L. ON THE EFFECTS OF VARIOUS SUBSTANCES (ELECTROLYTES, NON - ELECTROLYTES, ALKALOIDS, ETC.) UPON THE UREASE OF SOY-BEAN.

BY NAOSUKE ONODERA.

From the Institute of Physiology, University College, London.

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Musculus [1874] was the first to discover the ferment urease, which is capable of decomposing urea into NH_3 and CO_2 , in urine. Shibata found urease in *Aspergillus niger* [1904], and Kikkoji in *Cortinellus edodes* [1907]. Takeuchi was the first to discover urease in soy-bean in considerable amount [1909]. Various workers in succession [Keisel, 1911; Zemplén, 1912; and Annet, 1914] succeeded in finding urease in varying amounts in the seeds of various higher plants (wheat, lupin, some Indian seeds, etc.). Subsequently Marshall [1913, 1 and 2] and Plimmer and Skelton [1914] used the urease of soy-bean to estimate urea in urine, blood, etc., with extreme accuracy. These investigations were followed by the study of the mode of action of urease and the conditions which facilitate or retard its action.

Takeuchi endeavoured to ascertain the extent of the influence of salts, acids and caustic soda on urease. But we find his data of the concentration of the substances used by him too high, owing to the lack of an accurate method of estimation.

Armstrong and Horton [1912] and Armstrong, Benjamin and Horton [1913] studied the influence of various substances upon urease. They found that urea at a concentration of more than M/5 depressed the development of NH_3 , and all acids, even such relatively weak acids as carboxylic acids, acted on urease in an inhibitory manner, with the exception of amino acids and carbon dioxide which accelerated the action of urease. Boric acid was

inhibitory at M/50 and phenol at M/5. These observers also found that alcohols, aldehyde and glucose act as inhibitors; as regards alcohols, the lower their solubility, the greater was their inhibitory effect.

Marshall [1914] found that the velocity of action of urease is dependent on the hydrogen or hydroxyl ion concentration within rather narrow limits. Hydrochloric acid and caustic soda in sufficient amounts inhibited or destroyed urease. Ethyl alcohol had a moderate inhibitory effect but had apparently a slight destructive action upon urease. It markedly retarded urease action in concentrations over 20 volumes per cent.

Armstrong and his collaborators, and Marshall, used the water extract of soy-bean powder as urease. This extract contains a certain amount of buffer substances, such as albumin, which disturb the accurate neutralisation of the extract by means of titration. This was recognised more or less by these authors, and when they desired to ascertain the effect of dilute acids upon urease, these acids were mixed with urea and urease in the digestion bottle. It was found that the acids added in small quantities may be neutralised after a short time by the development of NH_3 .

To avoid the difficulty due to certain impurities, van Slyke and Cullen [1914, 2] tried successfully to obtain urease in a purer state by precipitating the water extract of soy-bean powder with acetone and drying the precipitate in vacuo over sulphuric acid. This dried precipitate, dissolved in distilled water before use, was used as urease. Van Slyke and his co-workers [van Slyke and Cullen, 1914, 1; van Slyke and Zacharias, 1914] also studied the effect of hydrogen ion concentration upon urease, altering the former as required by means of a phosphate mixture. They regarded the urease reaction as proceeding in two stages characterised by different velocities, the first being the combining velocity (c) of the urease and substrate, and the second the decomposing velocity (d) of the combined substance into NH_3 and CO_2 . The more alkaline the reaction of the medium, the greater was c , but d was maximal at the neutral reaction and less when the reaction was more alkaline or more acid. The total action of urease was depressed at the slight acid reaction of $\text{P}_\text{H} = 6.07$ and reached its maximum when P_H was close to 7. The authors ascribed the depressing effect of the product of the action to its hydrogen ion concentration, because the further addition of ammonia to the medium is no longer able to depress the urease action, since the hydrogen ion concentration reaches a certain limit and remains constant in spite of the increase of ammonia. Neutral salts, glucose and ethyl alcohol retarded c and at a high osmotic pressure retarded d as well.

Armstrong and Horton [1912] found that the velocity of action of urease proceeds independently of the amount of the substrate remaining unchanged, provided that the product with retarding effects on urease is neutralised.

Van Slyke and his collaborators introduced a formula for general use in enzyme action, making use of c and d . This formula is as follows:

$$t = \frac{1}{E} \left(\frac{1}{c} \log \frac{a}{a-x} + \frac{x}{d} \right).$$

They recommended this formula as the most accurate one because it corresponded exactly to the experimental data. They alleged that urease was not a contact catalyst but formed an intermediate labile compound with urea. They insisted that the action of urease was in accordance with the law of mass-action.

My experiments were directed towards ascertaining whether the hydrogen ion concentration alone is adequate to account for the effect of acids upon urease, as the custom is growing of stating results on enzyme action in terms of hydrogen ion concentration. This tendency has been increased by the work of Michaelis and his co-workers, and of Sørensen, etc. If enzymes are colloids and the reactions take place at the surface of colloids, it is reasonable to consider other physico-chemical properties at the same time as osmotic pressure and hydrogen ion concentration. Among the physico-chemical properties surface tension is the first to come into consideration. According to the Gibbs-Thomson theorem, substances which lower the surface tension of solutions accumulate at the surface of the second phase, e.g. at the boundary between air and solution or at the interface between suspended particles of solid and the solution, or between droplets of an immiscible fluid and the solution. Thus substances which actively affect surface tension may influence in one or other direction the actions which have some relation to surface. They may have an effect on enzyme particles in the sense of diminution or increase of active surface, i.e. aggregation or dispersion. They may also have effects on diffusion, absorption, adsorption, internal friction, etc. (see Traube's reports). To solve these questions various acids, alcohols, etc., which alter the surface tension of the solvent, were examined. I also endeavoured to ascertain whether the depressing effect of ammonium carbonate on urease should be ascribed to the hydroxyl ion concentration alone or in part to the reversible reaction of the hydrolysis of urea.

EXPERIMENTAL PART.

Methods.

Urease solution was mixed with urea in digestion bottles. After some hours, acid of considerable concentration was added in order to stop the urease action instantaneously. The ammonia developed on subsequent addition of sodium carbonate was driven off by means of an air current free from ammonia and caught in decinormal sulphuric acid. The absorbed ammonia was estimated by the usual method of titration by N/10 caustic soda.

The experiments were always carried out at room temperature. The digestion bottles were placed in a large wooden water bath in a position in the laboratory where the change of air temperature was as small as possible. During the period of several months in which the experiments were carried out, the temperature of the bath varied between 19° and 21°. The change of temperature during each experiment was very slight.

As digestion bottles I used the high cylinders used by Plimmer in his work on urea estimation by means of urease. The inner diameter of these cylinders is about 4.2 cm., and their height about 40 cm. Cylinders were selected which had the same thickness of wall approximately.

