Chemistry. — "*Urease and the radiation-theory of enzyme-action*". By Dr. H. P. BARENDRECHT.

(Communicated in the meeting of February 22, 1919).

I.

1. Since the discovery by TAKEUCHI of urease in the Soja-beans an exceptionally useful material for the study of enzyme-action has been at our disposal. The enzyme as well as the pure substrate, urea, are now readily obtainable in unlimited quantity. The estimation of the reaction products can be carried out easily and accurately, an important condition for success in pioneerswork, where innumerable analyses have to be made.

This chance of solving to some extent the great riddle of enzyme-action has therefore attracted many workers during the last few years.

MARSHALL (J. Biol. Chem. XVII, p. 351, 1914) has found, that in this case also the action is proportional to the concentration of the enzyme.

The ARMSTRONG'S, HORTON and BENJAMIN (Proc. Roy. Soc. 1912 and 1913) have made extensive empirical studies, from which they drew the conclusion, that ammonia retards, but carbonic acid accelerates the reaction, a surprising result, which others also state to have found. As will be seen from the present paper, pure chemical empiricism here leads to false conclusions.

A first endeavour to theoretical as well as to experimental study of the action of urease was made by DONALD VAN SLYKE and his collaborators (J. Biol. Chem. XIX, p. 141, 1914).

To clear the field it is necessary to pass some criticism on this work.

The theory of these authors and all their further work are based principally on three experiments. In experiments 1 and 2 "the effect of concentration of urea, enzyme concentration being constant" and "the effect of decreasing urea concentration on reaction, as the latter approaches completion" were investigated. As in these experiments the considerable changes in concentration of the hydrogen-ions were left out of consideration, notwithstanding the authors themselves have further on become aware, that the urease activity is dependent in a high degree on the H-ion concentration, it is no wonder, that they

tried to found the "formulation of the nature and course of the reaction" especially on experiment 3, in which phosphates acted as a buffer against large changes of the true acidity.

Since the results, as published, of this experiment, were incompatible with the experiments and theory of the present papers, the author has recalculated them on the basis of VAN SLIJKE's own theory.

The remarkable conclusion is, that even VAN SLIJKE's own basal experiment was not at all in accordance with his own theory, the c being clearly far from constant:

TABLE III of VAN SLIJKE.
$E = 0{,}1 \quad t = 60.$

Concentration urea.	a. 0,01 N NH_3 calc. for complete decomp. of urea.	x. 0,01 N NH_3 formed	$0{,}4343\ c = \frac{d}{dEt-x} log \frac{a}{a-x}$ acc to V. SLIJKE.	$0.4343\ c$ $\frac{d}{dEt-x} log \frac{a}{a-x}$ recalculated
per cent	c.c.			
0.0375	12.5	5.8	0.055	0.056
0.075	25.—	10.4	0 058	0.059
0.15	50.—	15.5	0.053	0.054
0.3	100.—	21.2	0.052	0.054
0.6	200.—	24.8	0.051	0.048
1 2	400.—	27.—	0.052	0.039
2.4	800.—	28.5	0.052	0.032

2. *The general equation of urease action.*

The investigations, published in this paper, were again based on the author's hypothesis, that an enzyme acts by radiation and that an enzyme particle contains the same molecule, which is liberated or acted upon by this enzyme, in some active state. In his first papers on enzyme action (Proc. K. Akad. Wetensch. Amsterdam 1904; Zeitschr. physikal. Chem. XL, p. 456, 1904; Biochem. J. VII, p. 559, 1913) the author has already suggested, that the radiation, by which enzymes exert their action, is due to the electrons, forming part of the atoms. The recent development of the electron theory of matter has revealed, that, every atom being a complex of positive and negative electrical units, all chemical action is in reality an electric phenomenon. In a general way it may be stated, that in an atom the electrons, moving round the positive nucleus, will have some effect e.g. of electromagnetic induction on other atoms in their neighbourhood.

If all the atoms, constituting a molecule, receive this radiation from a similar molecule at the same time in the required phase, the reactivity of the molecule as a whole can be expected to have changed. A small increase of the vibration of the molecule may increase its power to enter into combination (this point will be treated further on), a large elevation may tear it out of a compound with other molecules.

However, the author wishes not at present to lay much stress on the particulars of his hypothesis. The numerous experimental facts, recorded in these papers, for a great deal only revealed by the aid of this guiding hypothesis and which we have all coordinated by drawing their consequences, will prove its usefulness.

The radiation, by means of which urease acts on urea, thus originates from the enzyme molecule and is able to exert its hydrolysing effect to a certain distance, probably microscopically small.

When this urease-radiation strikes a urea-molecule, it is absorbed, just as for instance the specific radiation of a Na-atom is especially absorbed by a Na-atom.

