Chemistry. — *"Urease and the radiation-theory of enzyme action"*, II. By Dr. H. P BARENDRECHT. (Communicated by Prof. J. BÖESEKEN.)

(Communicated in the meeting of March 29, 1919).

In order to secure a more complete constancy of p_H a more extensive investigation, this time with 0,01 % of urea, was carried out some months later, when the technique of the estimations was more fully worked out and refined.

At least two series, with different p_H, were completed on the same day, starting from the same neutral phosphate extract of Soja-meal. On another day one of them was repeated together with a third one with a new p_H. In this way the uncertainty as to the comparability of the enzyme quantities, prepared on different days, was obviated.

For want of space these tables cannot be communicated here.

The value of m, now calculated with the formula $\frac{nc}{0{,}434} \log \frac{1}{1-y} + 0{,}01y = mt$, was constant in each table again, within the limits of the unavoidable experimental errors.

In continuing these investigations at still higher p_H a falling off of the constant m was generally observed towards the end of the reaction. This is, what might have been expected for different reasons.

From the well-known chart of the H-ion concentration data of SÖRENSEN it is clear, that the phosphate mixtures are only efficient buffers up to about $p_H = 8$.

It was for instance established by the present author, that, while 10 c.c. of a 9,6 % phosphate mixture, diluted with 2 c.c. of water, produced an 8 % phosphate mixture of $p_H = 8{,}11$, dilution with 2 c.c. of NH_3 $\frac{1}{50}$ N (i.e. the amount of NH_3 formed by the hydrolysis of 12 c.c. of 0,01 % urea solution) made $p_H = 8{,}25$.

At lower p_H this change in p_H is only about 0,01 or 0,02.

Evidently by the increase of alkalinity during the hydrolysis in a solution of 0,01 % urea the m already diminishes a little in the case of a large p_H.

Moreover, as indicated above, the radiation theory itself predicts a decrèase of the activity of the enzyme as soon as the total concentration of urea + H-ion (or more accurately, as soon as $m + nc$) has become so small, that the radiation does not all reach a urea

molecule or an H-ion before it has lost its power by spreading. In the very dilute solutions of 0,01 % urea and of low H-ion concentration this effect may certainly be expected, especially if a great deal of the urea has been hydrolysed.

As will be explained further on, a decrease of m in these alkaline solutions may also be brought about in the course of time by the reversed action, the synthesis of urea.

TABLE 12.

	Concentration of urease.	p_H	m, for unit of urease concentration.
May 24th, 1918	3	5.84	0.000205
May 24th, 1918	3	6.13	0.000221
May 24th, 1918	3	6.40	0.000267
March 2nd, 1917	3	6.40	0.00027
March 1st, 1917	3	6.40	0.000263
March 1st, 1917	3	6.67	0.000347
Febr. 26th, 1917	2	6.67	0.00036
Febr. 26th, 1917	2	7.0	0.000525
March 6th, 1917	1	7.0	0.00050
March 6th, 1917	1	7.21	0.00067
Jan. 22nd, 1917	$\frac{1}{2}$	7.21	0.00067
Jan. 22nd, 1917	$\frac{1}{2}$	7.52	0.000752
Jan. 22nd, 1917	$\frac{1}{2}$	7.64	0.000689
March 12th, 1917	$\frac{3}{8}$	7.64	0.000717
March 12th, 1917	$\frac{1}{4}$	7.80	0.00060
March 9th, 1917	$\frac{1}{2}$	7.80	0.000646
March 9th, 1917	$\frac{3}{10}$	8.03	0.000467
March 23rd, 1917	$\frac{6}{10}$	8.03	0.000479
March 23rd, 1917	$\frac{6}{18}$	8.13	0.000405
April 3rd, 1917	$\frac{6}{18}$	8.13	0.000431
April 3rd, 1917	$\frac{6}{18}$	8.65	0.000245
March 22nd, 1917	$\frac{3}{10}$	8.03	0.000453
March 22nd, 1917	$\frac{3}{18}$	8.13	0.000388
April 5th, 1917	$\frac{3}{18}$	8.13	0.000416
April 5th, 1917	$\frac{3}{18}$	8.65	0.000423

In the experimental verification of the formula $\frac{nc}{0{,}434} log \frac{1}{1-y} + ay = mt$ there is, however, besides a lowering of the H-ion concentration, another way to change the relation between the coefficients of $log \frac{1}{1-y}$ and of y.

If only p_H can be kept constant, we may raise a, the concentration of the urea, considerably. The realisation of this method will be communicated further on in this paper.

