mathematical expression of velocity to the inversion experiments described in Table II, the following results have been obtained:

TABLE II.—*Recalculated.*

Duration of time interval in minutes. θ.	Fraction of sugar inverted in θ. x.	$2k_1=\frac{1}{\theta}\log\frac{1+x}{1-x}$.
30	0·130	0·00376
64	0·256	0·00355
120	0·454	0·00356
180	0·619	0·00346
240	0·738	0·00343
300	0·831	0·00353
360	0·890	0·00343
420	0·935	0·00351
480	0·961	0·00354
540	0·983	0·00383
581	0·990	0·00395

It will be observed from the remarkably close agreement of the numbers in the third column, representing the values $2k$, that the author's experiments indicate a very similar velocity for inversion change to that which is represented by Henri's expression. This is of interest as further assisting to establish the fact that the progress of an inversion change is not ordered in conformity with the law of mass action.

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Such a method exists in causing a constant amount of invertase to act on varying amounts of cane sugar in constant volume of solution for a constant brief interval of time. Under these conditions, the variable in the actions is the amount of reacting substance (cane sugar) present, and in a simple mass action under these conditions, the amount of reacting substance changed in unit time is a *constant fraction* of the reacting substance present.*

In Table IV, the results of a series of five experiments are given in which a constant amount of invertase has acted on varying amounts of sugar under the conditions just named:

TABLE IV.—*Inversion changes by a constant amount of invertase acting in constant volume of cane sugar solutions of varying concentrations, in constant time.* 1 *c.c. of invertase solution added to* 100 *c.c. of cane sugar solution in each experiment. Temp.* 28°.

No. of experiment.	Grams cane sugar per 100 c.c.	Grams cane sugar inverted in 60 minutes.	Fraction of cane sugar inverted in 60 minutes $=x$.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
1	4·89	1·230	0·252	0·00210
2	9·85	1·355	0·138	0·00107
3	19·91	1·355	0·068	0·00051
4	29·96	1·235	0·041	0·00031
5	40·02	1·076	0·027	0·00020

When the law of mass action was evidenced by Ostwald's experiments on methyl acetate (*loc. cit.*) under conditions similar to those employed in the above experiments, he found that a *constant fraction* of the methyl acetate present in each solution was hydrolysed in unit time, and therefore, if the action of invertase is an instance of simple mass action, a *constant fraction* of the cane sugar present in each of the above experiments should be inverted. But it will be noticed that instead of a constant fraction, a constant (or approximately constant) *weight* of the cane sugar is inverted. The fraction inverted diminishes in inverse proportion to the amount of cane sugar present in the experiments, and, as a consequence, the value k, which is constant in a true mass action, varies to a very large extent.

Those experiments,† therefore, confirm the conclusion derived from the experiments given in Tables I and II, that the influence of

* For experimental confirmation of this necessary consequence of mass action, see Ostwald on the hydrolysis of methyl acetate by hydrochloric acid ("Outlines of General Chemistry," p. 353).

† Duclaux (*loc. cit.*) quotes some experiments with invertase derived from *Aspergillus niger* which fully confirm the author's experiments described in Table IV.

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mass action does not rule in inversion change. Moreover, when the velocity of the action of inversion determined in the manner described in Table IV is examined, it will be noticed that the action is similar in character to that of fermentation, referred to at the commencement of this paper. During alcoholic fermentation, a constant amount of yeast decomposes in unit time an approximately *constant weight* of sugar in equal volumes of solution containing varying amounts of sugar. Invertase is now found to invert approximately constant quantities of cane sugar under similar conditions. Therefore the supposed difference in character of the two actions of fermentation and inversion which led the author to commence the investigation described in this paper does not exist, for the action of both, if expressed graphically, is represented approximately by a straight line. So far, therefore, the first object of the investigation is attained.