The amount of urea, hydrolysed in a time-unit by an enzyme-molecule would therefore be independent of the urea-concentration, if the other coustituents of the solution had practically no absorbing power towards this radiation. Only with a very small concentration of urea, the radiation might be expected to be, at least partially, so much weakened by spreading before striking a urea-molecule, that it has lost the power of hydrolysing it. Hence for very dilute solutions of urea constancy of action of a given quantity of urease should not be expected; in these conditions the amount of action will be found smaller.

So far the theory is the same as that, put forward by the author previously for the sugar-enzymes.

A new point of dominating importance, at least in the case of urease, is, that the hydrogen-ions proved to be, besides urea, the only constituent in the solution, which absorbs this radiation.

It seems not improbable, that the way in which the H-ions were found to interfere with the urease-action will appear to play a part also in enzyme-action generally.

The mathematical formulation of this theory is very simple and gives at once the following differential equation for the reaction velocity at constant temperature and constant H-ion concentration:

$$-dx = m\frac{x}{x + nc}dt \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

In this equation x is the concentration of the urea (grams per

100 c.c.), c is the concentration of the H-ions (also in grams per 100 c.c.) and n is the coefficient of absorption of the H-ions, i.e., one gram of H-ions absorbs n times as much radiation as one gram of urea.

The velocity-constant m for a given temperature and H-ion concentration is proportional to the concentration of enzyme only, if both temperature and H-ion concentration are maintained really constant.

Calling the initial urea concentration a, expressed like x and c in grams per 100 c.c., putting

$$\frac{a-x}{a} = y,$$

substituting this in (1), we get

$$a\,dy = m\frac{a(1-y)}{a(1-y)+nc}dt \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

After integration and introduction of decimal logarithms the general equation for the reaction-velocity of urease at constant temperature and constant H-ion concentration becomes

$$\frac{nc}{0{,}434}\log\frac{1}{1-y} + ay = mt \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

3. *Determination of the constant n.*

For the estimation of the important constant n it was necessary, not only to determine accurately the H-ion concentration c, but also to take care, that c and thereby also m (as will be seen further on) remained unchanged from beginning to end of the reaction. Now, the hydrolysis of urea to ammonium-carbonate is in so far a difficult case for enzyme study, that here by the enzyme-action itself a distinctly alkaline substance is formed out of a neutral substrate. This production of alkali is so considerable, that even in presence of a buffer mixture of 8 % phosphate only 0.01 % or at the utmost 0.02 % of urea can be allowed to be transformed, if one wants to maintain anything like constancy of p_H.

A study of the kinetics of urease-action without the addition of a powerful buffer to keep the true reaction constant, is evidently as useless as working without a thermostat in a room of widely changing temperature.

In fixing the best conditions for the experimental determination of this constant n, two considerations determined the choice of the p_H of the regulating phosphate mixture.

As m had appeared to be a function of p_H with a distinct maximum, the p_H of this maximum would offer the advantage, that here a small variation of p_H would produce smaller change in m than elsewhere.

Secondly, to check the influence of the inavoidable experimental errors, the coëfficient $\frac{nc}{0{,}434}$ should not be much larger or smaller than a. For, if the coëfficient of $log\ \frac{1}{1-y}$ predominates largely, the reaction practically corresponds to the ordinary logarithmic line of the law of mass action. On the other hand, a being much larger, a nearly straight line will appear.

Therefore in these basal experiments a mixture of Na_2HPO_4 2 aq and KH_2PO_4 was used in such proportion, that the enzyme-action would proceed in an 8 % phosphate mixture of about $p_H = 7.5$.

The materials used were the following:

Ordinary yellow (probably Mantchourian) Soja-beans were powdered in a small American "Enterprise" mill, slowly turning the handle to avoid the heating by friction, which is otherwise soon perceptible. The powder was kept in a common stoppered bottle in the dark.

The KH_2PO_4 and Na_2HPO_4 2 aq were the purest compounds from KAHLBAUM, labelled "zu Enzym-studien nach Sörensen".

The urea, from KAHLBAUM, was recrystallised by the author from alcohol of 96 %.

All experiments in this research were made at a temperature of 27° C. This temperature is just high enough to allow without difficulty the use of a waterbath of constant temperature nearly the whole year round, and, on the other hand, low enough to avoid the deteriorating effect of higher temperatures on enzyme activity, within reasonable limits of time and true reaction.

7.28 g. of Na_2HPO_4 2 aq and 2.32 g. of KH_2PO_4 were dissolved in a stoppered flask to 100 c.c.

