XXXVII.—*The Velocity of Hydrolysis of Starch by Diastase, with some Remarks on Enzyme Action.*

By HORACE T. BROWN, LL.D., F.R.S., and T. A. GLENDINNING, F.I.C.

THE experimental work here described, on the rate of change during the hydrolysis of starch by malt diastase, was completed more than four years ago, but the results were temporarily put on one side, owing to the impossibility of reconciling them with the views then current with regard to the analogous changes induced by the action of invertase on cane sugar.

The investigations of C. O'Sullivan and Tompson (Trans., 1890, 57 834) and of J. O'Sullivan (Trans., 1892, 61, 926) had led these observers to conclude that the time rate of change during the inversion of cane sugar by the enzyme is the same as that observed in acid hydrolysis; that it conforms, in fact, to the logarithmic formula characteristic of a unimolecular reaction.

Our own observations on the hydrolysis of starch by diastase had convinced us that the rate of change certainly does not conform to this simple law of mass action, but that there is a progressive increase in the value of the "velocity coefficient" which appears at first sight to differentiate the mode of action of diastase from that of invertase. The recent work of Adrian Brown and of V. Henri has shown, however,

that in cane sugar hydrolysis, both as regards the time elements of change and the influence of varying concentration, the action of the enzyme differs materially from that of dilute acids.

This has led us to re-examine our experiments on starch hydrolysis, with the result that they are found to be in complete accord with the observations of the two last-mentioned chemists, thus rendering it probable that there will be found one fundamental law expressing the rate of change in all enzyme reactions which can be quantitatively studied with sufficient accuracy.

If we desire to follow the course of a starch transformation as a function of the time, there are two methods open to us, one based on the diminution of the optical activity of the solution, the other on the augmentation of the cupric reducing power of the mixed products of change.

It has been frequently shown by one of us (Brown and Millar, *Trans.*, 1899, 75, 315) that when soluble starch is transformed by an active diastase at temperatures below 60°, the hydrolysed solution speedily attains definite optical and reducing properties corresponding to a well-defined molecular decomposition of the original starch. This point is reached when the mixed products of hydrolysis have attained a specific rotatory power of $[\alpha]_D 150^\circ$, and a cupric reducing power of $R 80$; R being expressed in terms of maltose per cent. of the mixed products. This stage of the reaction is so well defined and permanent that it may be regarded, under ordinary conditions of starch transformation, as an indication that hydrolysis is complete. If, therefore, we ascertain either the total fall in rotation (which may be expressed either in degrees or in arbitrary scale units), or the increase in cupric reduction which a given solution of starch experiences in attaining this final resting stage, then the rotation or reduction at any intermediate stage will give us a measure of the amount of hydrolysis at that particular moment of time.

We have employed both these methods during the investigation, but since it is impracticable to use solutions of soluble starch of greater concentration than from 3 to 4 per cent., the total fall in optical activity is not large, and the errors of reading of the polarimeter consequently assume a very sensible magnitude relative to the total fall. Under these conditions, the optical method is far less accurate as a measure of the progress of the reaction than that based on cupric reduction, which is the one which latterly was exclusively employed.

The experiments we have cited in this paper were carried out as follows.

A 3 per cent. solution of soluble starch, prepared by Lintner's acid method, was maintained at a constant temperature in a thermostat, and to a known volume of the solution was added a small, definite

amount of a cold water extract of an actively diastatic malt, the time of this addition being accurately noted. In those cases in which the transformation was to take place at an elevated temperature the malt extract was previously heated for an hour at that temperature, or a little above.* The amount of malt extract was arranged with due regard to the temperature at which the transformation was to take place, and was so adjusted that the reaction did not progress too rapidly for the subsequent operations.

After the commencement of hydrolysis, and at carefully noted intervals, portions of the liquid amounting to exactly 25 c.c. were quickly taken out with a pipette, and were at once mixed with about double the volume of *boiling* water, the temperature of the mixture being sufficiently high to at once arrest further diastatic action. After boiling for a few minutes, the solution was evaporated and accurately made up to its original volume. The cupric reducing power was then determined gravimetrically with Fehling's solution under the standard conditions laid down in a previous paper (Brown, Morris, and Millar, Trans., 1897, 71, 94). Meanwhile a portion of the original starch solution had been hydrolysed completely down to the final stage by digestion for an hour at 50° with a very active malt extract added at the rate of 5 c.c. or more per 100 c.c. of the solution. This, of course, gave the maximum reducing power of the fully hydrolysed starch, and the ratios of the intermediate reductions to this amount afforded a measure of the hydrolysis at each stage. It is scarcely necessary to add that all the necessary corrections were introduced for the reducing power and the volume of the malt extract employed, for the small initial reducing power of the soluble starch itself, and for the changes of volume of the abstracted samples due to temperature.