Although experiments carried out in the manner just described show that the general character of the action of invertase resembles that of fermentation, they do not explain the apparent paradox that when the action of invertase is studied during a series of consecutive changes in a single solution, the velocity of the action is then represented, not by a straight line, but by a curve, showing that there is a decrease in the *absolute amount* of sugar inverted during each time interval (see the experiments in Tables I and II and foot-note to p. 378 which indicate that, although the curve of the action is not so pronounced as the logarithmic curve of mass action, it is still very marked).

Apparently there are two causes which may lead to the production of such a curve during the continued action of invertase in a solution of cane sugar. Either it may be due to a natural weakening of the invertase by continued work,* or it may be due, as Duclaux has suggested (*loc. cit.*), to the action of invertase being influenced prejudicially by the accumulation of its own products of inversion. From what is known regarding the very large amount of cane sugar which is capable of being hydrolysed by a very small amount of invertase, the former cause appeared to be far less probable than the latter, so the author turned his attention to the investigation of the possible retarding influence of inversion products on the action of invertase.

Action of Inversion Products on the Velocity of Inversion Change.

The method of experiment adopted by the author was to observe the action of a constant amount of invertase during a brief interval of time in equal volumes of solutions containing a constant amount of

* Some interesting experiments of Victor Henri (*loc. cit.*) indicate that sustained work does not weaken the action of invertase.

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cane sugar *and varying amounts of invert sugar.* The invert sugar used in the experiments was prepared by the action of invertase on a concentrated solution of cane sugar until complete inversion was obtained, the invertase being then destroyed by raising the temperature of the solution to 90°.

The following table gives particulars of a series of experiments in which different amounts of this solution of invert sugar were mixed with a constant amount of cane sugar solution, the total volumes of the solutions in the different experiments being made constant:

TABLE V.—*Influence of invert sugar on the action of invertase. Volume of each experiment,* 100 *c.c.* 1 *c.c. of invertase solution used in each experiment. Time of change,* 80 *minutes. Temp.* 30°.

No. of experiment.	Grams cane sugar present in 100 c.c.	Grams invert sugar present in 100 c.c.	Grams cane sugar inverted in 80 minutes.
1	4·06	none	2·27
2	4·06	1·47	2·21
3	4·06	5·39	1·99
4	4·06	11·38	1·66
5	4·06	17·87	1·25

In these experiments, if the presence of invert sugar exerted no influence on the action of invertase, the quantities of cane sugar inverted in constant time would be constant, for the same quantities of cane sugar and invertase were present in all the experiments. But an examination of the table shows that the amount of cane sugar inverted decreased as the quantity of added invert sugar increased, until, in the last experiment (No. 5), the quantity of cane sugar inverted has been reduced to nearly one-half in the presence of 17·87 grams of invert sugar.

The series of experiments indicate, therefore, that the presence of invert sugar has diminished the activity of invertase, and that the arresting influence has increased as the amount of invert sugar has increased. But it is possible that the arresting influence of invert sugar may be due, not to the presence of the sugar as such, but to the increased viscosity of the solution containing it, for, owing to the manner in which the experiments were conducted, the total amount of sugars in the different solutions is an increasing one from the first to the last experiment. In order to investigate this question, a series of experiments was conducted in a similar manner to those described in Table V, excepting that lactose was used in the place of invert sugar. Lactose is a sugar which is not changed by the action of invertase, but its solutions possess a viscosity almost identical with that of solutions

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of invert sugar of similar concentrations. Therefore in a series of experiments with lactose in place of invert sugar, the factor of increasing viscosity is introduced apart from any special influence possessed by invert sugar alone.

The results of a series of experiments with lactose are given below:

TABLE VI.—*Influence of lactose on the action of invertase. Volume in each experiment,* 100 *c.c.* 1 *c.c. of invertase solution used. Time of change,* 60 *minutes. Temp.* 28°.