Into this solution 0.4 gram of Soja-meal was introduced, the flask was shaken thoroughly and left in the waterbath of 27° for one hour. After addition of 0.4 gram of kiezelgur, which had been repeatedly washed and then dried, the extract was filtered off easily and perfectly clear through an ordinary pleated filter. In the mean time there had been prepared a solution of 14.4 grams of Na_2HPO_4 2 aq in 150 c.c. of water in a larger stoppered flask. To this were now added 75 c.c. of the clear Soja extract, by which a diluted, still perfectly clear, extract resulted, which will be indicated by the letter E.

Ten test-tubes of Jena-glass, about 20 cm. long and 2.3 cm. wide, had before been placed in the bath. These test-tubes were (as in VAN SLYKE's experiments) closed by rubber stoppers with two borings. Through one of these a glass tube passed, about 30 cm. long and 4 or 5 m.m. outside diameter, ending near the bottom in a little bulb with pinholes. The second boring held a small pipette-like tube, with some cottonwool in the narrow end at the top, which was meant to prevent the passage of any splashes of the liquid with the air-current.

Each of these test-tubes received 10 c.c. of the extract E. Together with the tubes a flask with 0.150 gram of urea, dissolved in 250 c.c. water, was placed in the thermostat.

After equilibrium of temperature had been established, 2 c.c. of urea solution were introduced in each test-tube with an accurate pipette. Like all the pipettes used in these experiments, this one was calibrated for blowing out one minute after the liquid had run out, which gives the greatest accuracy, provided of course, the inside is cleaned beforehand with a mixture of sulphuric acid and bichromate. A moment's stirring through the long tube with air, freed from carbon dioxide, ensured complete mixing. Both tubes were closed by pieces of rubber tubing and clips.

The moment the 2 c.c. had run out of the pipette and the contents of the test-tube had been provisionally mixed by shaking, was taken as the starting-point of the enzyme-action. As the 2 c.c. ran out of the pipette in a few seconds this point could be determined with sufficient accuracy.

In a wooden block with two rows of holes (see Figure 1) the necessary number of thickwalled glass tubes were kept ready, each containing a carefully measured quantity, between 5 and 12 cc., of sulphuric acid $^1/_{50}\,N$, and filled up with water to a height of about 7 cm. These tubes were also closed by a rubber stopper, through which passed a long tube with pinholes and a short one.

At the end of the fixed time-interval (or rather about $^3/_4$ minutes before it, as this was within a few seconds, the time required for the next operation till the reaction was considered to have stopped) the test-tube was taken out of the thermostat and put into the wooden block. The rubber tubing B being connected with the glass tube, the clip was removed, the closing of the tube A was taken off and replaced by a piece of rubber tubing, in the open end of which was then put a drop of octylalcohol to prevent foaming. Immediately after this the point of a pipette with about 25 cc. of saturated potassium carbonate solution was introduced into this rubber tubing

and by blowing out its contents rapidly and then blowing through air for a moment, the potassium carbonate solution was mixed