The Allihn bottles, into which the developed ammonia was led to combine with decinormal sulphuric acid, are about 17 cm. high, with a content of 400 cc. The tubes which lead the ammonia to the sulphuric acid have six small holes at the closed end, each hole having a diameter of about 1.5 mm. Air with ammonia is bubbled through these holes into the sulphuric acid. The faster the air containing ammonia is drawn through these holes, the smaller are the bubbles and the more accurate is the ammonia estimation, as Folin, van Slyke and Plimmer ascertained.

In my experiments the urea was used in a concentration of 0.15 to 0.12 % of the whole mixture in the digestion bottle. This concentration of urea is the most favourable for urease action (van Slyke).

In order to check the urease action instantaneously sufficient N/5 sulphuric acid was added to the bottles to make the whole at least decinormal after neutralisation of the ammonia present. This checking action was complete in half an hour, but the bottles were always left for one or two hours. The mixture was then neutralised by the addition of 2N caustic soda and crystalline sodium carbonate added to make a 4 % solution. Folin's aeration was carried out in the water bath at a temperature of 40°.

The N/10 sulphuric acid which was to catch the ammonia was made up with distilled water to 250 cc. and alizarin red was used as indicator. To prevent frothing a small quantity of lubricating oil was poured into the bottles. The escape of ammonia was prevented during the urease action by the use of rubber corks or ordinary corks and wax. Unless specially mentioned the total quantity of acting medium was always 50 cc. Each digestion bottle contained 5 cc. of 1.5 % solution of urea, with the exception of one series of experiments to be mentioned later in the experimental part. If the action of urease is complete with this amount of urea, then theoretically 25 cc. of N/10 ammonia should be evolved.

Two different preparations of urease were used, one being the 1-10 % water extract of soy-bean powder. This powder was digested carefully in distilled water, left half an hour, with occasional stirring, and filtered through linen. Only the fresh filtrate was used. The other solution was that of precipitated urease prepared by van Slyke's method. This solution was always made immediately before use and the undissolved flocculent substance was filtered off.

The reaction of the water extract of soy-bean was faintly acid compared with that of the standard neutral solution of Prideaux [1912], alizarin red being used as indicator. This extract did not turn phenolphthalein red, as Armstrong stated. Marshall, however, found the water extract of soy-bean alkaline to methyl orange.

0.1 cc. of N/10 ammonia was evolved from 10 cc. of 10 % water extract of soy-bean after standing for 20 hours at room temperature. This correction is made in the experimental data.

The reaction of the solution of the precipitated urease was found by van Slyke to be acid, but in my preparation it was quite neutral.

In order to make certain of my technique of ammonia estimation I carried out the following preparatory experiments.

I made up a N/5 NH_4Cl solution and by Kjeldahl's method I found 19.6 cc. N/10 ammonia in 10 cc. of this NH_4Cl solution. Folin's method was carried out in the water bath at a temperature of 15° for three hours, and the amount of N/10 NH_3 obtained was only 16.98 and 18.20 cc. instead of 19.6 cc.

In the next experiment Folin's distillation of ammonia was carried out in the water bath at a temperature of 40° for an hour, an hour and a half, and two hours respectively. The amounts of N/10 NH_3 obtained were 19.65, 19.69 and 19.66 cc. respectively. We know thus that all the ammonia was

driven out under these conditions within an hour. In all the following experiments the distillation lasted for an hour and a half to two hours.

To check the urease action instantly, I always used sulphuric acid in my work, and avoided the use of heat. It is known that boiling of urea solution with acid or alkali develops ammonia to a small extent [see Armstrong and Horton, 1912].

To 5 cc. of 1 % bean extract N/5 H_2SO_4 was added to make N/10 and N/50 solution *in toto*. The acid was allowed to act on urease fifteen minutes, then the mixture was neutralised with N/5 NaOH. Urea was then added, the mixture was kept at room temperature the whole night, and NH_3 was determined in this mixture exactly as described previously. The control mixture without acid developed 23.83 cc. of N/10 NH_3 . The urease inhibited by N/10 H_2SO_4 developed only 0, 0.03, 0.11, 0.05 cc., an average of 0.05 cc. of N/10 NH_3 , and urease inhibited by N/50 H_2SO_4 developed only 0.06, — 0.02 cc., an average of 0.02 cc. of N/10 NH_3 . Notwithstanding this fact, in order to check urease action, I always added to the digestion bottles N/5 H_2SO_4 so as to make the whole mixtures stronger than N/10 and left them for half an hour to one hour.

Urease mixed with 4 % crystalline sodium carbonate developed no ammonia at 40° in several hours.

(1) *Effects of acids on urease.*

Marshall found that the velocity of urease action is dependent upon the hydrogen or hydroxyl ion concentration within rather a narrow limit. Van Slyke found that slight acidity ($P_{\text{H}} = 6.07$) exercised a depressing effect on urease action and that the velocity reached its maximum at the neutral point ($P_{\text{H}} = 7$).

I made my first experiments with a water extract of soy-bean as did Armstrong and Marshall. Before use the bean extract had been neutralised with N/20 NaOH, being compared with the standard solution of Prideaux.

The acids employed were prepared by titration against N/10 NaOH, using phenolphthalein as an indicator. Only phosphoric acid was prepared by weighing from Merck's pure preparation, and this was estimated by weight after being converted into magnesium pyrophosphate.

The first experiment was commenced with sulphuric acid; the acid was mixed in digestion bottles with 5 cc. of 1.5 % urea solution and 10 cc. of a 2.5 % extract of the bean and left for nearly 20 hours at room temperature. Urease action was then checked and NH_3 estimated.

We see from this experiment that sulphuric acid is capable at a concentration of N/100 and N/500 of totally inhibiting urease, while at a concentration of N/1000 and N/1500 it has practically no effect.

In order to ascertain to what extent the enzyme was inactivated by the prolonged action of acid, the urease and acid were mixed, left for some time and then neutralised with NaOH before the addition of urea.

TABLE I.

H ₂ O in cc.	H ₂ SO ₄ in cc.	Conc. of acid	N/10 NH ₃ developed in 17 hrs in cc.	
35	—	—	24.21	24.14
35	—	—	24.06	
30	N/10 5	N/100	0.02	0.12
30	N/10 5	N/100	0.11	
30	N/50 5	N/500	0.29	0.25
			0.20	
10	N/500 25	N/1000	24.08	
10	N/750 25	N/1500	23.98	

Sulphuric acid was allowed to act on urease for 16.5 hours, then neutralised, mixed with urea and left for 22.5 hours at room temperature. The urease action was then checked and the NH₃ estimated. The control urease developed 24.42 cc. of N/10 NH₃. N/500 sulphuric acid was found to have totally inactivated the urease; and N/1000 sulphuric acid had almost completely inactivated the urease, only 0.5 cc. of N/10 NH₃ being formed.

In order to find out the lower limit of the inactivating effect of sulphuric acid the experiment recorded in Table II was carried out. The columns of this table read from left to right show the order in which the various substances were added to the digestion bottles. In every experiment the procedure was carried out as Tables I and II.

TABLE II.