No. of experiment.	Grams cane sugar present in 100 c.c.	Grams lactose present in 100 c.c.	Grams cane sugar inverted in 60 minutes.
1	7·0	none	2·072
2	7·0	5·0	2·052
3	7·0	10·0	2·052
4	7·0	20·0	1·893

The results given in this table show that the influence on the action of invertase of the viscosity (or any other property) of the lactose used in the experiments is comparatively insignificant. In experiments 2 and 3, the retarding influence of 5 per cent. and 10 per cent. of lactose lies almost within the limits of experimental error, and in 4, in which the large amount of 20 per cent. lactose is present, the reduction in the amount of cane sugar inverted is only 9 per cent. On the other hand, it has already been shown (Table V, No. 4) that 17·8 per cent. of invert sugar under similar conditions reduced the amount of cane sugar inverted to the extent of 45 per cent. The major part of this reduction, therefore, is not due to viscosity, but must be occasioned by the arresting influence of invert sugar as such.

When the arresting influence of invert sugar on the action of invertase is thus established, there is then no difficulty in explaining the apparent paradox that the true action of invertase, which is indicated graphically by a straight line, is expressed by a curve when the action is determined for a series of progressive changes in one solution. Under the latter conditions, as the action of inversion proceeds, the products of inversion accumulate, and these consequently exert an increasing retarding influence on the action of inversion, and thus compel the action to follow the course of a curve.

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The Inversion Functions of Living Yeast Cells.

So far, when discussing the action of invertase, the author has referred more especially to C. O'Sullivan and Tompson's experiments and conclusion regarding the velocity of its action. It now remains to discuss J. O'Sullivan's experiments, alluded to at the commencement of this paper as supporting C. O'Sullivan and Tompson's conclusion.

It will be remembered that J. O'Sullivan, when studying the velocity of the inversion change produced by living yeast in solutions of cane sugar (*loc. cit.*), found that the value k derived from the expression $\frac{1}{\theta}\log\frac{1}{1-x}$, was constant, or nearly so, during the progression of the changes, and from this he concluded that the velocity of change followed the law of mass action.

There is no doubt that J. O'Sullivan's determinations—like those of C. O'Sullivan and Tompson—indicate a velocity approximating to that of mass action, when the progress of an inversion change is followed in one solution; but J. O'Sullivan has overlooked the fact—rendered evident by his own determinations—that, although the velocity in each separate change approximately follows the law, the value k found for comparable experiments in which varying amounts of sugar have been used, shows that there is no conformity with mass action, but, on the contrary, indicates that a *constant amount* of sugar is inverted—an action similar to that which has been shown for free invertase.

For instance, in J. O'Sullivan's paper four comparable experiments are described, in which equal amounts of yeast were used in equal volumes of solution during equal intervals of time, the only variable being the quantity of cane sugar present in the solutions. The results of these experiments are given in the table on p. 384.

It will be noticed, on examining this table, that J. O'Sullivan has determined the progression of inversion in each of the four solutions at three time intervals, and the values of k for the changes in each separate solution are fairly constant; but the values k should also be constant for all four of the solutions if the velocity of change follows the law of mass action, because the solutions only differ in containing varying quantities of sugar. On the contrary, however, the value k varies inversely as the amount of sugar present, in a similar manner to the value k in the author's experiments with invertase, given in Table IV.

A similar conclusion may also be derived from the numbers in the column in Table VII showing the fractions of cane sugar inverted during the experiments. If the first numbers in each series are

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TABLE VII.—*Velocity of inversion by living yeast cells (J. O'Sullivan).*

Grams cane sugar per 100 c.c.	Grams of yeast used.	Time of action in minutes. θ.	Fraction of sugar inverted. x.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
5	0·5	30	0·1636	0·0025
		60	0·3164	0·0027
		120	0·5442	0·0028
10	0·5	30	0·1042	0·0016
		60	0·1544	0·0012
		120	0·2780	0·0012
20	0·5	30	0·0627	0·0009
		60	0·0850	0·0006
		120	0·1467	0·0006
30	0·5	30	0·0366	0·0005
		60	0·0495	0·0003
		120	0·0862	0·0003

compared, it will be noticed that the fractions inverted are, approximately, in inverse proportion to the amounts of cane sugar present in the solutions—or, in other words, the actual *quantity* of sugar inverted is the same for all the experiments.