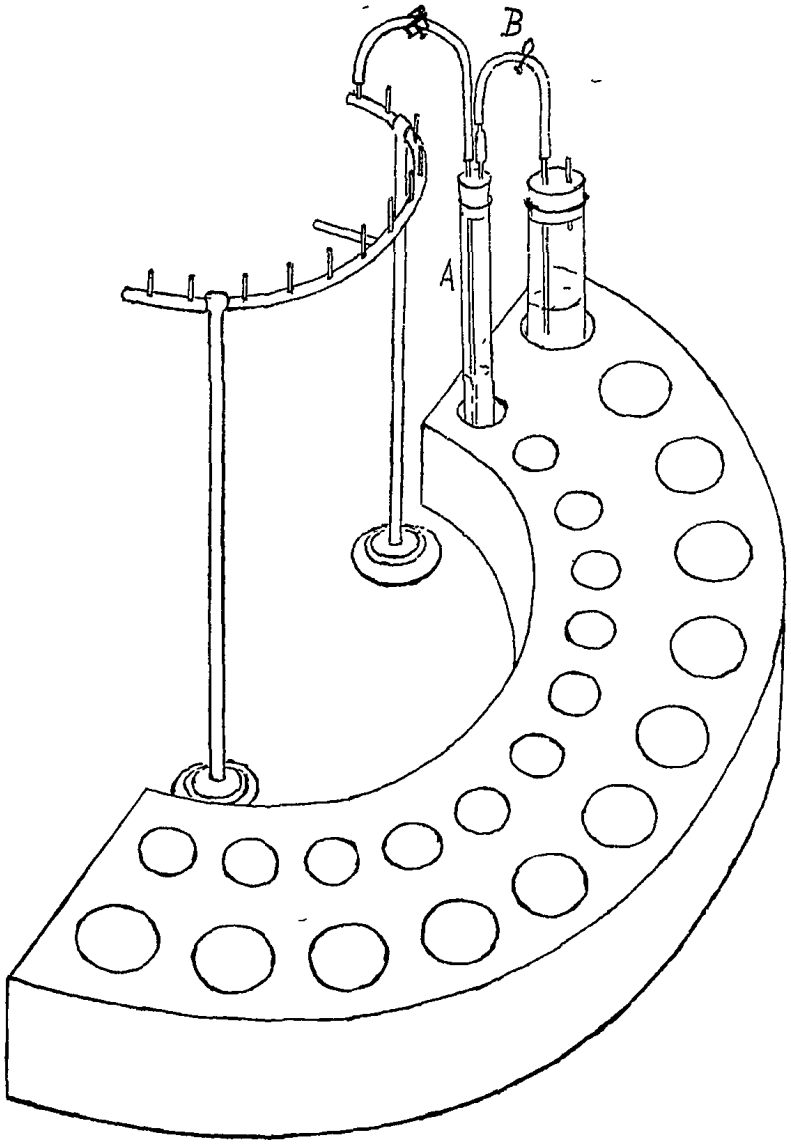

Fig. 1.

within a few seconds with the liquid in the reaction tube, stopping the enzyme-action abruptly.

The tube *A* was then connected with the air-supply and the ammonia blown over by a vigorous current of air, washed through sulphuric acid. Two hours was proved to be amply sufficient for the quantities of liquid used.

Larger volumes would have been difficult to handle.

In order to obtain sufficient accuracy in estimating these very small quantities of NH_3 single determinations were not sufficient. On two consecutive days identical series of experiments were carried

out in duplo, without changing the sulphuric acid tubes. In this way each absorbing tube got four times the amount of NH_3 of one test-tube. On each day the Soja extract was freshly prepared as described above.

The necessary correction for the traces of NH_3, which might have been given off by the Soja-meal, the phosphate or the potassium carbonate, was determined by placing each day 3 times 10 cc. of extract *E* into 3 empty test-tubes and after the addition of 25 cc. of potassium carbonate, blowing over the NH_3 in the same manner into absorbing tubes, filled with 5 cc. of H_2SO_4 $^1/_{50}$ N and water. Each of these absorbing tubes thus received 6 times the amount of this correction.

The estimation of p_H was made electrometrically in the air-thermostat of 27°, as described further on in this paper.

In the present case for 10 cc. extract *E*, mixed with 2 cc. water $p_H = 7.515$.

10 cc. of extract *E*, mixed with 2 cc. of urea solution (0.06 %) after 4 hours standing at 27° gave $p_H = 7.525$.

As the p_H on a total hydrolysis of 0.01 % urea in 8 % phosphate showed the slight increase of 0.01, it was taken here as 7.52.

The titration was carried out directly in the wide absorbing tube with $^1/_{50}$ N NaOH, prepared shortly before with distilled water, freed from carbon dioxide and $^1/_{10}$ N NaOH solution, prepared and and kept free from CO_2.

A very dilute solution of sodium alizarin sulphonate proved again to be the best indicator for NH_3 estimations. Cleaning of burettes and pipettes with bichromate and sulphuric acid directly before use is absolutely necessary in this kind of work.

Jan. 17—18 1917. TABLE 1. (Fig. 2 *A*)

0.01 % urea. $p_H = 7.52$

t - minutes.	c.c. $\frac{1}{50}$ N H_2SO_4	c.c. $\frac{1}{50}$ N NaOH	c c. $\frac{1}{50}$ N NH_3	c.c. $\frac{1}{50}$ N NH_3 correct.	*y*	$m = \frac{0{,}0327 \log \frac{1}{1-y} + 0{,}01y}{t}$
20	10	8.1	1.9	1.78	0.223	0.000290
30	10	7.3	2.7	2.58	0.323	0.000292
50	10	6.2	3 8	3.68	0.46	0.000267
70	10	4.8	5.2	5.08	0.635	0.000295
90	10	4.0	6.0	5.88	0.735	0.000291
110	12	5.37	6.63	6.51	0.814	0.000291

As will be also seen in Fig. 2 *A* the point for $t = 50$ falls outside the curve and is evidently erroneous.