H ₂ O in cc.	1 % bean extract in cc.	N/200 H ₂ SO ₄ in cc.	Conc. of acid	Added after 17 hours		1.5 % urea in cc.	N/10 NH ₃ developed in 20.5 hrs. in cc.	
				H ₂ O in in cc.	N/200 NaOH in cc.			
40	10	—	—	10	—	5	24.05	24.16
40	"	—	—	"	—	"	24.27	
30	"	10	N/1000	—	10	"		0.10
33	"	7	N/1429	3	7	"		0.62
35	"	5	N/2000	5	5	"		13.86
36	"	4	N/2500	6	4	"		23.98
37	"	3	N/3300	7	3	"	24.12	24.03
37	"	"	"	"	"	"	23.93	
38	"	2	N/5000	8	2	"	24.15	24.04
38	"	"	"	"	"	"	23.93	

When the added sulphuric acid was not neutralised by the NH_3 developed, a markedly depressing effect of N/2000 H_2SO_4 in 17 hours was observed, N/2500 acid showed some depressing effect, whilst N/3330 had no effect.

It is to be noticed here that acids, whether organic or inorganic, caused in urease solution an opacity or fine flocculent precipitation. Even N/5000 H_2SO_4 caused a marked opacity, which disappeared on neutralisation. If this neutralised mixture of acid and urease still contains some effective urease, and consequently ammonia is developed, the mixture goes into the turbid state again.

By the same method as that mentioned above, the influence of hydrochloric acid, acetic acid and butyric acid on urease was examined.

TABLE III.

Concentration of acid	N/10 NH_3 developed in 20·5 hrs in cc.		
	HCl	Acetic acid	Butyric acid
N/1000	—	15·87	18·99
N/2000	16·09	24·06	23·81
N/2500	24·17	24·29	23·50
N/3330	24·12	24·28	23·78
N/5000	—	24·38	23·82
Control urease without acid	24·25	24·38	23·76

By these experiments it was proved that all acids employed at a strength of N/2500 did not affect urease.

If we want to compare the effect of more diluted acids on urease it is necessary to use precipitated urease instead of water extract of soy-bean, because the latter contains much regulatory substance, which may protect urease from being inhibited by diluted acid.

All the following experiments, unless there is some note otherwise, were carried out with a solution of precipitated urease. At first I endeavoured to obtain some idea of the relative strength of various acids against urease.

N/500 acids were allowed to act on 5 cc. of 0·5 % urease for 17 hours, when they were neutralised, mixed with urea and left at room temperature for 22·5 hours. The amount of NH_3 evolved from these mixtures is shown in Table IV.

The NH_3 developed was very small in amount, proving that even weak acids are able to destroy urease in process of time.

In order to find out the length of time necessary for the action of acids on urease, N/500 acids were allowed to act on 5 cc. of 0.5 % urease for 15,

TABLE IV.

Name of acid	Developed N/10 NH ₃ in cc.	
0.5 % urease alone	23.62	23.60
" "	23.59	
HCl	0.18	0.19
"	0.19	
Oxalic	0.22	0.22
"	0.21	
Formic	0.22	0.21
"	0.20	
H ₂ SO ₄	0.33	0.28
"	0.22	
H ₃ PO ₄	0.33	0.27
"	0.21	
Acetic	0.36	0.36
"	0.35	
Butyric	0.41	0.37
"	0.32	

30, 60 and 90 minutes, the acids were then neutralised, urea was added and the mixture left at room temperature for 16.5 hours. Urease action was then checked and NH₃ estimated.

TABLE V.

Acids neutralised after	Developed N/10 NH ₃ in cc.	
	H ₂ SO ₄	CH ₃ COOH
15 minutes	0.87	—
30 "	0.61	5.64
60 "	0.26	4.50
90 "	0.20	1.65
0.5 % urease alone	23.81	

We see here a very distinct difference between a strong and a weak acid in regard to the velocity of their inhibitory effect. Regarding these facts, I varied the length of time of exposure of urease to acids and the strength of acids employed. A number of experiments were carried out, the following acids being used: HCl, HNO₃, H₂SO₄, H₃PO₄, formic, acetic, butyric, oxalic, trichloroacetic, benzoic and salicylic.

TABLE VI.

N/1000 acids were allowed to act upon 5 cc. of 0.5 % urease for 10 minutes, then neutralised, mixed with urea and left at room temperature for 16 hours.

Name of acid	Developed N/10 NH ₃ in cc.
0.5 % urease alone	23.85 } 23.82
" "	23.79 }
Trichloroacetic	0.48 } 0.54
" "	0.60 }
Oxalic	0.38 } 0.56
" "	0.73 }
HCl	0.61 } 0.61
" "	0.61 }
Formic	2.40 } 2.29
" "	2.17 }
Butyric	23.69 } 23.47
" "	23.24 }
H ₃ PO ₄	23.56 } 23.63
" "	23.69 }
Acetic	23.80 } 23.76
" "	23.72 }

TABLE VII.

N/1000 acids were allowed to act upon 5 cc. of 0.5 % urease for one hour, then neutralised, mixed with urea and left at room temperature.

Name of acid	Developed N/10 NH ₃ in 22 hrs in cc.
H ₃ PO ₄	3.06
Butyric	11.40
Acetic	15.02
	Developed N/10 NH ₃ in 21 hrs in cc.
Salicylic	0.66
Benzoic	1.14
Acetic	15.19

TABLE VIII.

N/2000 acids were allowed to act upon 5 cc. of 0.5 % urease for one hour, then neutralised, mixed with urea and left at room temperature for 20.5 hours.

Name of acid	Developed N/10 NH ₃ in cc.
0.5 % urease alone	23.55
HNO ₃	0.49 } 0.42
" "	0.34 }
Trichloroacetic	0.48 } 0.43
" "	0.38 }
HCl	0.50 } 0.50
" "	0.49 }
H ₂ SO ₄	0.65 } 0.83
" "	1.01 }
Salicylic	1.27
Formic	3.90
Benzoic	19.02

TABLE IX.

N/3000 acids were allowed to act upon 5 cc. of 0.5 % urease for an hour, then neutralised, mixed with urea and left at room temperature for 18 hours.

Name of acid	Developed N/10 NH ₃ in cc.
0.5 % urease alone	23.87
Oxalic	21.47
"	20.18
Trichloroacetic	21.15
HCl	23.15
"	23.36
HNO ₃	23.37
"	23.62
H ₂ SO ₄	23.58
"	23.66
Formic	23.92
"	23.60

TABLE X.

N/4000 acids were allowed to act upon 5 cc. of 0.5 % urease for an hour, then neutralised, mixed with urea and left at room temperature for 20.5 hours.

Name of acid	Developed N/10 NH ₃ in cc.
0.5 % urease alone	24.35
Oxalic	21.22
H ₂ SO ₄	23.57
HNO ₃	23.63
HCl	23.72
Trichloroacetic	23.77
Formic	23.78

If we arrange the acids according to their inhibitory effect upon urease, we get the following order.

The inhibitory effect decreases passing from left to right.

TABLE XI.

According to Table VI:

Trichloroacetic, oxalic, hydrochloric > formic > butyric, phosphoric, acetic.