For the above mentioned reasons at high p_H only the first values of mt have been used for the main present purpose: the determination of m at different H-ion concentration.

For the purpose of comparing the values of m obtained, they are all reduced to the same enzyme concentration, the unit of which is again arbitrarily chosen as resulting when 1 g. of Soja is extracted in 100 c.c. of water + 7.28 g. of Na_2HPO_4 2 aq + 2,32 g. of KH_2PO_4 and 50 c.c. of filtrate of this is mixed with 100 c.c. of water + 9,6 g. of phosphate.

m as a function of p_H.

According to the mathematical formulation of the radiation theory, $-dv = m \frac{x}{x + nc} dt$, the constant m is only dependent on the concentration of urease present.

When equal concentrations of urease are compared or the effect has been reduced to equal concentrations, i. e. when the enzyme concentration is kept constant as well as the temperature, m will be proportional to the activity of the same ureáse concentration.

From table 12 it is clear, that the activity m changes with p_H in a peculiar manner.

In figure 3 the mean values of m, in arbitrary units, are plotted against the values of p_H as abscissae. The strikingly regular curve, thus obtained, allows a further mathematical treatment to elucidate the nature of urease.

MICHAELIS [1]) had already shown, that enzyme activity, represented as a function of p_H gives in many cases a curve somewhat similar to that of the undissociated part of an amphoteric electrolyte. The vagueness and irregularity of the curves of MICHAELIS and of those of SÖRENSEN for invertase excluded, however, all further comparison and analysis.

[1]) Die Wasserstoffionen-Konzentration. Verlag von JULIUS SPRINGER, 1914.

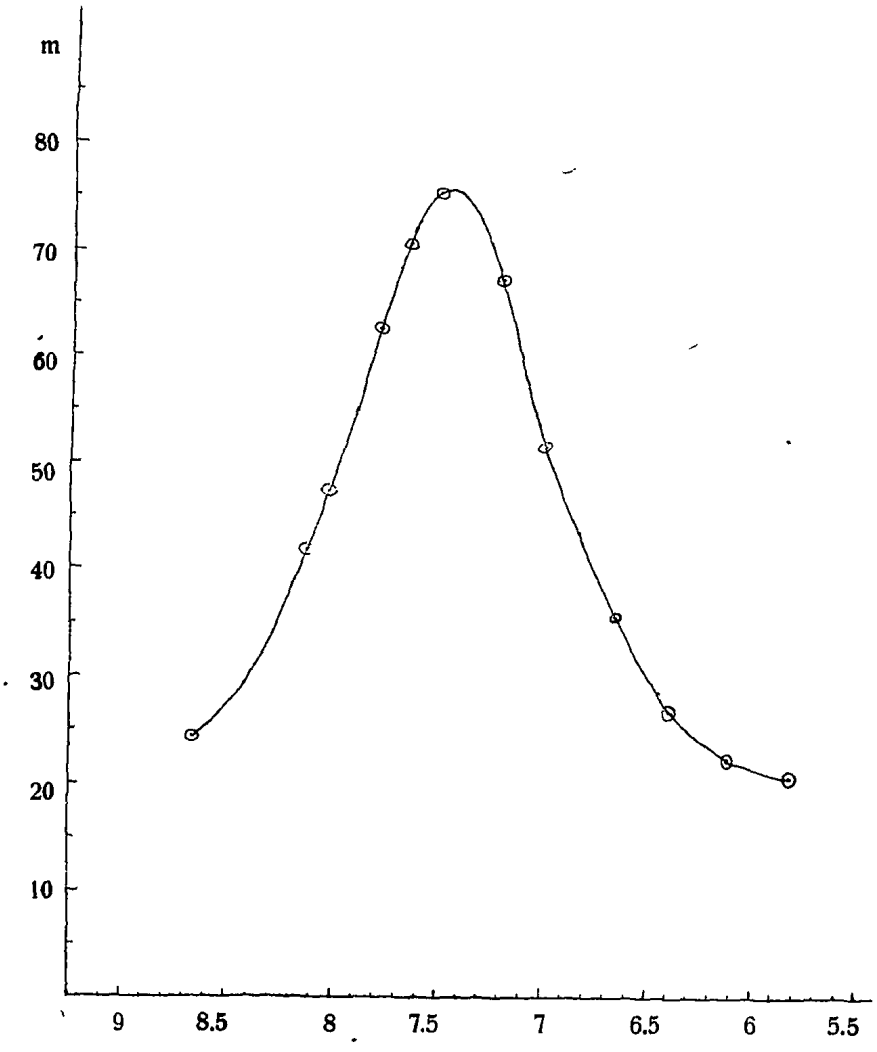

Fig. 3.