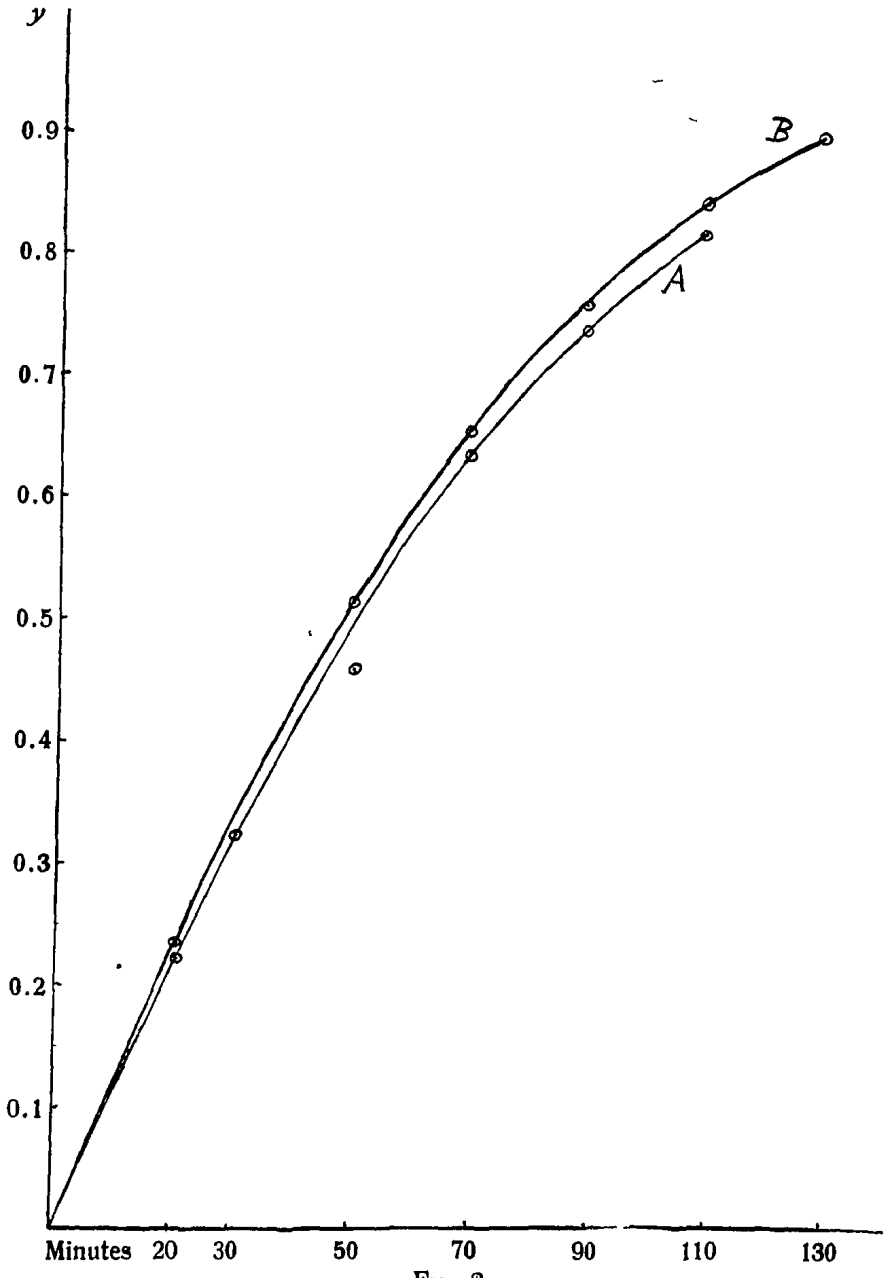

Fig. 2.

By combining the pairs of values, which are sufficiently wide apart on this curve, the equation $m = \frac{1}{t}\left(\frac{nc}{0{,}434}\ log\ \frac{1}{1-y} + 0{,}01y\right)$ gives the following figures for $\frac{nc}{0{,}434}$. (Table 2).

The concentration of the hydrogen-ions in this equation had to

TABLE 2.

t	$\frac{nc}{0.434}$
20 and 90	0.0314
20 and 110	0.0318
30 and 90	0.0335
30 and 110	0.0334
	mean 0.0327

be expressed in the same units as the concentration of the urea, in grams per 100 c.c. As $p_H = 7.52$ means a hydrogen-ion concentration of $10^{-8} \times 3{,}02$ in the usual units, grammolecules per Litre, we have here $10^{-8} \times 0{,}302$ g. H. in 100 c.c.

From this $n = 0{,}047 \times 10^8$.

In order to show, that in these estimations a high accuracy is wanted, but is hardly to be expected in the result, and that the deviations are within the limits of experimental errors, we may, for instance, calculate $\frac{nc}{0{,}434}$, assuming that for $t = 20$ the titration had given 8.05 instead of 8.1.

From

$$\frac{1}{20}\left(\frac{nc}{0{,}434}\,0{,}5768 + 0{,}00735\right) = \frac{1}{90}\left(\frac{nc}{0{,}434}\,0{,}1128 + 0{,}00229\right)$$

would then follow $\frac{nc}{0{,}434} = 0{,}0426$.