From the experimental data so obtained, the 'coefficient of velocity' was either directly calculated or, as was generally the case, the results were plotted out on a system of rectangular coordinates, representing on the one hand equal time intervals, and on the other the proportion of hydrolysable substance still left in solution. A perfectly even curve was then drawn through the experimental points with the aid of a flexible lath.

If we denote the cupric reduction of unit volume of a completely hydrolysed starch solution as *unity*, and take x as the ratio of the reduction observed at any given time reckoned from the commencement of the reaction, then, if the time curve representing the course of the hydrolysis is logarithmic,

$$\frac{1}{\theta} \log \frac{1}{1-x} = k,$$

* This is a necessary precaution, in order to avoid any slow changes in the activity of the extract after it has been added to the hot solution of starch.

where k is a constant representing the 'coefficient of velocity' of the hydrolysis, and θ is the time elapsed.

The results of a few typical examples of experiments of this kind are given in the Tables appended to the paper, the values of k deduced from the above formula being given in the fourth column.

Table I records a transformation carried out at a temperature of $51-52^\circ$ for more than $2\frac{1}{2}$ hours, during which time, as will be seen from the value of $1-x$ at the close of the experiment, 95 per cent. of the full hydrolytic change had taken place.

It will be noted that throughout the reaction there is a steady augmentation in the value of k . This is an invariable feature of all our numerous experiments, and proves that the course of the hydrolysis does not conform to the above logarithmic expression.

If we assume that the normal curve expressing the rate of change is logarithmic, but that its form has been modified by secondary disturbing causes, then the constant augmentation of k points to a set of conditions which is producing a constant acceleration in the velocity of change. In other words, within any given time interval there is somewhat more of the residual substance hydrolysed than there should be according to the logarithmic formula.

In an early stage of the inquiry, when we still had reason to believe that this was an exceptional instance of departure from the "mass law," we were led by certain considerations to search for the disturbing cause in the complex intermediate products of hydrolysis. We certainly cannot account for the results by assuming that they are due to the gradual accumulation of the products of change, with a consequent tendency to chemical reversion, since any influence of this kind will tend to diminish, not to increase, the velocity coefficient k .

Whatever differences of opinion may still exist as to the exact nature of some of the intermediate products of starch transformation, it is quite certain that, in course of its hydrolysis with diastase, soluble starch, unlike cane sugar under the action of invertase or acids, does not at once split up into its final products, maltose and a well characterised dextrin. This stage is reached only through a series of intermediate substances of the amylo-dextrin and malto-dextrin class. Now it is quite conceivable, although it has never been proved, that these intermediate substances may show a differential resistance to hydrolysis. If the hydrolysis of the lower members of the series is more readily effected than that of the products which stand in closer genetic connection with starch itself, these differential properties of the intermediate products would almost certainly show themselves by some such augmentation of the velocity coefficient as we have observed.

We attempted to solve this question experimentally in the following manner.

Two solutions, A and B, were prepared, containing the same amount of hydrolysable starch products, as measured by the amount of cupric reduction which the solutions gave on complete hydrolysis with an active malt extract. Solution A, however, contained starch products which had been only very slightly hydrolysed, whereas B contained more highly converted products, that is to say, a much larger proportion of those intermediate substances which, on the above assumption, ought to undergo hydrolysis more rapidly. To equal volumes of these solutions were added exactly equal quantities of a cold water malt extract, and the course of each reaction was followed by the time method already described, care being of course taken to keep all the conditions exactly the same for the two solutions. The result certainly did *not* confirm the above hypothesis of the more ready hydrolysis of the lower members of the series.

It was this failure to discover any reasonable explanation of the augmenting value of k in starch transformations, and our consequent inability to bring our observations into line with the analogous hydrolysis of cane sugar, which caused us to delay publishing our results four or five years ago.

If we critically examine the results of the two starch transformations given in Tables I and II appended to this paper, we see that the time-curve expressing the rate of hydrolysis is approximately represented by a straight line until from 30 to 40 per cent. of the total hydrolysis is complete. This is well shown in the last columns of Tables I and II, where we have given the increased amount of cupric reduction for equal intervals of time. Within the limits mentioned above, the amount of hydrolysis is *approximately proportional to the time*.