It is to be remembered, that the activity m, as calculated according to the radiation theory, is quite different from what had hitherto been empirically determined as activity.

This is immediately clear from the formula

$$m = \frac{\frac{nc}{0{,}434} log \frac{1}{1-y} + ay}{t}$$

The observed effect y in a given time t is evidently by no means proportional to m

The above mentioned regularity in m and the results, connected with it, which will be recorded further on, therefore adduce considerable experimental evidence for the radiation theory.

In the work of MICHAELIS attention is drawn to the fact, that a representation of the undissociated part of an amphoteric electrolyte as a function of the H-ion concentration, taking as abscissae the values of p_H, instead of those of the H-ion concentration itself, presents the advantage of producing curves of far more characteristic type.

His "rest-curves" are derived as follows:

Calling (A) the total concentration of the amphoteric electrolyte, $(A+)$ that of the kation, $(A-)$ that of the anion, the concentration of the undissociated rest (x) is:

$$(x) = (A) - (A+) - (A-).$$

According to the law of mass action we have in the solution the two equations of equilibrium

$$(A+)\,(OH) = k_b(x)$$
$$(A-)\,(H) = k_a(x).$$

Therefore

$$(x) = (A) - (x)\frac{k_b}{(OH)} - (x)\frac{k_a}{(H)},$$

from which

$$(x) = \frac{(A)}{1 + \frac{k_a}{(H)} + \frac{k_b}{(OH)}}$$

The undissociated fraction $\varrho = \frac{(x)}{(A)}$ becomes

$$\varrho = \frac{1}{1 + \frac{k_a}{(H)} + \frac{k_b}{(OH)}}.$$

For the sake of comparison the curves, drawn by MICHAELIS for different values of the dissociation-constants k_a and k_b, are reproduced in Figure 4.

The resemblance of our diagram of urease activity m to these curves is obvious.

It is to be borne in mind, however, that the relative dimensions of p_H and ϱ are, of course, arbitrary in these figures

Evidently, at least with decreasing p_H, where the experiments could be pushed farther than on the other side, m tends not to zero, but to a value of about 18.

The interpretation of these results is therefore as follows:

Urease is an amphoteric electrolyte, whose activity is greatest when undissociated When the asymptote, to which m approaches,

is drawn as a new axis of abscissae, the curve represents the excess of activity of undissociated over dissociated urease.

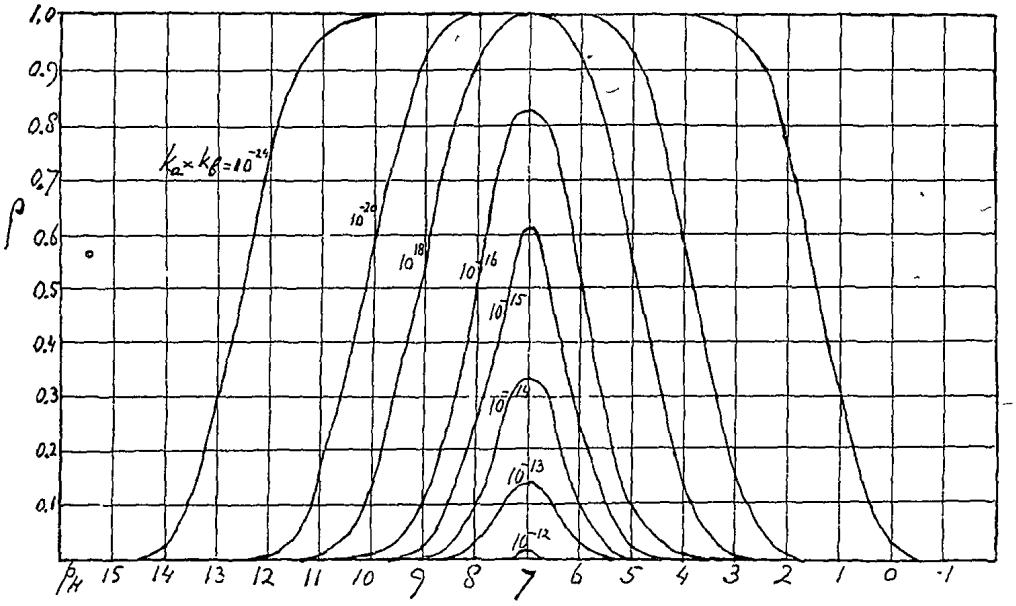

Fig. 4.