Considering, that the two small samples of Soja-meal, weighed

Jan. 31st 1917. TABLE 3. (Fig. 2 *B*)

t minutes.	c.c. $\frac{1}{50}$N H_2SO_4	c.c. $\frac{1}{50}$N NaOH	c.c. $\frac{1}{50}$N NH_3	c.c. $\frac{1}{50}$N NH_3 correct.	y	$m = \frac{0{,}0302 \log \frac{1}{1-y} + 0{,}01y}{t}$
20	5	4.03	0.97	0.94	0.235	0.000293
50	5	2.92	2 08	2.05	0.513	0.000291
70	5	2.35	2.65	2.62	0.655	0.000293
90	5	1.95	3.05	3.02	0.755	0.000289
110	5	1.62	3.38	3.35	0.838	0.000293
130	5	1.40	3.60	3.57	0.892	0.000293

off on two consecutive days might not have been absolutely equal, the author made a new set of experiments, in which only the two series of the same day were combined. (Table 3).

From this table was calculated:

TABLE 4.

t	$\frac{nc}{0.434}$
20 and 50	0.0343
20 and 110	0.0301
20 and 130	0.0302
50 and 110	0.0282
50 and 130	0.0287
70 and 110	0 0298
70 and 130	0.0300

mean 0.0302

The measurement for 90 minutes contains, as will be seen in the m-column of table 3 a comparatively large experimental error. Therefore the values, calculated with the aid of this estimation have been discarded from table 4.

Since of the many experiments of this kind that the author has carried out, this series was the most sucessful one, as to regularity, and in view of the smallness of the numbers, which had to be determined by titration, had given also a perfectly satisfactory result, the final value of $\frac{nc}{0{,}434}$ was taken to be 0,0302.

From this it follows, that

$$n = 0{,}043 \times 10^8$$

which value is used throughout in the course of this study and is confirmed indirectly by the important numerical relations, which will be developed with the aid of it in the following parts.

4. *Experimental verification of the general equation of urease-action.*

Activity of enzyme dependent on true reaction of the solution.

Experimental evidence will be brought forward in this part to show, that the formula

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

is really the general equation of urease-action at constant temperature and constant H-ion concentration.

If $\frac{nc}{0{,}434}$ is small, compared to a, evidently the reaction curve must be expected to be practically a straight line. On the other hand the logarithmic curve of the simple law of mass action will appear to represent the course of the reaction in more acid solutions, where $\frac{nc}{0{,}434}$ predominates largely over a small value of a.

By changing the proportion of Na_2HPO_4 2 aq and KH_2PO_4 in the 8% phosphate mixtures a great range of constant H-ion concentrations could be covered. To secure the constancy of p_H throughout the course of the reaction, it was necessary to work always with 0.02 %, or better still with 0.01 % urea solutions. Since 12 c.c. of 0.01 % contain only 1.2 mg. of urea, this means, that in all these experiments the degree of hydrolysis of 1.2 mg. urea had to be determined by single measurements, a serious disadvantage, which, however, had to be put up with in view of the dominating importance of constant H-ion concentration.

The same high degree of accuracy, as was absolutely necessary in the determination of the constant n, is not to be expected here, nor, happily, is it required.

A second object of these experiments was to determine m in the solutions of different acidity, when equal or comparable amounts of enzyme were present or in other words to investigate m as a function of p_H.

To get comparable amounts of enzyme in the solutions the following simple method proved to be efficient.

Some 500 grams of powdered Soja-beans were kept stored for this purpose in a stoppered bottle, shut off from the influence of light in a cupboard, and simply mixed now and then by shaking in the course of these experiments, which lasted several months.

The quantity of Soja-meal required was always weighed off and extracted on the day of the experiment with the same nearly neutral solution of 7.28 gr. of Na_2HPO_4 2 aq + 2.32 gr. of KH_2PO_4 per 100 cc. of water. This extraction was performed by mixing Soja-meal and phosphate-solution in a stoppered flask, shaking through thoroughly, leaving it for one hour in the water-thermostat at 27°, adding kiezelgur of the same amount as the Soja-meal, and filtering rapidly through an ordinary pleated filter. Invariably, without any difficulty, a clear solution was obtained, slightly opalescent if large quantities of Soja-meal had been employed. The working solution

was then prepared by mixing this filtrate with the required volume of 9.6 % solution of Na_2HPO_3 2 aq and KH_2PO_4. A row of Jena test-tubes, each with 10 cc. of this liquid, was placed in the thermostat together with a 250 or 500 cc. flask with a 0.12 % or a 0.06 % urea solution, in short, the same methods were followed as described above in the determination of the constant n.

Some preliminary experiments had shown, that m, as calculated with our formula, was small at low and at high H-ion concentration, and that two hours' standing at 27° was already somewhat destructive to the enzyme in distinctly alkaline solution, not, however, in acid ones.