Thus J. O'Sullivan's experiments show that the velocity of action of the inversion function of yeast falls into line with the action of free invertase, and the action of fermentation,* previously demonstrated by the author.

Time and Molecular Change.

It was stated at the commencement of this paper that the author's object was to examine, and, if possible, bring together, certain conclusions regarding the nature of enzyme action which seemed to be contradictory. Experimental evidence appeared to show that on the one hand the action of invertase, both in the free state and confined within the living cell, followed the law of mass action; and, on the other hand, that the action of the enzyme of alcoholic fermentation followed a different law. The author has now shown that these supposed differences in character of action do not exist, and that the actions of both inversion and fermentation follow approximately

* It is interesting to note the agreement in character of action of the inversion and fermentation functions of the *living yeast cell*, as it tends to strengthen the conclusion that fermentation is a true enzyme action.

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the same order of progression—an order which is not that of mass action.

But this conclusion, that the actions of the two enzymes exhibit an exceptional order of progression differing from that of mass action, introduces a question which requires explanation.

It appears impossible to believe that enzyme change, however produced, is independent of mass action. According to our present conception of matter and its mechanics, such an idea appears to be inconceivable. Therefore, in looking for some explanation of the exceptional character of the actions of inversion and fermentation, the author concludes that the influence of mass in these actions, as they have been studied so far, must be limited or concealed by some other influence.

If such an influence is looked for, consideration shows that it may be due to the existence of a time factor in certain forms of complex molecular change.

When the law of mass action regulating simple chemical change has been confirmed by direct experiment, the reactions investigated have been changes such as the hydrolysis of methyl acetate by hydrochloric acid (Ostwald, *loc. cit.*) and the inversion of cane sugar by acids. In such experiments, the molecular change following collision of the reacting molecules takes place with extreme rapidity and the existence of a time factor is not in evidence in experimental determinations of the velocity of change. But it is quite conceivable, with regard to such a change as that of enzyme action, that the time elapsing during molecular union and transformation may be sufficiently prolonged to influence the general course of the action.

There is reason to believe that during inversion of cane sugar by invertase the sugar combines with the enzyme previous to inversion. C. O'Sullivan and Tompson (*loc. cit.*) have shown that the activity of invertase in the presence of cane sugar survives a temperature which completely destroys it if cane sugar is not present, and regard this as indicating the existence of a combination of the enzyme and sugar molecules. Wurtz (*Compt. rend.*, 1880, **91**, 787) has also shown that papain appears to form an insoluble compound with fibrin previous to its hydrolysis. Moreover, the more recent conception of E. Fischer with regard to enzyme configuration and action, also implies some form of combination of enzyme and reacting substance.

Let it be assumed, therefore, that one molecule of an enzyme combines with one molecule of a reacting substance, and that the compound molecule exists for a brief interval of time during the further actions which end in disruption and change. Let it be assumed also that the interval of time during which the compound molecule of enzyme and reacting substance exists is 1/100 of a time unit.

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Then it follows that a molecule of the enzyme may assist in effecting 100 completed molecular changes in unit time, but that this is the limit to its power of change.

Again, let it be assumed that the number of molecular collisions between the active and reacting molecules which lead to their combination bears some proportion to the number of possible completed molecular changes in unit time. Let the number of collisions be 20, then there may be 20 complete molecular changes; if 40, there may be 40 changes. In fact, the action of the mass law is observed, for other conditions being equal, the average number of molecular collisions must depend on the number of molecules, or mass, of the matter present.

But now assume that the mass of reacting substance is increased, so that the number of molecular collisions in unit time exceeds 100; let it be 150, 1000, or any other number larger than 100. Then, although the number of molecular collisions may exceed 100 by a number following the law of mass action, 100 molecular changes cannot be exceeded, for the compound enzyme and sugar molecule is only capable of effecting 100 complete changes in unit time.