Thus

$$m - \beta = \frac{\alpha}{1 + \frac{k_a}{(H)} + \frac{k_b}{(OH)}}$$

The constant α, expressing the proportionality between the activity as determined (in arbitrary units) and the undissociated fraction, had to be calculated from the experiments as well as the constants k_a and k_b.

The term β, which appears to be about 18 from a provisional survey of the curve, had, in point of fact, also to be determined more accurately in the same way.

These calculations required the knowledge of the hydroxyl-ion concentration (OH) as well as of the hydrogen-ion concentration (H).

In water or dilute solutions this value is immediately given by the dissociation-equation of water:

$$(H)\,(OH) = k_w$$

Since an 8% phosphate solution, however, is not to be regarded as a dilute solution, special determinations of the hydroxyl-ion concentration were indispensable.

The experiments, by which these were carried out, are described in the last part of this paper.

The simplest method of calculation appeared to be giving provi-

sionally a definite value to β, for instance 18. By then combining the equations, say for $p_H = 7.52$ and for $p_H = 7$, α was eliminated directly. The same process, applied to the equations for $p_H = 6.40$ and for $p_H = 8.03$, afforded a second equation, in which only k_a and k_b were unknown. From these two equations k_a and k_b were calculated.

In table 13 are summarised the values found for p_H and p_{OH}, the concentration of the H-ions and OH-ions (multiplied by 10^8) determined and those calculated on the basis of three different values of β.

TABLE 13.

p_H	p_{OH}	$10^8 (H)$	$18^8 (OH)$	$10^5\ m$ determined	$10^5 m$ calculated for:		
					$\beta = 18$	$\beta = 17.9$	$\beta = 19$
5.84	7.94	144.5	1.15	20.5	20.6	20.5	21.6
6.13	7.65	74.13	2.24	22.1	23.—	22.9	24.—
6.40	7.38	39.81	4.17	26.7	27.2	27.1	28.2
6.67	7.11	21.38	7.76	35.4	35.—	34.7	35.7
7.0	6.78	10.—	16.6	51.3	51.3	51.3	51.3
7.21	6.57	6.17	26.92	67.—	64.8	64.9	64.—
7.52	6.26	3.02	54.95	75.2	75.2	75.2	75.2
7.64	6.14	2.29	72.44	70.3	72.4	72.2	73.7
7.80	5.98	1.59	104.7	62.3	64.—	63.6	66.9
8.03	5.75	0.93	177.8	47.3	49.—	48.7	53.—
8.13	5.65	0.74	223.9	41.7	43.4	43.1	47 3
8.65	5.13	0.22	724.4	24.4	26.—	25.9	28 —

Taking β to be 18 or 17.9, the differences of m determined and m calculated are not larger than, according to table 12, the different values of m at the same p_H determined on different days; hence not larger than the uncertainty, left in their experimental estimation.

For $\beta = 19$ the deviations are distinctly larger.

For $\beta = 17.8$ the calculation from the above four values of m produced a negative k_a. Hence the minimum value of β would be about 17.9.

The results of these calculations, as regards k_a, k_b and α are summarised as follows:

TABLE 14.

β	k_a	k_b	σ
17.9	$10^{-8} \times 1293$	$10^{-8} \times 20880$	46356
18.—	$10^{-8} \times 132.6$	$10^{-8} \times 2170$	4828
19.—	$10^{-8} \times 10.8$	$10^{-8} \times 206.5$	469.3

It is to be borne in mind, that the equation

$$m - \beta = \frac{\alpha}{1 + \frac{k_a}{10^{-p_H}} + \frac{k_b}{10^{-p_{OH}}}},$$

from which these constants had to be calculated, is an exponential one. Slight variations in p_H must therefore be expected to have a large influence.

However, as the deviations between the experimental curve and that, representing the calculated values of m for, say $\beta = 18$, in table 13, are within the limits of accuracy, set by the experimental methods employed, it may be concluded, that the equation for the undissociated part of an amphoteric electrolyte represents fairly well the activity of urease as a function of p_H.

An important consequence of this is the possibility of obtaining at least an approximate knowledge of the dissociation constants of the enzyme urease. It is evident from table 14, that k_a and k_b appear to be about 1.3×10^{-6} and 2.2×10^{-5} or even higher.

The dissociation constants of carbonic acid [1]) and ammonia at 27° are respectively 4.4×10^{-7} and 1.9×10^{-5} [2]).

The approach of these constants to those of urease is in a line with the author's view [3]) that enzymes generally contain in some active state the same molecule, which is liberated or acted upon by them.