Unless the acidity has been too high, the diminution of the urease-activity by acids is a reversible process, like the neutralisation of a basic substance.

This fact was established by experiments, the particulars of which will be omitted here for want of space.

In Sept. 1916 the following series of experiments was made with 0.02 % urea. The correction for the traces of NH_3, developed from the materials employed, was estimated in the ordinary way by collecting these small quantities from 3 tubes, each with 10 cc. extract, in the same absorbing tube with 5 cc. H_2SO_4 $^1/_{50}$ N.

In order to meet the possible objection, that the enzyme might have suffered by the influence of time, temperature and true reaction, in both the last experiments a tube with 10 c.c. of the same mixture as contained in the other ones, was left for 4 hours in the bath before introducing into it 2 c.c. of urea solution. After 60 minutes the same amount of urea was found to have been hydrolysed as recorded in tables 10 and 11. As mentioned before and as will be demonstrated more extensively further on, the stability of urease is still greater at lower p_H.

These experiments already confirm the theory. At high H-ion concentration the course of the reaction, as seen by comparison of the last columns, is practically identical with that which can be represented by the law of mass-action. The lower this concentration, the more it deviates from it and approaches to a straight line, just in the same degree, as predicted by the formula:

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

By taking as the unit of urease concentration 1 gram of Soja to 150 cc. total 9.6 % phosphate solution and reducing to this the

TABLE 5.

3 gr. Soja in 100 c.c. { 7.28 gr. Na_2HPO_4 2 aq. / 2.32 gr. KH_2PO_4

50 c.c. filtrate mixed with 100 c.c. water + { 1.92 gr. Na_2HPO_4 2 aq. / 7.68 gr. KH_2PO_4

$p_H = 6.13$

t minutes.	c.c. NaOH $\frac{1}{50}$ N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}74\ log\ \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{log\ \frac{1}{1-y}}{t}$
60	9.3	0.6	0.15	0.00090	0 00118
90	9.1	0.8	0.20	0.00082	0.00108
120	8.9	1.—	0.25	0.00079	0.00104
150	8.65	1.25	0.31	0.00082	0.00107
180	8.45	1.45	0.36	0.00082	0.00108
210	8.2	1.7	0.425	0 00087	0.00114
240	8.05	1.85	0.46	0.00084	0.00111
270	7.8	2.1	0.525	0.00090	0.00120
315	7.7	2.2	0.55	0.00083	0.00110
370	7.5	2.4	0.60	0.00081	0.00107
				Mean 0.00084	

$\frac{1}{3} \times 0.00084 = 0.00028.$

TABLE 6.

3 gr. Soja in 100 c.c. { 7.28 gr. Na_2HPO_4 2 aq. / 2.32 gr. KH_2PO_4

50 c.c. filtrate mixed with 100 c.c. water + { 3.84 gr. Na_2HPO_4 2 aq. / 5.76 gr. KH_2PO_4

$p_H = 6.40$

t minutes.	c.c. NaOH $\frac{1}{50}$ N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}394\ log\ \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{log\ \frac{1}{1-y}}{t}$
30	9.3	0.6	0.15	0.00103	0.0023
60	8.8	1.1	0.275	0.00101	0.0023
90	8.4	1.5	0.375	0.00098	0.0023
125	7.95	1.95	0.488	0.00100	0.0023
150	7.6	2.3	0.575	0.00105	0.0025
180	7.4	2.5	0.625	0.00101	0.0024
215	7.1	2.8	0.70	0.00102	0.0024
240	6.95	2.95	0.738	0.00102	0.0024
270	6.75	3.15	0.788	0.00104	0.0025
300	6.6	3.3	0.825	0.00105	0.0025
				Mean 0.00102	

$\frac{1}{3} \times 0.00102 = 0.00034.$

TABLE 7.

0.75 gr. Soja in 100 c.c. { 7.28 gr. Na_2HPO_4 2 aq. / 2.32 gr. KH_2PO_4

50 c.c. filtrate mixed with 100 c.c. water + { 8.64 gr. Na_2HPO_4 2 aq. / 0.96 gr. KH_2PO_4.

$p_H = 7.21$

t minutes.	c.c. NaOH $\frac{1}{50}$ N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}0611 \log \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{\log \frac{1}{1-y}}{t}$
20	9.05	0.95	0.238	0.00060	0.0060
40	8.3	1.7	0.425	0.00058	0.0060
60	7.7	2.3	0.575	0.00057	0.0062
80	7.22	2.78	0.695	0.00057	0.0064
100	6.85	3.15	0.788	0.00057	0.0067
120	6.55	3.45	0.86	0.00058	0.0071
150	6.35	3.65	0.91	0.00055	0.0070
180	6.25	3.75	0.94	0.00052	0.0068
210	6.1	3.9	0.975	0.00056	0.0076

Mean 0.00057

$\frac{4}{3} \times 0.00057 = 0.00076.$

TABLE 8.