It follows, therefore, that if, in a series of changes like the imaginary ones described, a constant amount of enzyme is in the presence of varying quantities of a reacting substance, and in all cases the quantity of reacting substance present ensures a greater number of molecular collisions in unit time than the possible number of molecular changes, then a *constant weight* of substance may be changed in unit time in all the actions.

When invertase acts in solutions of cane sugar of varying concentrations, an approximately *constant weight* of sugar is inverted in unit time, and the yeast cell, under similar conditions, ferments an approximately *constant weight* of sugar; it appears, therefore, that the exceptional character of these changes may be satisfactorily accounted for by the theory advanced.

Experimental evidence may also be brought forward in support of this theory.

In Table IV, the results of the author's experiments show that approximately constant quantities of cane sugar are inverted in unit time in solutions varying in concentration from 5 to 40 per cent. If the results of these experiments are looked at in the light of the author's theory, the number of molecular collisions in unit time in each experiment must have equalled, or exceeded, the possible number of changes by the compound molecule of enzyme and sugar. But this has happened in solutions containing 5 per cent. and upwards of cane sugar. It must, however, be possible to make solutions of varying quantities of cane sugar so dilute that the number of molecular collisions taking place in unit time between the sugar molecules and a

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constant number of invertase molecules will fall below the possible number of changes. Then, if the author's theory be correct, the progress of inversion in a series of these dilute solutions of cane sugar of different concentrations will exhibit an action in accordance with the law of mass action, for the time interval of change no longer restricts its effect.

It seemed very possible when commencing the attempt to demonstrate this experimentally that it might prove that the solutions of cane sugar required for the purpose were too dilute to use for experimental purposes. But when the attempt was made, it was found that the necessary dilutions are within the limit of experiment, as the results given in the following table (VIII) show:

TABLE VIII.—*Velocity of action of invertase in very dilute solutions of cane sugar.* 100 *c.c. of cane sugar solution and* 1 *c.c. of diluted invertase solution employed for each experiment. Time of change,* 60 *minutes. Temp.* 31°.

No. of experiment.	Grams cane sugar per 100 c.c.	Grams cane sugar inverted in 60 minutes.	$k=\frac{1}{\theta}\log\frac{1}{1-x}$.
1	2·0	0·308	0·00132
2	1·0	0·249	0·00219
3	0·5	0·129	0·00239
4	0·25	0·060	0·00228

The results given in the above table furnish very strong evidence in support of the view that in the dilute solutions of cane sugar employed the number of contacts of the sugar molecules with the invertase molecules in unit time have been reduced to a less number than the possible number of molecular changes. In experiment No. 1, in which a concentration of 2 grams of sugar per 100 c.c. has been used, the dilution appears to have been hardly sufficient to reach the desired point. In Nos. 2, 3, and 4, however, the quantities of sugar inverted in unit time are no longer constant quantities—as was found in the experiments with concentrations of 5 per cent. and upwards, given in Table IV., and decrease in direct proportion with the concentrations.

Moreover, the value k in these experiments is a constant number.

These observations indicate a change in accordance with mass action, which, according to the author's theory, should be evidenced in solutions of sufficient dilution. There is, therefore, reason to believe from the results of the above experiments that the exceptional action of inversion in all but very dilute solutions of cane sugar is due to a time

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factor accompanying molecular combination and change which limits the influence of mass action.

It has been shown in this paper that the action of alcoholic fermentation follows approximately the same order of progression as that of inversion, and the work of Kastle and Loevenhart (*Amer. Chem. J.*, 1900, **24**, 491) shows that the action of lipase progresses in the same manner; it therefore appears probable that both these enzyme actions are regulated, like inversion, by a time factor accompanying complex molecular change.

It will be noticed that the author's theory demands, not only the formation of a molecular compound of enzyme and reacting substance, but the existence of this molecular compound for an interval of time previous to final disruption and change. Various speculations regarding the conditions ruling such an effect suggest themselves, but the author does not at present attempt to discuss this question.

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