Ammonium-carbonate + carbonic acid as a buffer-mixture.

In the beginning of this study it soon became clear to the writer, that the commonly accepted statement as to the accelerating action of CO_2 was not only not sufficiently borne out by experiment, but, as a matter of fact, might be totally erroneous.

[1]) MICHAELIS und RONA, Biochem. Zeitschr. 1914, 67, 182.
[2]) LUNDEN, Affinitätsmessungen an schwachen Säuren und Basen.
[3]) BARENDRECHT, Biochem. J. 1913, 7, 549.

The action of urease on a solution of urea soon produces so considerable a lowering of the H-ion concentration, that the course of the hydrolysis is seriously checked. To regard the accelerating effect of a stream of CO_2, passed through this solution, as a proof of the specially favourable influence of CO_2 is an unnecessary assumption, as long as full account is not taken of the power, CO_2 has to compensate the depression of the H-ion concentration. This was not done by previous authors.

It was, however, known, that nature often makes use of bicarbonates and carbonic acid as well as of phosphate mixtures as buffers to maintain the necessary constancy of the true reaction in the living cell. The buffer action of bicarbonates in blood is a case in point, which has attracted much attention of late.

Before reaching the point of view, that the urease radiation is only absorbed by the substrate urea and the H-ions, the author assumed, that the products of the enzyme-action — here ammonia and carbonic acid — also absorbed the radiation to some extent and in this way interfered with the rate of hydrolysis.

At the same time it was taken into consideration, that by passing a continuous and abundant stream of CO_2 through a urease solution containing much ammonium-carbonate and not too large an amount of urea, the H-ion concentration might easily be maintained constant; for generally the true reaction of a solution of a bicarbonate, saturated with carbonic acid, is not changed by some variation in its concentration.

It was therefore that in 1915 and 1916 a considerable amount of experimental work was carried out with ammonium-carbonate and carbonic acid as buffer-mixture, a short account of which will now be recorded and explained by the theory afterwards developed.

The ammonium-carbonate employed was KAHLBAUM's Ammonium-carbonat, "zur Analyse mit Garantie-Schein".

By dissolving ammonium carbonate in water, as FENTON [1]) has shown, an equilibrium of ammonium-carbonate and ammonium-carbamate is obtained. FENTON's method of estimating both these compounds and urea in one solution by the use of sodiumhypochlorite and sodiumhypobromite was tried by the present author with a view to establish what was the original product of the hydrolysis of urea by urease.

The velocity with which both ammonium-carbonate and ammonium carbamate tend to equilibrium, is, however, too great. These efforts

1) Proc. Roy. Soc. 1886. 39. 386.

were stopped the sooner as the question of what is the original product of change is not of much importance in these experiments. The change of carbamate to carbonate is generally quicker than the enzyme action and the carbonic acid converts all carbamate as well as carbonate into bicarbonate.

The powdered ammonium carbonate had practically the composition NH_4HCO_3. A solution of ammonium carbonate (= 2 % urea) will therefore mean in this paper a concentration of about $2 \times 2{,}63$ % ammonium carbonate.

The required amount of Soja-meal was digested at 27° with a solution of ammonium carbonate, through which a stream of carbonic acid was maintained during about one hour. After mixing with some kiezelgur a clear filtrate was very easily obtained, only slightly opalescent, if large quantities of Soja-meal had been used.

It is obvious, that the electrometric estimation of p_H is impossible in a solution of ammonium carbonate, which is to be kept saturated with carbonic acid. The much less accurate colorimetric method had therefore to be applied here. By using Tropaeolin 00 in order to give the SÖRENSEN phosphate solutions as nearly as possible the same colour as the ammonium carbonate extract of Soja-meal and kiezelgur and with rosolic acid as indicator, the p_H of an ammonium carbonate solution (= 2 % urea) with 1,36 g. of Soja per 100 c.c., through which carbonic acid had been passed at 27°, could be estimated to be about 7,0. By adding ammonium carbonate (= 0,5 % urea) and passing carbonic acid again no distinct shifting of the p_H was observed.

As will be seen, no great accuracy is required in these experiments, where $\frac{a}{\frac{nc}{0{,}434}}$ is so much larger than above in the phosphate mixtures, that the curves all approach to straight lines.

As types of the numerous experiments only the following will be recorded here.

In a round bottomed flask of $\frac{3}{4}$ Litre, placed in a waterbath of 27°, 15,125 g. of ammonium carbonate, dissolved to 250 c.c., and 6 g. of Soja-meal were introduced. A few drops of octylalcohol were added to prevent foaming.