According to Table VII:

Salicylic > benzoic > phosphoric > butyric > acetic.

According to Table VIII:

Nitric, trichloroacetic, hydrochloric > sulphuric > salicylic > formic > benzoic.

According to Table IX:

Oxalic > trichloroacetic > hydrochloric, nitric, sulphuric, formic.

According to Table X:

Oxalic > sulphuric, nitric, hydrochloric, trichloroacetic, formic.

If we arrange the above-mentioned acids according to the strength of their inhibitory effects on urease, we get a general order of acids from Table XI as follows:

Oxalic > Trichloroacetic > Hydrochloric, Nitric > Sulphuric > Salicylic > Formic > Benzoic > Phosphoric > Butyric > Acetic.

According to Ostwald, if one gram-molecule of each of these acids is dissolved in 1024 litres of distilled water, they are dissociated at 25°, as follows:

Trichloroacetic acid	...	...	99.40 %
Salicylic acid	...	...	62.80 %
Formic acid	...	...	35.80 %
Benzoic acid	...	...	21.61 %
Acetic acid	...	...	12.61 %
Butyric acid	...	...	11.41 %
Oxalic acid	...	...	94.70 % (gram-molecule in 256 litres).

This order of hydrogen ion concentration of nearly N/1000 acids corresponds with the order of acids which I obtained in Tables VI, VII and VIII. But there is one important exception, namely, butyric acid (Tables VI and VII). This, in spite of a lower hydrogen ion concentration than acetic acid, had an inhibitory effect on urease greater than that of acetic acid. This fact corresponds precisely with the result of J. Loeb [1909], who found butyric acid to have a stronger influence on membrane construction of sea-urchin eggs even than mineral acids. He remarked that acids in their physiological action are not dependent only upon their hydrogen ion concentration, and he is of opinion that the undissociated part of an acid plays the main rôle concerning its relative permeability through the cell membrane of eggs, and that the process may be of a purely physical nature.

According to Ostwald the equivalent conductivity of N/1000 acids at 18° is as follows:

HCl	375 practically totally dissociated,
HNO ₃	373,
1/2H ₂ SO ₄	355,
1/3H ₃ PO ₄	106,
CH ₃ COOH	41.

If we compare the order of acids obtained in this work with the degree of dissociation of acids given above, we see that the former coincides with the latter fairly well.

At a dilution of N/3000 and N/4000 it is considered that the strong mineral acids such as HCl, HNO₃ and H₂SO₄ are almost entirely dissociated. Organic

acids with high dissociation coefficients, as trichloroacetic acid and oxalic acid, will be in the same dilution also almost entirely dissociated. Yet I have confirmed, by Prideaux's colorimetric method, that they have a smaller hydrogen ion concentration than HCl in the same dilution. Notwithstanding, we find a markedly stronger inhibitory effect of trichloroacetic and oxalic acid than mineral acids. These exceptional cases force us to take other considerations than the exclusive idea of hydrogen ion concentration into account.

It is true that the hydrogen ion concentration of acids plays the main rôle in many purely chemical reactions. According to Ostwald, the inversion order of acids is: HCl, HNO_3 , H_2SO_4 , tri-, di-, mono-chloroacetic acid, formic acid, acetic acid. It is just the order of hydrogen ion concentration. On the other hand there are many contradictions with regard to the physiological actions of acids.

Paul, Birnstein and Reuss obtained the order of acids as follows, classified by their bactericidal action [see Traube, 1912]:

Trichloroacetic acid, HNO_3 , HCl, oxalic acid, H_2SO_4 , H_3PO_4 , formic acid, acetic acid, butyric acid.

Barratt [1904] found that univalent aliphatic acids are more toxic than mineral acids on *Paramoecium*. Fühner and Neubauer [1907] showed that the limiting concentration of acids capable of causing haemolysis does not correspond to their hydrogen ion concentration.

Pauli [1910] investigated the order of acids in regard to their influence on the internal friction and the precipitability of solutions of albumin by alcohol, and found it to be:

Trichloroacetic acid, dichloroacetic acid, H_2SO_4 , HNO_3 , HCl, monochloroacetic acid, acetic acid.

He ascribed these effects not to the strength of acids, but to the nature of their anions.

Loeb's experiments on parthenogenesis have been mentioned already. Here again the order of hydrogen ion concentration is not the order of activity. As to the results of the present work it shows us that the effect of acids on urease is mainly dependent upon hydrogen ion concentration, but not upon negative ions, because the influence of salts of these acids is not apparently so strong, as my later experiments and van Slyke's "salt effect" show. But on the other hand my experiments show us that there are some exceptions to the order of hydrogen ion concentration, viz. butyric acid, trichloroacetic acid and oxalic acid.

By these facts we are forced to consider as important the properties of acid radical ions, or undissociated acids, which may act in the way of facilitating the contact of enzyme particles and hydrogen ions.

Among the physical properties of acids or acid radical ions the surface tension is perhaps the most important, assuming that enzyme action takes place at the surface of enzyme particles.

According to Traube's [1909] measurements the constant of surface tension (γ) of molecular solutions of acids is as follows. The constant $\frac{rhs}{2}$ is given in mg./mm. and was measured at 15°. The constant (γ) for water was taken as 7.30:

acids: $\frac{1}{2}$ H ₂ SO ₄	HCl	HNO ₃	formic	acetic	propionic	butyric	trichloroacetic
γ : 7.285	7.23	7.19	6.84	6.05	4.82	3.31	4.82

It is known that the less the surface tension the greater is the partition coefficient and adsorption coefficient. In adsorption, the adsorbed substances can carry other molecules or ions with them. According to these considerations the inhibitory action of acids on urease is, I assume, a function of hydrogen ion concentration and of surface tension, which latter gives the ions more opportunity of combining with urease particles in the diluted condition. The greater the lowering of surface tension, the more will the relatively smaller hydrogen ion concentration of the weaker acids be compensated by having more opportunity to come into touch with the urease particles.

By means of these considerations the exceptional cases of butyric and trichloroacetic acids are explained. Many results concerning the biological effects of acids on bacteria, haemolysis, paramoecium, colloidal precipitation and parthenogenesis will be found to receive a common explanation in this way.

There is still only one difficulty to explain, that is, the superior efficiency of oxalic acid. This acid is not one with a very strong surface-tension activity as is trichloroacetic acid. Some other factors, probably physical ones, remain to be detected to explain this exception.

(2) *Effects of caustic soda and ammonia on urease.*

Takeuchi, Armstrong and Marshall reported the inhibitory effects of alkalis on urease. Van Slyke and Zacharias [1914, p. 208] allege that the hydroxyl ion retards *d* but facilitates *c*. In accordance with this the optimum of urease action in diluted urea solution is more on the alkaline side, because in the diluted urea solution the combination of urease and of urea is retarded.

At first I experimented with neutralised 2 % watery extract of soy-bean. To 5 cc. of this extract caustic soda and urea were added, bringing the content of each bottle to equal volumes, the mixture left at room temperature for twenty hours, the urease action then stopped and ammonia estimated.