That the amount of transformation is, up to a certain point, a linear function of the time, is also well shown in the following experiment, where the observations have been restricted to a transformation of only 15·5 per cent. of the total hydrolysable products :

Transformation of a 3 per cent. soluble starch solution with 1 c.c. of malt extract per 100 c.c. Temperature 21°.

Time in minutes.	x . Amount transformed. Total hydrolysis = 1.	Amount which should have been transformed if hydrolysis is directly proportional to the time.
5	0·038	—
10	0·077	0·076
20	0·155	0·152

In this case, the linear expression is almost strictly correct, that is to say, up to a point at which 15·5 per cent. is hydrolysed, we have equal amounts of transformation in equal times.

We may go further than this, and ascertain the nature of the curve after the linear elements of it have been passed. For this purpose, we have taken the results given in Table I at the end of the paper, and have commenced from the stage of the hydrolysis which was reached in 40 minutes, when 0·4355 of the complete hydrolysis had been attained, that is to say, when 0·5645 of hydrolysable substance was left. Making this last value the new starting point, and taking it therefore as unity, we obtain the following results on recalculation :

Old time units, minutes.	New time units.	k .
40	—	—
50	10	0·00842
60	20	0·00831
70	30	0·00821
80	40	0·00837
90	50	0·00818
100	60	0·00807
110	70	0·00822
120	80	0·00840
130	90	0·00855

The values of k no longer show the steady increase which they do if we take the commencement of the transformation as our starting point, and their approach to equality shows that this part of the curve is approximately *logarithmic*.

We are able therefore to analyse our time curves and divide them into two parts, an earlier one which is *linear*, and a later one which is approximately *logarithmic*, the change from one expression to the other not being abrupt, but gradual. In his paper on enzyme action, Adrian Brown (this vol., p. 373) has brought forward a striking number of facts showing that the time-curve representing the action of invertase on cane sugar is not logarithmic, as had been previously believed, but that the value of k steadily augments during the reaction, just as we have found it do in the case of starch transformations. He has also shown that with varying concentrations of sugar, all other conditions remaining the same, approximately equal masses of the sugar are hydrolysed in equal times, providing the comparisons are made at an early stage of the hydrolysis; in other words, the inversion up to a certain point is a linear function of the time.

Still more recently, V. Henri (*Compt. rend.*, 1901, 133, 891) has also shown that the velocity of inversion of cane sugar with invertase

increases more rapidly than is expressed by the ordinary logarithmic formula of a unimolecular change.

He proposes the following empirical formula as more accurately representing the course of the reaction :

$$\frac{1}{\theta} \log \frac{1+x}{1-x} = 2k_1.$$

We have applied this formula to the results obtained in our experiments on starch hydrolysis by diastase, and find that the values of k_1 so obtained are much more nearly constant than those of k derived from the original logarithmic expression, as will be seen on looking at the values of k_1 given in the fifth column of the appended tables. From what has been said, however, about the composite nature of the time-curve, it is improbable that any single mathematical expression will be found which is strictly applicable to all parts of the curve.

It is now perfectly clear that as regards the general course of the reactions there is a close parallelism between starch hydrolysis with diastase and inversion of cane sugar by invertase, and that the observed changes in the velocity coefficient of starch hydrolysis are not necessarily conditioned by the exceptional nature of the intermediate products. Both reactions are linear in the early stages, and both are influenced by variations in the concentration of the hydrolyte. The influence of decreasing concentration is to cause a larger proportion of the remaining substance to be hydrolysed than would be expected from the application of the 'mass law' of a unimolecular change, provided always the time units are reckoned from the commencement of the reaction. In the case of starch transformations, the simple logarithmic formula is fairly well applicable in dilute solutions when from 30—40 per cent. of the hydrolysis is complete. The same will probably be found to hold good with regard to cane sugar inversion.

Henri's formula is equally well applicable to both reactions.

Adrian Brown (*loc. cit.*) explains the results which he obtained in his experiments with cane sugar by assuming that an appreciable time elapses between the molecular union of the sugar and enzyme and the actual hydrolysis, and that this time interval necessarily limits the amount of work which the enzyme can perform, so that when the ratio of sugar molecules to enzyme molecules is large, a certain maximum amount of hydrolytic work is accomplished by the latter which cannot be exceeded, and that no increased effect is consequently produced by increasing the concentration of the sugar, all other conditions remaining the same. He obtained apparent confirmation of this by gradually decreasing the concentration of the sugar, the enzyme remaining constant in amount. He then found, when a certain low point of concentration had been reached, that the reac-

tion approximately conformed to the mass law, that is, was independent of concentration.