The carbonic acid from a steel cylinder was first passed through a narrow copper tube of about 150 c.m. length, placed in the bath and then through two wash-bottles, filled with water, also in the bath of 27°. The stream of carbonic acid, in this way brought to the required temperature and saturated with watervapour, was passed

through the mixture in the flask for one hour, after which a rapid filtration with 2 g. of kiezelgur through a pleated filter gave a clear filtrate.

175 c.c. of this liquid were introduced into a similar round bottomed flask, closed by a rubber stopper, carrying two glass tubes, one of which, reaching to the bottom, admitted the carbonic acid, while the second short one was connected with a tube, filled with 10 c.c. of H_2SO_4 $\frac{1}{5}$ N, to allow an estimation of the ammonia, which might have been blown over.

After saturation with carbonic acid the current was stopped, the controlling tube with H_2SO_4 was exchanged for another, the stopper of the flask was lifted a moment and 25 c.c. solution, containing 1 g. of urea were quickly introduced. This solution had been brought before to 27° in the same bath. After replacing the stopper and again admitting the carbonic acid the reaction was allowed to proceed at constant temperature and constant p_H and its progress measured from time to time by interrupting the current of carbonic acid for a moment, taking out a sample of 5 c.c. with a pipette and running this quickly into 25 c.c. of H_2SO_4 $\frac{1}{5}$ N. After dilution with some water the contents of this flask were boiled to expel the carbonic acid and titrated with NaOH $\frac{1}{10}$ N and lacmoid (or later with Sodium alizarin sulphonate) as indicator. Owing to phosphate and proteins of the Soja, this titration was not very sharp, leaving an uncertainty of one or two drops of NaOH $\frac{1}{10}$ N.

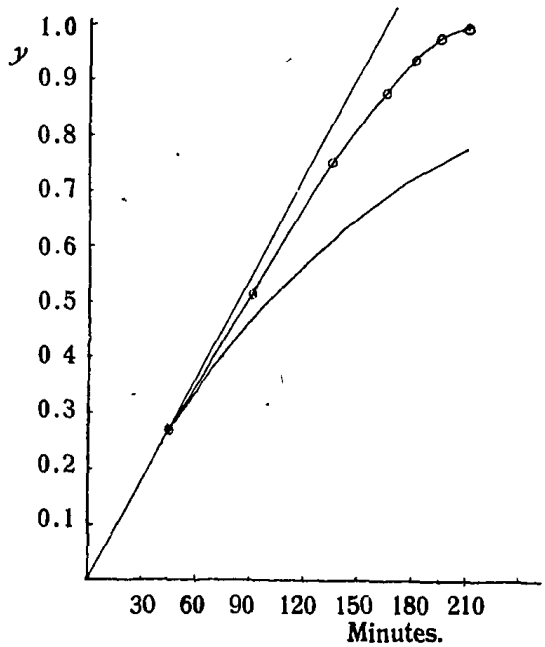

Fig. 5.

The ammonia in the original 175 c.c. solution was estimated in the same way as well as the small quantities, which might have been blown over with the CO_2 in the controlling tubes.

The results are represented in table 15 and in fig. 5, in which for the sake of comparison also the straight line and the logarithmic curve for $log \frac{1}{1-y} = kt$, are drawn, both through the origin and the first point, determined for y.

TABLE 15.

3 gr. of Soja on 286 c.c.
ammonium-carbonate (= 2 % urea)

0.5 % urea $\qquad p_H = 7$, hence $\frac{nc}{0.434} = 0.1$

t (minutes)	y	$m = \frac{0{,}1\ log \frac{1}{1-y} + 0{,}5y}{t}$	$k = \frac{log \frac{1}{1-y}}{t}$
45	0.269	0.0033	0.0030
90	0.514	0.0032	0.0035
135	0.746	0.0032	0.0044
165	0.869	0.0032	0.0054
180	0.931	0.0032	0.0064
195	0.97	0.0033	0.0078
210	0.984	0.0032	0.0085

Repeated on many different ways these experiments always produced similar results.

For instance: Febr. 3rd, 1916. 3 g. of Soja extracted with 200 c.c. of ammonium carbonate solution.