TABLE XII.

Concentration of NaOH	Developed N/10 NH ₃ in cc.
2 % bean extract alone	19.93
N/10	0.18
N/50	0.26
N/100	1.47
N/500	0.95

We see here that caustic soda inhibited urease action even at a concentration of N/500.

By the same method an experiment was made with ammonia. From the quantity of ammonia obtained the known quantity of ammonia added was subtracted with the following result.

TABLE XIII.

Concentration of NH ₃	Developed N/10 NH ₃ in cc.
2 % bean extract alone	24.32
N/20	12.35
N/50	21.46
N/100	24.05
N/200	24.50

We see here that the inhibitory action of ammonia is much inferior to that of soda. N/50 ammonia had a slight depressing effect.

I have also examined the effect of soda on precipitated urease.

TABLE XIV.

NaOH was allowed to act upon 5 cc. of 0.5 % urease for 1.5 hours, then neutralised, mixed with urea and left at room temperature for 22 hours.

Concentration of NaOH	Developed N/10 NH ₃ in cc.
0.5 % urease alone	22.85
N/50	0.16
N/100	8.60
N/250	18.67
N/500	21.00
N/1000	22.77

It was thus found that N/50 soda totally destroyed the urease in 1.5 hours, while N/500 soda had a slight action and N/1000 soda had no effect.

If we compare the results obtained in Tables XIII and XIV we see that the inhibitory effect of N/500 soda and N/50 NH_3 are about equal. But soda was allowed to act on urease only 1.5 hours, while N/50 NH_3 was left for 20 hours with urease. Thus we can assume with certainty that the effect of N/500 soda must be greater than that of N/50 NH_3 .

Marshall, and van Slyke and his co-workers, ascribed the depressing effect of alkalis and ammonia on urease to their hydroxyl ion concentration. But there is another possibility that the hydrolysis of urea by urease may be a reversible one.

According to Kohlrausch, the equivalent conductivity at 18° is nearly 204 for N/500 NaOH, only 3.3 for N/10 NH_3 and 9.6 for N/100 NH_3 . It is evident that we cannot explain the effect of alkali and ammonia on urease only by hydroxyl ion concentration. The difference of surface tension of soda and ammonia is also not enough to afford the required explanation.

We may therefore suggest that the effect of soda may be the effect of hydroxyl ion concentration and the effect of ammonia be partly due to the reversible reaction of hydrolysis of urea by urease, but this latter possibility is very doubtful.

(3) *Effects of neutral salts and alkaloids on urease.*

As is seen in the previous experiments the depressing effect of ammonia was far below that of soda. We may expect therefore that if neutral salts of strong alkalis be added to the digestion bottle and their bases be displaced by ammonia, the more or less depressing effect on urease, according to the kind of alkali, can be seen.

Van Slyke and his coadjutors found that neutral salts retard *c* and, if their concentration exceeds a 2 M solution, also retard *d*. If my assumption is right, van Slyke should have obtained the result that neutral salts retard *d* and facilitate *c*, because in my opinion alkalis displaced by ammonia will have an influence on urease.

In order to investigate this matter the following experiments were made.

At first neutral salts were mixed with 5 cc. of 0.5 % urease and urea, and after standing at room temperature for 20 hours urease action was checked and ammonia was estimated. It was noticed that the mixture showed a fine flocculent precipitation directly after adding CaCl_2 , BaCl_2 , and HgCl_2 to urease.

TABLE XV.

N/10 salt solution	Developed N/10 NH_3 in cc.
0.5 % urease alone	22.73
" "	22.82
NaCl	20.32
"	19.38
CaCl_2	22.03
"	22.32
BaCl_2	22.68
"	21.72

As I supposed N/10 NaCl has an apparent depressing effect while N/10 CaCl_2 and N/10 BaCl_2 , in spite of precipitation, showed only a slight depressing effect. We can understand the marked depressing effect of 2 M solutions of neutral salts on urease as observed by van Slyke.

In the salt solutions of the present experiment the number of chlorine ions are equal, therefore these different depressing effects of neutral salts must be ascribed to the nature of the metallic ions. If the action of metallic ions is a tendency to colloidal precipitation of urease, we should expect more effect of the multivalent ions as Ba^{++} and Ca^{++} than of the univalent Na^+ ions. This was not the case.

It was concluded therefore that the metallic bases of these neutral salts were displaced by ammonia hydrolysed from urea, owing to the law of mass-action. Metallic bases are able to dissociate more hydroxyl ion than their equivalent of ammonia. As to the degree of dissociation, sodium stands of course far beyond barium and calcium.

Van Slyke reported that neutral salts disturb the combination of enzyme and substrate, so that, when the concentration of urea becomes smaller at the end of the reaction, the depressing effect of neutral salts on the combination of urea and enzyme becomes more manifest.

If the urease action is checked at an early stage, then one would see less effect of neutral salts. The data of Table XVI agree with this assumption.

TABLE XVI.

N/10 salt solution	Developed N/10 NH_3 in 17.5 hours in cc.
0.4 % urease alone	12.10
NaCl	5.73
BaCl_2	5.52
CaCl_2	5.62
HgCl_2	0.00

But we can explain this result otherwise, assuming that hydroxyl ion concentration is effective on urease only when it exceeds some limit.

In an early period of the reaction the hydroxyl ion concentration would not perhaps reach the effective limit. That is the reason why we do not see in Table XVI a distinct difference between Na, Ba and Ca ions in their effects.

HgCl₂ totally destroyed urease, as Hata [1909] found in the case of various ferments; it is to be ascribed to the special nature of the mercuric ion. I did not try to restore the lost urease action by K₂S.

Necessarily we are forced to ask, What will be the result if we use salts of weaker alkaline substances than ammonia? We can expect with reason to see accelerating effects. I used for this purpose the salt of an alkaloid (quinine hydrochloride).

TABLE XVII.

H ₂ O in cc.	1 % quinine hydrochloride in cc.	0.5 % urease in cc.	1.5 % urea in cc.	N/10 NH ₃ developed in 17 hrs in cc.
40	—	5	5	10.65
35	5	"	"	13.25
37.5	2.5	"	"	11.74
39	1	"	"	11.49

After standing for 17 hours the mixture in the digestion bottles became turbid. It indicates that quinine was precipitated, being freed from its salt by the action of the ammonia developed.

We find in this experiment that 0.02–0.1 % quinine hydrochloride has a marked accelerating effect on urease, as I supposed. But if I continued this experiment, I should no longer find this effect, because the free quinine base is toxic for urease. Table XVIII shows us this fact. Quinine base is difficult to dissolve in water. Therefore 0.06 g. of quinine was dissolved at first in about 2 cc. of absolute alcohol and was diluted with distilled water to 100 cc. Such a small quantity of ethyl alcohol has no effect on urease as is shown later on.

TABLE XVIII.