It appears to us that the time-curves representing enzyme action can be explained in a somewhat different manner, and without postulating any differences in the time intervals between the successive stages of the reaction other than those due to variations in the respective masses of the reacting substances existing in unit volume.

It will simplify matters if we consider the case of the inversion of cane sugar, although the same argument, with a little modification, is equally applicable to the hydrolysis of starch.

When cane sugar is inverted with dilute *acids* of different kinds, but of the same *molecular concentration*, the velocity coefficients of hydrolysis not only vary in the same order as the electric conductivities of the dilute acids, but there is also a remarkable numerical agreement in the values representing the invertive action on the one hand, and the electric conductivity on the other.

The striking general agreement between these two properties has been emphasised by Ostwald, who has shown that it exists for a large number of acids (see "Outlines of General Chemistry," p. 360).

This can be explained by assuming that the velocity coefficient of inversion is a function of the number of molecules of electrolytically dissociated *ions* per unit volume of the solution.

The number of free hydrogen ions in unit volume of the most dilute acids which have hitherto been employed in such experiments must be very large compared with the number of cane sugar molecules, and under these conditions it might be expected that the course of the reaction would be expressed, as it actually is, by the logarithmic formula of a simple mass action, since for all practical purposes the cane sugar is the only substance of which the mass is changing. In the case of *enzyme* hydrolysis, such as that effected by invertase, the apparent active agent is a non-electrolyte, incapable of any appreciable dissociation, and at first sight it would appear that notwithstanding the identity of final products, there is some essential difference between acid and enzyme hydrolysis. We believe this is only apparent, and that enzyme hydrolysis is also brought about either by active water ions, or by active water molecules dissociated from the inactive and large aggregates of which the liquid mainly consists.

Pure water is in itself not a perfect non-electrolyte, but even if it were, we are never dealing with pure water in such experiments, and the remarkable influence of very minute but sensible amounts of acid in intensifying the action of invertase and diastase is so well established as in itself to suggest that water dissociation in some form or

ether is intimately bound up with the action of these and other enzymes.*

The ordinary conditions of feeble acidity necessary for the complete development of these enzyme actions are not sufficient to produce any appreciable direct hydrolytic action on the cane sugar within the time ordinarily occupied by such an experiment and within the limits of temperature necessarily imposed. In such cases, however, there can be little doubt that hydrolysis is preceded by a combination of the hydrolyte with the enzyme, and that this combination is much more unstable and less able to withstand the action of the active ions or dissociated molecules of the electrolyte than the original cane-sugar.†

According to this view these active ions are the true hydrolysts, not the enzyme itself, which has only an intermediate function, and it is now necessary to consider how this way of looking at the facts will affect our conception of the rate of change of the hydrolyte.

A solution of cane sugar undergoing hydrolysis by invertase must contain the following substances :

A—the unaltered cane sugar.

a—the cane sugar in combination with the enzyme.

b—the added enzyme, a portion of which, *b'*, is at any one moment in combination with *a*.

$(a + b')$ —the combination of cane sugar and enzyme.

*a*₁—the products of hydrolysis of *a*.

x—the free water ions, or dissociated water molecules, which act as the true hydrolysts.

It is assumed that, owing to favourable conditions, the number of active ions, *x*, is very large indeed compared with that of the other reacting molecules, and further that there is no difference in the respective velocities of the other reactions except those conditioned by the varying masses of the reacting substances in unit volume.

If we wish to follow an inversion experimentally, it is always

* On the question of the influences of mere traces of acid on the action of diastase and invertase, see Baranetzky, *Die Stärkeumbildenden Fermente in den Pflanzen*, Leipzig, 1878 ; O'Sullivan and Tompson, *Trans.*, 1890, **57**, 855 ; Brown and Morris, *Trans.*, 1890, **57**, 511 ; Fernbach, *Journ. Fed. Inst. Brewing*, 1896, 128.

The extreme sensitiveness of both diastase and invertase to minute quantities of alkali in the solution becomes easy to understand if the hydrolytic action is conditioned by the presence of electro-positive ions.