A row of test tubes, each with 10 c.c. of filtrate and one drop of octylalcohol in the bath at 27°. Each tube connected with a wide tube (over the rim of the bath), containing 25 c.c. of H_2SO_4 $\frac{2}{5}$ N and some water. Hence current of CO_2 first passing test-tube and then wide absorption-tube. 1 c.c. of a 2,75 % solution of urea, out of flask in the same bath, added to each test-tube. Reaction stopped, without opening tubes or loosing connections, by running 25 c.c. of saturated potassium carbonate solution into test-tubes and blowing over the ammonia during the whole night. Next day titrated directly in the wide tube with NaOH $\frac{1}{10}$ N and sodium alizarin sulphonate as indicator. Two test-tubes with 10 c.c. of extract, without urea, treated in the same way.

TABLE 16.
3 g. of Soja on 220 c.c.
ammonium-carbonate (= 2 % urea)
0.25 % urea $p_H = 7$

t (minutes)	y	$m = \frac{0{,}1 \log \frac{1}{1-y} + 0{,}25\, y}{t}$
40	0.495	0.0038
60	0.685	0.0037
70	0.800	0.0039
80	0.876	0.0039
90	0.936	0.0039
95	0.945	0.0038
100	0.968	0.0039
105	0.974	0 0038
110	0.985.	0.0039

These results show clearly, that the formula $\frac{nc}{0{,}434} \log \frac{1}{1-y} + ay = mt$ represents equally well the course of the reaction in urea solutions of far greater concentrations than above in the phosphate mixtures.

The nearly straight line, found generally in the hydrolysis of urea by urease, when a is not small, or at any rate large in comparison with $\frac{nc}{0{,}434}$, is equally well in accordance with the radiation theory as the logarithmic curve, the ordinary representation of the law of mass action, predicted by the same theory for dilute urea solution, if c is relatively large.

Initial velocity of urease action in urea solutions of different concentration.

Using phosphates as buffers it is, as shown above, impossible to study the course of the reaction in urea solutions, whose concentration exceeds about 0,02 %. If, however, we allow the same quantity of enzyme to act under the same conditions on urea-solutions of different concentration only to such an extent that no more than about 0,02 % urea concentration is hydrolysed, the phosphate mixtures can maintain a constant p_H in these initial periods of the process.

The experiments on this line were all arranged in the following manner:

A flask of 250 c.c. was filled to the mark with a solution of a mixture of Na_2HPO_4 2 aq and KH_2PO_4, calculated to produce a concentration of phosphate of 8% during the reaction. A small quantity of Soja-meal was added, mixed with this solution, and the flask was left for one hour in the bath at 27°. After addition of kiezelgur (the same weight as that of the Soja-meal) the solution was rapidly run through a pleated filter. From the perfectly clear filtrate portions of 10 cc. were introduced into test-tubes (as above) and placed in the same bath, in which a series of stoppered flasks with urea solutions were brought to 27°. As 2 cc. of these urea solutions were to be added to 10 cc. of enzyme-extract, all the urea-solutions had 6 times the required final concentration. The three highest concentrations of 4%, 6% and 8% were obtained by preparing a solution of 4.8 g. of urea to 10 cc. and bringing 1 cc. of this with 1 cc. of water into the 4% tube, 1.5 cc. with 0.5 cc. of water into the 6% tube and 2 cc. into the 8% tube.

The reaction was allowed to proceed for a fixed time, usually 2 hours, after which the NH_3 formed was estimated by connecting the tubes with wider ones, into which had been brought 10 cc. of H_2SO_4 $\frac{1}{50}$ N and some water, running 25 cc. of saturated potassium carbonate and a drop of octylalcohol into the reaction-tube and passing a current of air for two hours.

The p_H was determined with the electrometer in 10 cc. phosphate-enzyme-solution + 2 c.c. water at 27°.

The quantity of Soja-meal was usually 0.2 gram. Only at the lowest p_H more enzyme and different reaction times had to be taken. The results are then reduced to 0.2 g. of Soja and 120 minutes. (See tables 17 and 18 on following page).

The conclusions, to be drawn from these results, are the following:

The amount of action, produced under the same conditions, as to temperature and p_H, by the same quantity of urease in urea solutions of different concentrations becomes never really constant, not even in highly concentrated solutions.

The higher the acidity of the solutions, the more the amount of action increases with increasing concentration.

These facts are in agreement with the fundamental formula

$$-dx = m\frac{x}{x+nc}dt$$

For the initial velocity, when x is still equal to a, this formula gives the mathematical expression

$$-\frac{dx}{dt} = m\frac{a}{a+nc}$$

TABLE 17.

c.c. NH_3 $\frac{1}{50}$ N, formed in 120 minutes in 12 c.c.