H ₂ O in cc.	0.06 % quinine in cc.	0.5 % urease in cc.	1.5 % urea in cc.	N/10 NH ₃ developed in 22 hrs in cc.
40	—	5	5	6.84
—	40	"	"	2.87
20	20	"	"	4.01
30	10	"	"	4.65
35	5	"	"	5.01

It is evident that free quinine base inhibits urease action apparently even in a concentration of 0.006 %. This effect is not due to the hydroxyl ion

because 0.006 % quinine is only about mol./6300; it is apparently the effect of the alkaloid ion.

Laqueur [1906] examined the effect of quinine hydrochloride on various ferments. In accordance with many other workers he observed that quinine hydrochloride, generally speaking, inhibited enzymes, but sometimes in its diluted concentration accelerated the ferment action. As indicated by Traube and the author [1914], and as the present work suggests, it is desirable to distinguish between the results obtained by quinine salt and quinine base. It would be wrong to ascribe the effect of "quinine salts" to an effect of "quinine," as is sometimes done.

The same inhibitory results were observed with free atropine and pilocarpine base. Mixing of atropine and pilocarpine had only an additive effect but not an antagonistic effect on urease. The experiments of this section show that the effects of neutral salts are due to the alkalinity of metal bases and alkaloid bases to a certain limit. They do not agree with van Slyke's results concerning *c* and *d*.

(4) *Effects of alcohols on urease.*

Armstrong, Marshall, and van Slyke reported the retarding effect of alcohols on urease. Armstrong showed that alcohols which have less solubility retard the urease action more strongly than alcohols with greater solubility. Van Slyke stated that ethyl alcohol in 30 volumes per cent. (5.7 mol.) retarded both *c* and *d*, especially the latter. He ascribed this effect to the high osmotic pressure of the alcohol solution. He stated that a 2 M solution of alcohol is the limiting concentration for retardation of *d* as well as of *c*.

Alcohols are substances which are able to lower the surface tension of water considerably and we may therefore expect here some considerable

TABLE XIX.

H ₂ O in cc.	5 M alcohol in cc.	0.5 % urease in cc.	1.5 % urea in cc.	Conc. of alc. in M	N/10 NH ₃ developed in 5 hrs in cc.
20	—	5	5	—	9.43
"	—	"	"	—	9.39
—	Methyl 20	"	"	3.3	8.75
—	"	"	"	"	8.93
—	Ethyl 20	"	"	"	7.65
—	"	"	"	"	6.95
—	Propyl 20	"	"	"	7.62
—	"	"	"	"	7.00
—	M/8 amyl 20	"	"	1/12	8.80
—	"	"	"	"	8.76
30	M/2 paraldehyde 20	"	"	1/6	1.15

effects of strongly active substances, like alcohols, on urease. Can it be justifiable to take into consideration here only the osmotic pressure-capacity factor of osmosis of Traube?

My experiments were directed at first to confirm the results obtained by others. Concentrated alcohol solutions were employed (Table XIX).

Addition of paraldehyde caused immediate precipitation of urease solution.

We see from this table an obvious depressing effect of methyl, ethyl, and propyl alcohol in 3·3 M and amyl alcohol in M/12. Paraldehyde in M/6 inhibited urease considerably.

This order of alcohols in relation to their depressing effect is the order of decrease of solubility, surface-tension activity and "attraction pressure" of Traube. The reason why the difference of effect between ethyl and propyl alcohol is not so distinct as is expected from the surface-tension activity, may be the too high concentration of alcohols used, or the time during which the digestion bottles were left at room temperature which was not suitable for the observation of this difference (see later).

Experiments were next carried out to examine the effect of diluted alcohols. The results were contradictory to the reports of previous investigators. In order to attain certainty the experiments were repeated carefully.

TABLE XX A.

Concentration of alcohol	N/10 NH ₃ developed in 18 hours in cc.
0·5 % urease alone	23·52
M methyl	23·63
M ethyl	23·87
M/10 amyl	23·87
M/16 amyl	23·83

TABLE XX B.

Concentration of alcohol	N/10 NH ₃ developed in 7 hours in cc.
0·5 % urease alone	6·66
M methyl	7·69
M ethyl	7·98
M/10 amyl	8·09

TABLE XX C.

Concentration of alcohol	N/10 NH ₃ developed in 6·7 hours in cc.
0·5 % urease alone	7·25
M methyl	7·24
M ethyl	8·86
M propyl	8·97
M/20 amyl	8·75
"	8·55
"	8·53
"	8·75
"	8·50
"	8·34

Alcohols in diluted condition accelerate the urease action. It is beyond question; methyl, ethyl, and propyl alcohol in a concentration of 1 M and amyl alcohol in one of M/20 per litre.

Another experiment with more dilute alcohols was carried out.

TABLE XXI.

Concentration of alcohol	N/10 NH ₃ developed in 7 hours in cc.
0.5 % urease alone	6.00
" "	5.97
M/5 methyl	5.97
" "	6.18
M/5 ethyl	6.06
M/5 propyl	5.90
" "	6.51
M/40 amyl	6.47
" "	6.44

It is evident that methyl, ethyl and propyl alcohol in M/5 had only a feeble accelerating effect, but M/40 amyl alcohol still had a somewhat more recognisable accelerating action.

It is necessary to notice here that, in order to find the accelerating or retarding effect of some substance on ferments, it is very important that the conditions of duration of ferment action and relative concentration between ferment and substrate must be carefully adjusted.

In Table XX A we cannot see distinctly the accelerating effect of alcohol. The reason for this is the longer duration of the digestion, so that the process of hydrolysis went on nearly to the end, both with and without the addition of alcohols. On the other hand, to confirm the above assumption, I have experimented with relatively concentrated alcohol solutions. In this case the concentration of alcohol was the same as in Table XX—1 M, but the urease solution was relatively less concentrated, 0.4 % being used instead of 0.5 %. The digestion bottles were also left a little longer at room temperature.

TABLE XXII.

Concentration of alcohol per litre	N/10 NH ₃ developed in 22 hours in cc.
0.4 % urease alone	22.99
" "	22.80
M methyl	22.78
" "	22.66
M ethyl	22.63
" "	22.71
M propyl	22.72
M butyl	22.79
" "	22.76
M/40 amyl	22.80
" "	22.80

As is seen, there was a slight depressing effect produced by 1 M solutions of the alcohols on urease. That is quite contradictory to the result where this same concentration of the alcohols was able to accelerate the urease action.

Provided that the conditions mentioned above are fulfilled, we learn that concentrated alcohols inhibit urease, while diluted alcohols, on the contrary, accelerate its action.

It is probable that many previous investigators did not succeed in observing this accelerating effect of alcohol on the urease action, because the conditions mentioned above were not satisfied. The alcohol solutions may have been absolutely or relatively too concentrated or the duration of urease action too short.

There are still some points of interest to be considered. It is strange that here alcohols facilitate the enzyme action, while we are taught that alcohols usually paralyse various chemical and biological reactions. My result however is not the only one of the kind, since Giselt [1905] has observed the accelerating effect of alcohol on the action of pancreatic lipase.

Now again, the question arises: To what properties of alcohols are their inhibitory and accelerating effects on urease due?