† There is a considerable amount of experimental evidence in favour of there being a real combination of hydrolyte and enzyme prior to hydrolysis. The recent work of Emil Fischer, showing that there is some sort of stereochemical relationship between hydrolyte and enzyme, seems to point to the possibility of the enzyme itself holding the combined hydrolyte with some definite orientation, which facilitates the action of the active water ions upon it. The enzyme may be regarded therefore as the *vice* which holds the sugar molecule in a position favourable for the splitting agent to act.

necessary to restrict its velocity, and in order to do this the concentration of b , the added enzyme, must be very small in relation to the initial concentration A of the cane sugar. In the earlier stages of the hydrolysis therefore, A in unit volume will be very large compared with $(a+b')$, the combination of cane-sugar and enzyme present at any moment. But $(a+b')$ must strictly speaking be considered the starting point from which the hydrolysis commences, the true hydrolyte, in fact, and the velocity of the inversion will depend on the concentration of $(a+b')$. So long as the concentration of the unaltered cane sugar A remains very large compared with $(a+b')$, this latter will remain almost constant, and equal amounts of inversion will take place in equal times: the time 'curve' will in fact be *approximately a straight line*. When, however, the concentration of A is materially reduced and begins to approach that of $(a+b')$ in the order of magnitude, then, by the ordinary laws of mass action, $(a+b')$ will gradually get smaller, and the rate of inversion will more nearly approach the logarithmic expression.*

This explanation accords very well with all the known facts, as will be seen from the following considerations.

We have seen from the results obtained by the hydrolysis of starch by diastase that the first part of the time reaction is represented by a straight line. This has also been found to be the case by Adrian Brown as regards the inversion of cane sugar by invertase; not only do the first portions of the curves approach very nearly to straight lines, but he also finds that, with equal concentration of invertase, considerable variations in the initial concentration of the sugar up to a certain stage of the reaction do not materially affect the absolute amount of sugar inverted in a given time. This we should expect from what has already been said: when a certain point is reached, any increase in A no longer sensibly influences the value of $(a+b')$, which is really the regulating factor of the reaction, and so long as these conditions exist we have equal amounts of hydrolysis in equal times.

Our hypothesis also affords an explanation of Kjeldahl's empirical "law of proportionality," which may, in fact, be deduced from it. Kjeldahl found that the relative diastatic power of two solutions is expressed by the cupric reducing power produced in a given time when the enzyme solutions act on the same weight of starch at the same temperature, provided always the reducing power is not allowed to go

* We have here, for the sake of simplicity, omitted any reference to the disturbing influence produced by the accumulation of a_1 , the products of inversion. Towards the close of a hydrolysis of a fairly concentrated solution this effect is unquestionably noticeable, but we doubt whether it has much influence in determining the form of the curve of dilute solutions, such as we used for starch transformations.

beyond R 40—49.* This corresponds to the hydrolysis of from 50 to 60 per cent. of the starch. It is clear, however, from an inspection of Kjeldahl's own curves, expressing the amount of hydrolysis with varying quantities of diastase, that the reaction is not by any means rectilinear as far as R 40, although it may be sufficiently so for all practical purposes of diastasimetry. It is, however, very nearly straight as far as R 29, at which point about 36 per cent. of the full hydrolysis is complete. This corresponds very closely with our own observations, already referred to, on the straight part of the curve.

The conditions of Kjeldahl's experiment amount to increasing the concentration of b , and consequently of $(a + b')$, leaving everything else the same. Under these circumstances, the hydrolysis effected in a given time will be approximately proportional to the concentration of $(a + b')$, provided the reaction is not allowed to go so far that the concentration of A , the untransformed hydrolyte, is no longer able to maintain the combination of the enzyme and hydrolysable substance practically constant. This is guarded against by making the comparisons for cupric reduction within the time during which the course of the reaction is practically rectilinear, and when, consequently, a large amount of hydrolysable material still remains.

We can also make another important deduction from our hypothesis, and predict that by largely increasing the relative amount of enzyme to hydrolyte, that is to say, increasing the value of $(a + b')$ in relation to A , the more completely will the linear element of the time-curve be eliminated, and the more nearly will the course of the whole reaction be represented by the unimolecular formula $\frac{1}{\theta} \log \frac{1}{1-x} = k$.