Concentration urea a	$p_H =$ 5.83	$p_H =$ 6.68	$p_H =$ 6.81	$p_H =$ 6.89	$p_H =$ 7.14	$p_H =$ 7.47	$p_H =$ 7 83	$p_H =$ 8.10
0.03	0.068	0.58	0.95	1.2	1.65	3.2	3.2	2.9
0.05		0.9	1.4	1.7	2.25	3.45	3.75	3.2
0.08	0.164	1.3	1.9	2.3	2.7	4.05	3.55	3.2
0.1	0.21	1.6	2.15	2.5	3.—	4.15	4.1	3.4
0.2	0.375	2.3		3.4	3.55	4.65	4.3	3.7
0.5	0.85	3.3	3.9	4.3	4.1	5.—	4.5	3.8
1.—	1.5	3.9	4.5	4.65	4.3	5.05	4.25	3.9
2.—	2.75	4.45	4.9	5.2	4.5	5.15	4.75	4.1
4.—	3.2	4.8	5.15	5.4	4.45	5.25	4 6	3.9
6.—	4.15	4.85	5.2	5.3	4.45	4.85	4.35	3.65
8.—	4.65	4.8	5.—	5.15	4.25	4.6	4.—	3.25

TABLE 18.

Values of:

$$m = \frac{\frac{nc}{0{,}434} \log \frac{1}{1-y} + ay}{t} \quad \text{or:} \quad m = -\frac{dx}{dt} \left[\frac{x+nc}{x} \right]^{x=a}$$

multiplied by 1000.

Concentration urea a	$p_H =$ 5.83	$p_H =$ 6.68	$p_H =$ 6.81	$p_H =$ 6.89	$p_H =$ 7.14	$p_H =$ 7.47	$p_H =$ 7.83	$p_H =$ 8.10
0.03	0.068	0.103	0.135	0.153	0.140	0.23	0.174	0.139
0.05		0.114	0.142	0.157	0.160	0.19	0.181	0.144
0.08	0.066	0.128	0.150	0.168	0.160	0.20	0.161	0.140
0.1	0.067	0.136	0.153	0.166	0 167	0.20	0.184	0.147
0.2	0.066	0.143		0.181	0.172	0.21	0.185	0.157
0.5	0.08	0.165	0.184	0.199	0.184	0.21	0.189	0.159
1.—	0.102	0.178	0.194	0.199	0.182	0.21	0.177	0.163
2.—	0.152	0.185	0.208	0.220	0.190	0.22	0.198	0.171
4.—	0.154	0.205	0.218	0.226	0.186	0.22	0.192	0.163
6.—	0.19	0.205	0.219	0.221	0.186	0.20	0.181	0.152
8.—	0.20	0.203	0.209	0.214	0.177	0.19	0.167	0.135

If nc is large, compared with a, the initial velocity is small; a larger a gives a greater velocity. On the other hand, if nc is small, then even for low urea concentrations $\frac{a}{a+nc}$ is not small and will sooner approximate to a constant value.

The values of m were calculated in the tables, either, when y had an appreciable value, from the integrated equation, or from the differential equation for the initial velocity as soon as the urea concentration was high enough to make these equations give the same value.

The inconstancy of m will now be shown to afford favourable evidence to the radiation theory.

For in surveying the columns which give the c.c. NH_3 $\frac{1}{50}$ N, formed in equal times of action, a remarkable feature will be observed.

For low p_H these values increase continually from 0.03 up to 8 % urea concentration.

For higher p_H there is first an increase and then, in the most concentrated urea solutions, a decrease.

This is exactly, what the theory would lead us to expect.

A urease particle being the centre of a sphere of action and the action in this case producing an alkaline substance, the H-ion concentration around the enzyme particle will be lowered and kept low by the enzyme action itself. This process will be negligible in dilute urea solutions, but in concentrated ones, where the sphere of action is concentrated into a small volume, a marked diminution of the H-ion concentration may be expected.

Bearing in mind the dependence of urease activity m on p_H (see Fig. 3), it will be evident, that in solutions of low p_H a decrease of the H-ion concentration around the enzyme particles, i.e. a diminution of c, means a rise of m. Hence for two reasons considerably more action is found here in high urea concentrations. For, besides the increase of $\frac{a}{a+nc}$, there is also an increase in m, because the p_H, though constant as far as can be estimated in the solutions as a whole, is increased in the small sphere around the enzyme, to which the action is confined.

If p_H is not very low, the production of an alkaline substance around the enzyme particle may raise p_H above the optimum in these phosphate solutions. Hence in the concentrated urea solutions of a p_H near or above this optimum the p_H may soon be raised so far, that m is diminished.

(To be continued.)