Two equal experiments, one on Sept. 18th, 1916, another with freshly prepared solution on Sept. 19th, 1916.

0.75 gr. Soja in 100 c.c. { 7.28 gr. Na HPO_4 2 aq. / 2.32 gr. KH_2PO_4.

50 c.c. filtrate mixed with 100 c.c. water + 9.6 gr. Na_2HPO_4 2 aq.

$p_H = 7.52$

t minutes.	c.c. NaOH $\frac{1}{50}$ N		c.c. NH_3 $\frac{1}{50}$ N corrected (mean).	y	$m = \frac{0{,}0302 \log \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{\log \frac{1}{1-y}}{t}$
20	8.55	8.50	1.45	0.36	0.00065	0.0097
40	7.4	7.4	2.575	0.64	0.00065	0.0111
60	6.75	6.7	3.25	0.81	0.00063	0.0120
80	6.3	6.25	3.7	0.925	0.00066	0.0141
100	6.15	6.1	3.85	0.96	0.00061	0.0140

Mean 0.00064

$\frac{4}{3} \times 0.00064 = 0.00085$

TABLE 9.

0.5 gr. Soja in 100 c.c. { 7.28 gr. Na_2HPO_4 2 aq. / 2.32 gr. KH_2PO_4

50 c.c. filtrate mixed with 150 c.c. water + 14.4 gr. Na_2HPO_4 2 aq.

$p_H = 7.64$

t minutes.	c.c. NaOH $\frac{1}{50}$ N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}0227 \log \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{\log \frac{1}{1-y}}{t}$
20½	9.2	0.8	0.20	0.00030	0.0048
40	8.4	1.6	0.40	0.00032	0.0055
60	7.72	2.28	0.57	0.00033	0.0061
80	7.3	2.7	0.675	0.00031	0.0061
100	6.8	3.2	0.80	0.00032	0.0070
120	6.5	3.5	0.875	0.00032	0.0075
150	6.2	3.8	0.95	0.00032	0.0087
				Mean 0.00032	

$2 \times \frac{4}{3} \times 0.00032 = 0.00085.$

TABLE 10.

0.5 gr. Soja in 100 c.c. { 7.28 gr. Na_2HPO_4 2 aq. / 2.32 gr. KH_2PO_4

50 c.c. filtrate mixed with 200 c.c. water + 19.2 gr. Na_2HPO_4 2 aq.

$p_H = 7.75$

t minutes.	c.c. NaOH $\frac{1}{50}$ N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}0176 \log \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{\log \frac{1}{1-y}}{t}$
20	9.27	0.73	0.18	0.00026	0.0043
40	8.65	1.35	0.34	0.00025	0.0045
60	8.1	1.9	0.475	0.00024	0.0047
80	7.7	2.3	0.575	0.00023	0.0047
100	7.2	2.8	0.70	0.00025	0.0062
120	6.85	3.15	0.79	0.00023	0.0056
150	6.45	3.55	0.89	0.00023	0.0064
180	6.2	3.8	0.95	0.00023	0.0072
210	6.1	3 9	0.975	0.00023	0.0076
				Mean 0.00024	

$2 \times \frac{5}{3} \times 0{,}00024 = 0{,}00080.$

TABLE 11.

Repetition of the experiment of Sept. 25th.

$p_H = 7.75$

t minutes.	c.c. NaOH $\frac{1}{50}$N	c.c. NH_3 $\frac{1}{50}$ N corrected.	y	$m = \frac{0{,}0176 \log \frac{1}{1-y} + 0{,}02y}{t}$	$k = \frac{\log \frac{1}{1-y}}{t}$
20	9.3	0.7	0.175	0.00025	0.0042
40	8.57	1.43	0.358	0.00026	0.0048
60	8.12	1.88	0.47	0.00024	0.0046
80	7.65	2.35	0.588	0.00022	0.0048
100	7.2	2.80	0.70	0.00025	0.0062
120	6.9	3.1	0.775	0.00022	0.0054
150	6.4	3.6	0.90	0.00024	0.0066
180	6.15	3.85	0.963	0.00025	0.0079
216	6.05	3.95	0.988	0.00025	0.0089
				Mean 0.00024	

calculated mean of m (as done at the foot of each table), we get for equal enzyme concentration at different p_H the following list:

p_H	Activity of the same quantity of urease
6.13	0.00028
6.40	0.00034
7.21	0.00076
7.52	0.00085
7.64	0.00085
7.75	0.00080