In actual practice, it is not possible to go very far in this direction, since the velocity of the action then becomes so great that the course of the hydrolysis cannot be followed with sufficient accuracy. The results of Adrian Brown's experiments on the inversion of very dilute solutions of cane sugar with fixed amounts of enzyme point, however, to the correctness of this deduction, for he found under these conditions that the "velocity-coefficient" was much less influenced by varying concentration; in other words, there was a much nearer approach, even in the earlier stages of the reaction, to the simple logarithmic expression of a unimolecular change.

We must express our thanks to Mr. D. McCandlish for assistance in the experimental part of this inquiry.

* In his original paper (*Meddelelser f. Carlsberg Lab.*, 1, French *Résumé*, p. 117), Kjeldahl has employed an older form of notation for expressing the reducing powers. We have converted them into the usual form, which expresses the specific reducing powers in terms of percentage of apparent maltose.

In the following tables :

θ = the time units elapsed from the commencement of the experiment.

x = the proportion of substance hydrolysed when 1 = total hydrolysable substance.

k = "velocity-coefficient" from formula $\frac{1}{\theta} \log \frac{1}{1-x} = k$.

k_1 = velocity-coefficient from Henri's formula $\frac{1}{\theta} \log \frac{1+x}{1-x} = 2k_1$.

TABLE I.—Transformation of a 3 per cent. starch solution with 0.25 c.c. of malt-extract per 100 c.c. of solution. Temperature, 51–52°.

θ . Time in minutes.	x .	$1-x$.	k .	k_1 .	Amount of trans- formation in equal time intervals.
10	0.1084	0.8916	0.00498	0.00472	
20	0.2250	0.7750	0.00553	0.00497	0.1166
30	0.3350	0.6650	0.00590	0.00504	0.1100
40	0.4355	0.5645	0.00620	0.00506	0.1005
50	0.5350	0.4650	0.00650	0.00518	0.0995
60	0.6150	0.3850	0.00690	0.00518	0.0800
70	0.6800	0.3200	0.00706	0.00514	0.0650
80	0.7385	0.2615	0.00728	0.00514	0.0585
90	0.7800	0.2200	0.00730	0.00504	0.0415
100	0.8150	0.1850	0.00732	0.00495	0.0350
110	0.8500	0.1500	0.00749	0.00495	0.0350
120	0.8800	0.1200	0.00762	0.00497	0.0300
130	0.9030	0.0970	0.00779	0.00497	0.0230
140	0.9220	0.0780	0.00791	0.00497	0.0190
150	0.9400	0.0600	0.00814	0.00503	0.0180
160	0.9500	0.0500	0.00813	0.00492	0.0100

TABLE II.—Transformation of a 3 per cent. starch solution with 1 c.c. of malt-extract per 100 c.c. of solution. Temperature, 21°.

θ . Time in minutes.	x .	$1-x$.	k .	k_1 .	Amount of trans- formation in equal time intervals.
10	0.095	0.905	0.00433	0.00413	
20	0.191	0.809	0.00460	0.00419	0.096
30	0.285	0.715	0.00485	0.00424	0.094
40	0.378	0.622	0.00515	0.00431	0.093
50	0.465	0.535	0.00543	0.00437	0.087
60	0.548	0.452	0.00574	0.00445	0.083
70	0.628	0.372	0.00613	0.00457	0.080
80	0.708	0.292	0.00668	0.00479	0.080

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TABLE III.—*Transformation of a 3 per cent. starch solution with 1 c.c. of malt-extract per 100 c.c. Temperature, 21°.*

θ . Time in minutes.	x .	$1 - x$.	k .	k_1 .
10	0.081	0.919	0.00366	0.00352
20	0.163	0.837	0.00386	0.00357
30	0.238	0.762	0.00393	0.00351
40	0.308	0.692	0.00399	0.00345
44	0.334	0.666	0.00410	0.00342
50	0.378	0.622	0.00412	0.00345
60	0.440	0.560	0.00419	0.00341
70	0.506	0.494	0.00437	0.00345

TABLE IV.—*Transformation of a 3 per cent. starch solution with 1 c.c. of malt-extract per 100 c.c. Temperature, 21°.*

θ . Time in minutes.	x .	$1 - x$.	k .	k_1 .
5	0.034	0.966	0.00298	0.00295
10	0.072	0.928	0.00323	0.00313
20	0.145	0.855	0.00339	0.00317
40	0.337	0.663	0.00446	0.00380
60	0.480	0.520	0.00473	0.00378
80	0.579	0.421	0.00469	0.00358
100	0.660	0.340	0.00468	0.00344
120	0.720	0.280	0.00460	0.00328
140	0.760	0.240	0.00442	0.00309