If we try to explain these facts only by osmotic pressure, then it is rather curious that one property can act in two contrary directions.

Traube alleged long since that osmotic pressure is only a capacity factor of the mechanics of osmosis—number of the dissolved particles—and another important driving factor of osmosis—intensity factor—is neglected. Traube called the latter attraction pressure or “Haftdruck.” The substance which has less attraction pressure against water accumulates on the surface of the second phase in contact with water, and lowers the surface tension of water. This fact has long been known as Gibbs’ theorem and has been experimentally proved.

We do not therefore exhaust all the possibilities when we take into consideration only the osmotic pressure in dealing with substances which have a large effect on surface-tension and with colloidal particles having immense surface development.

With regard to the cause of the accelerating effect of dilute alcohols on urease action there are two possibilities in reference to the considerations mentioned above.

1. Alcohol molecules carry urea molecules with them together to the surface of urease particles, when alcohol molecules accumulate on the surface of enzyme. This process facilitates the contact between enzyme and substrate.

2. Change of surface-tension of urease solution disperses the urease particles and thus increases their active surface.

With regard to the depressing effect of concentrated alcohols on urease there are also two possible causes.

1. Alcohol molecules accumulating on the surface of enzyme particles disturb the contact between urease particles and urea molecules or drive off urea molecules from the surface of enzyme particles.

2. Enormous change of surface-tension favours aggregation of enzyme particles.

Supposing that the first consideration might be the case, I planned my experiment as below.

We learned that formic acid had a moderate inhibitory effect on urease. If we add urease to a mixture of alcohol and formic acid we may expect the inhibitory effect of formic acid to be increased by dilute alcohol, and we may expect with the concentrated alcohol, on the contrary, the diminution of the inhibitory effect of formic acid.

Formic acid was mixed and shaken with ethyl alcohol, and water and 0.5 % urease were then added. In the whole mixture the formic acid was N/2000 and alcohol was 1 and 3.3 molar respectively. The acid was allowed to act on the urease for 15 minutes, and was then neutralised. Urea was added, each bottle was brought to an equal quantity of content and left for 22 hours at room temperature.

TABLE XXIII.

H ₂ O in cc.	N/200 formic acid in cc.	5 M ethyl alcohol in cc.	0.5 % urease in cc.	Concentra- tion of alcohol M	} neutralised by addition of	M/200 NaOH in cc.	1.5 % urea in cc.	H ₂ O in cc.	N/10 NH ₃ developed in 22 hrs in cc.
40	—	—	5	—		—	5	5	23.18
35	5	—	„	—	} neutralised by addition of	5	„	—	10.35 } 10.6
„	„	—	„	—		„	„	—	9.76 } 10.6
25	„	10	„	1		„	„	—	10.14 } 10.15
„	„	„	„	„		„	„	—	10.16 } 10.15
2	„	33	„	3.3		„	„	—	13.08 } 12.54
„	„	„	„	„		„	„	—	11.99 } 12.54

The bottles with 1 M ethyl alcohol and acid developed practically as much ammonia as the bottles with acid alone. But if we consider that 1 M ethyl alcohol is able to accelerate the urease action we come necessarily to the conclusion that 1 M ethyl alcohol facilitated the inhibitory effect of formic acid. In the case of 3.3 M ethyl alcohol we may expect the additional result of the inhibitory effect of acid and the depressing effect of concentrated alcohol if we do not take into consideration the influence of the change of

surface-tension. In fact, we find in this experiment a distinct increase of ammonia in the bottles with acid and 3·3 M ethyl alcohol. It shows us that the action of the urease particles has been relieved from acid inhibition by concentrated alcohol. It has over-compensated the loss caused by the depressing effect of concentrated alcohol.

Let us consider the second possibility mentioned above. If the effect of 1 M alcohol was merely in the dispersion of enzyme particles, but not in facilitating the contact between substrate and urease particles, we must have a larger yield of ammonia from the bottles with acid and 1 M ethyl alcohol than from the bottles with acid alone. But this was not the case.

For the same reason, if 3·3 M alcohol had no effect in driving off acid from the surface of urease particles, but only the aggregating effect on urease, we must get a smaller yield of ammonia from the bottles with 3·3 M ethyl alcohol and acid than from the bottles with acid alone. But this was also not the case.

The question next arises, Does not alcohol combine with formic acid to deprive the latter of its inhibitory effect?

As compared with the concentration of N/2000 formic acid, 1 and 3·3 M ethyl alcohol are very concentrated. If the process concerned is only ester formation between alcohol and formic acid, then 1 and 3·3 M alcohol are strong enough to combine with all the formic acid. We may expect then in Table XXIII only the accelerating effect of diluted alcohol and the retarding effect of concentrated alcohol. But this also was not the case, the result was the reverse.

Further, if the effect of dilute alcohol is in the facilitation of combination between urease and urea, and the effect of concentrated alcohol is in the driving off urea from the surface of urease particles, we can assume as follows. If concentrated urea solution is used as substrate, then the combination of urea molecules with urease particles will be so easy that addition of 1 M alcohol has no effect. On the other hand in such conditions urease particles will be surrounded by so many urea molecules that addition of concentrated alcohol will not be able to drive off the urea molecules from the surface of urease particles in order to take their place.

For this purpose I used 5 % urea solution instead of the usual 0·15 %.

This result proves the above assumption to be true. 3·3 M ethyl alcohol even accelerated the urease action a little. Compared with the high concentration of urea, 3·3 M ethyl alcohol is a relatively diluted solution, so the driving power of alcohol is not stronger than the adhering power of

TABLE XXIV.

H ₂ O in cc.	5 M ethyl alcohol in cc.	0.5 % urease in cc.	25 % urea in cc.	Concentra- tion of alcohol M	N/10 NH ₃ developed in 7 hours in cc.
35	—	5	10	—	7.14
"	—	"	"	—	7.25
25	10	"	"	1	7.60
"	"	"	"	"	7.19
2	33	"	"	3.3	7.53
"	"	"	"	"	7.90

7.20

7.40

7.72

urea to urease. This must be the reason of the acceleration. 1 M ethyl alcohol accelerated only a little. These results teach us also that the effect of alcohol is not in a dispersion or aggregation of urease particles.

There is still one more question. According to Traube the lowering of surface-tension by methyl, ethyl, propyl, butyl and amyl alcohol increases in the proportion of 1:3:3²:3³:3⁴. But in my series of experiments I could not see such enormous differences in the effect of these alcohols. I am of opinion that this was due entirely to unsatisfactory experimental conditions. I tried, therefore, to resolve this question from the other side. I prepared isocapillary alcohol solutions and examined their effect on urease.

Traube's stalagmometer was used for this purpose. It contained 56.9 drops of water at 20°; 5 M methyl alcohol showed 79.1 drops. The number of drops of alcohol solutions and the data of the experiments are as follows. The dilution of alcohols to make isocapillary solutions corresponded nearly to the law of Traube mentioned above.

TABLE XXV.

H ₂ O in cc.	10 cc. of alcohols		1.5 % urea in cc.	0.5 % urease in cc.	N/10 NH ₃ developed in 6-7 hours in cc.
	Name	No. of drops water			
40	—	56.9	5	5	14.30
"	—	"	"	"	14.16
30	Methyl	79.1	"	"	15.46
"	"	"	"	"	15.50
"	Ethyl	79.0	"	"	14.74
"	"	"	"	"	15.44
"	Propyl	78.9	"	"	14.62
"	"	"	"	"	14.28
"	Butyl	79.0	"	"	14.68
"	"	"	"	"	14.87
"	Amyl	78.9	"	"	14.61
"	"	"	"	"	14.72

14.23

15.48

15.09

14.45

14.78

14.67

The results were quite decisive. All the alcohols employed accelerated nearly in the same degree. The alcohol solutions were not quite isocapillary but the number of drops of these solutions varied between 78.9 and 79.1.

The results obtained were very instructive, because the yield of ammonia was exactly proportional to the number of drops, i.e. to the surface-tension. A difference of 0.1 drop in nearly 80 drops showed itself distinctly in the effect on urease action.

It is necessary to emphasise once more the fact that, in the discussion of the effect of substances, which have a large effect on surface-tension, on ferments, warning must be given earnestly against taking only osmotic pressure into consideration; another important factor, surface-tension, must be remembered. The present series of experiments proved this idea to be quite justified.

(5) *On the diminution of action of urease during preservation.*

Traube and the author [1914] proved by means of dark ground illumination that the diminution of toxicity of atropine in a watery solution during preservation is to be ascribed to the aggregation of colloidal particles of atropine. We supposed that this process would follow the formula of unimolecular reactions and in fact we found the (k) of this process fairly constant.

Van Eck [1911] found that the destruction of peroxidase in milk by heat proceeds as a reaction of the first order and the (k), obtained by using the formula of a unimolecular reaction, was constant, and this was confirmed by Zilva [1914].

It is not without interest, therefore, to investigate urease in this direction.

1 % urease solution was kept under toluene in a room where the change of temperature was not great. The bottle with urease was exposed to diffuse light. During 52 days' preservation the efficiency of the urease was estimated at intervals.

5 cc. of the urease solution were mixed with 5 cc. of 1.5 % urea and brought up to 50 cc. with distilled water. The digestion bottle was kept in the water bath at 16° for 7 hours; then the urease action was checked and ammonia was estimated.

During preservation some amount of precipitation took place.

In order to find out the relation between the concentration of urease and the development of ammonia by urease I performed the following experiment.

At the start of this experiment out of the above-mentioned 1 % urease solution 5, 3.5, 2.5, 1.67, 1.25 and 1.0 cc. had been measured and the amount of NH_3 developed by these quantities of urease under the same conditions as stated above was determined. The relation of urease concentration and yield of ammonia was the following:

TABLE XXVI.

Concentration of enzyme	N/10 NH_3 developed in cc.
1.0 (5 cc. of 1 % urease)	21.65
0.7	17.17
0.5	12.20
0.33	5.96
0.25	3.01
0.2	2.02

These data are shown graphically in Curve I.

The following curve and table show the amount of ammonia developed by preserved urease and the calculated concentration of urease on the various days of experiment. This calculation was carried out by making use of Curve I and the quantity of developed ammonia.

TABLE XXVII.

Days of preservation	N/10 NH ₃ developed in cc.	Calculated concentration of urease	<i>k</i>
<i>t</i> = 0	21.65	1.00 = <i>a</i>	
2	21.37	0.97	0.0152
5	20.10	0.88	0.0256
9	17.96	0.74	0.0334
13	14.16	0.55	0.0460
16	12.16	0.48	0.0459
20	9.80	0.41	0.0446
23	8.71	0.38	0.0421
29	5.26	0.32	0.0465
33	4.10	0.29	0.0375
40	2.90	0.25	0.0346
44	2.55	0.23	0.0333
52	1.72	0.17	0.0341

If a unimolecular reaction is not reversible we have the well-known formula:

$$k = \frac{1}{t} 2.302 \log \frac{a}{a-x}.$$

I took here 24 hours as a unit of t and obtained k as shown above.

A steady change of k was here observed; it reached its maximum on the 29th day and then began to fall.

SUMMARY.

1. The effect of various acids, alkalis, alcohols, aldehyde, neutral salts and alkaloids on the urease of soy-bean has been studied.

2. In the inhibitory effects of inorganic and organic acids on urease the hydrogen ion concentration plays the main rôle. These inhibitory effects are influenced by the physical properties, and especially the surface-tension, which the acids possess. Total inhibitory effects are considered to be the combined effects of chemical and physical properties. If the dissociation of the acids is equally strong, then the inhibitory effects of acids with greater effect on surface-tension obtain the upper hand. Even the deficiency of hydrogen ion concentration can be over-compensated by great effect on surface-tension. The acids with great effect on surface-tension facilitate the adsorption of hydrogen ions on the surface of urease particles.

3. The inhibitory effects of caustic soda and ammonia do not coincide in reference to the hydroxyl ion concentration. Soda inhibited urease in far lower concentration than ammonia. But in equally effective soda and ammonia solutions the hydroxyl ion concentration is far greater in soda solution. The inhibitory effect of soda is to be ascribed to the hydroxyl ion concentration but ammonia has some further action.

4. Provided that certain conditions are fulfilled, it has been proved that 1 M alcohols (methyl, ethyl, propyl) and M/40 amyl alcohol accelerate the urease action, but 3.3 M of the former group and M/12 of the latter retard the urease action.

5. Isocapillary alcohol solutions manifest equal effects on urease.

6. The cause of this acceleration or retardation is not to be understood merely in the light of osmotic pressure—Traube's capacity factor of the mechanism of osmosis. The intervention of Traube's intensity factor—attraction pressure, represented here by the surface-tension—makes clear the explanation of these phenomena.

7. The dilute alcohols which lower the surface-tension of the solvent accumulate on the surface of the second phase in contact with the solvent. By this action they facilitate adsorption between urease and urea. This is probably the cause of the acceleration of the urease action. Concentrated alcohols disturb the adsorption between urease and urea by displacement. This is the cause of retardation of the urease action.

8. In this work it is not found that the effects of alcohols on urease were caused by the dispersion or aggregation of urease particles.

9. Aldehyde inhibits urease notably.

10. The effects of neutral salts are merely those of their metallic bases. They retard in N/10 strength the urease action, because metallic bases displaced by ammonia produce a greater hydroxyl ion concentration than the equivalent of ammonia.

11. In accordance with this, alkaloid salts (quinine is a weaker base than ammonia) accelerate urease action in the first stage of hydrolysis. But alkaloid bases themselves inhibit urease action markedly (quinine, atropine and pilocarpine). The effects of the atropine and the pilocarpine on the urease are additive but not antagonistic.

In conclusion, I have pleasure in expressing my thanks to Prof. Starling, Prof. Bayliss and Dr Plimmer, for their kindness in allowing me to work in their institute and for their advice. The theme of this work was suggested by Prof. Bayliss and I worked under his supervision.

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