XCVIII. UREASE. PART II. THE MECHANISM OF THE ZYMOLYSIS OF UREA.

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IN a previous communication [Fearon, 1923] an account was given of the detection and estimation of cyanic acid in solutions of urea undergoing zymolysis, and it was suggested that cyanic acid and ammonia are the intermediate stages in the enzymic decomposition of urea:

Since this paper was submitted for publication, a paper has appeared on the mechanism of urease by Mack and Villars [1923]. In this paper the authors agree with the present writer in accepting the Werner formula for urea. They have detected and estimated cyanate in solutions of urea undergoing zymolysis, and also have shown that the cyanate rises to a maximum and falls again towards the end of the zymolysis. Their maximum values expressed as percentage cyanic acid agree closely with several maximum values obtained by the present writer (0.003 %).

On the other hand, they arrive at the conclusion, by a process of elimination, that the transformation

is the stage catalysed by the enzyme, and they do not appear to consider cyanic acid as a possible intermediate product in the zymolysis.

This conclusion is based on indirect evidence; carbamic acid or carbamates have not been shown to be present in the urea/urease system. The work of Yamasaki [1918] is inconclusive in this respect, since the experimental method he adopts will not discriminate between carbamates and cyanates.

The method employed by Mack and Villars in estimating cyanic acid consists in precipitating the samples with excess of silver nitrate, washing the precipitate free from ammonia, digesting with dilute nitric acid to convert the cyanate into ammonium nitrate, and estimating the ammonia so formed by nesslerisation.

When silver nitrate is added in excess to a mixture of ammonium carbonate, carbamate, and cyanate in solution, a precipitate is thrown down consisting of the silver salts of the three acids. If the solution be now set to p_H 5 by means of dilute nitric acid the carbonate and carbamate dissolve leaving the silver cyanate. This is the basis of the method adopted by the present writer in estimating cyanic acid [1923].

The method of Mack and Villars is open to the objection that any carbamate present would be included in their result as cyanate; this defect can be overcome by setting the solutions to a suitable p_H before filtration. Thus modified, their method is much more convenient than that formerly adopted by the present writer, especially now that a preparation of urease containing a minimum amount of protein is available (*urease Dunning*).

To determine the exact significance of the Mack and Villars results, and to obtain information as to the part played by carbamate in the urea/urease system, the cyanate and carbamate in solutions of urea undergoing zymolysis were determined simultaneously.

Estimation of the Carbamate and Cyanate in the Urea/Urease System.

Solutions of pure urea and urease (*Dunning*) of such proportions as to produce 0.1 *M* urea and 0.1 % urease were mixed and incubated at 20°. At definite intervals duplicate samples of 50 cc. were withdrawn and treated with excess of *N*/1 $AgNO_3$. One sample was filtered at once, this gave the carbamate and cyanate residue. The other sample was set to p_H 5 by means of 0.6 *N* HNO_3 , using methyl-red and a comparator, and then filtered, this gave the cyanate residue only. The residues were washed until free from ammonia, and then were digested on a water-bath with 20 cc. of 2 % HNO_3 . After cooling, they were treated with 15 cc. of saturated K_2CO_3 , diluted to 50 cc. and the ammonia determined by aspiration into 100 cc. of *N*/500 HCl , and subsequent nesslerisation. The results are expressed as nitrogen derived from both carbamate and cyanate and from cyanate alone in the 50 cc. samples. The difference between these values represents carbamate alone. By titration of samples from time to time with 0.6 *N* HNO_3 , using methyl-red, the progress of the zymolysis was roughly determined.

Table I.

Time in minutes	Carbamate and cyanate N in 50 cc. $\times 10^4$ g.	Cyanate N in 50 cc. $\times 10^4$ g.	Cyanic acid %	Urea % decomposed
0	0	0	0	0
30	1.1	1.0	0.0006	18.6
60	2.3	2.5	0.0015	—
130	5.2	5.0	0.0031	52
240	2.0	2.3	0.0014	91
1320	0.1	?	0	99.9
<i>Substrate</i> 0.1 <i>M</i> urea.		<i>Temperature</i> 20°.		
<i>Enzyme</i> 0.1 % urease <i>Dunning</i> .				

From these results it will be seen that the cyanate N and the cyanate N plus carbamate N agree; and consequently there is no indication of the presence of carbamate at any stage during the zymolysis of urea. The results of Mack and Villars may be taken as representing cyanate, as will be seen from a comparison of their figures with the above table.

Table II. (*From the data of Mack and Villars [1923]*)

Time in minutes	N in 50 cc. $\times 10^4$ g.	Cyanic acid %
5	2.33	0.0014
10	2.75	0.0016
20	3.46	0.0021
80	5.59	0.0034
165	3.99	0.0024
Substrate	0.1 M urea.	Temperature 25°.
Enzyme	0.1 % urease.	

Mack and Villars conclude that the cyanate in the urea/urease system is due to the spontaneous dissociation of the urea, and is uninfluenced by the presence of the enzyme.

That this is not so was demonstrated by a control solution of urea alone, which showed no indication of cyanate formation during the time of the experiment, nor for several days unless it had become infected.

The Urea-Ammonium Cyanate Equilibrium.

Urea in aqueous solution when kept *sterile* appears to be stable at ordinary temperatures for an indefinite period. Werner [1918] has found $N/2$ solutions of urea in toluene water free from cyanate or carbonate after nine months. On the other hand, a similar solution of urea in plain water showed signs of decomposition after 14 days at room temperature. A 5 % solution of urea kept by the author under ordinary laboratory conditions for one year showed on analysis a conversion of 0.2 % of the urea into ammonium carbonate. A mould with urealytic properties was found to have developed in the liquid. The solution contained no cyanate; the activities of the mould had presumably been inhibited by the increasing alkalinity of the solution, and the cyanate had been hydrolysed to carbonate.

Werner explains the stability of urea in sterile solutions below 40° as being due to the fact that it is not dissociated into ammonia and cyanic acid under such conditions. If, however, these solutions are heated to about 60° dissociation occurs with production of cyanic acid and ammonia. This dissociation has been very fully investigated by Walker and Hambly [1895], who have shown that, after one hour at 100°, 4 % of a $N/10$ urea solution had come into an equilibrium with ammonium cyanate produced by the transformation of the urea.

The equilibrium concentration for 39° found by incubating $N/10$ solutions containing 95 % urea and 5 % ammonium cyanate, is open to several experimental objections. Part of the cyanate is converted into urea and part is hydrolysed to ammonium carbonate.

The urea-ammonium cyanate equilibrium is not stable as the cyanate is gradually hydrolysed to ammonium carbonate until all the urea is removed from solution by this double process of dissociation and hydrolysis.

This change is slow if the ammonia be prevented from leaving the system. Fawsitt [1902] found that 450 hours were required to bring about the de-

composition of 98.9 % of the urea originally present in a semi-normal solution kept at 99° in a sealed tube.

The Formation of Ammonium Carbamate from Urea.

The direct formation of ammonium carbamate by the hydration of urea has not been demonstrated experimentally. Its possible occurrence is open to doubt. The various syntheses of urea from ammonium carbonate and from ammonium carbamate have been shown by Werner to involve an intermediate formation of cyanic acid from the dehydration of carbamic acid. It is hoped to discuss the very interesting subject of the enzyme synthesis of urea from ammonium carbonate and carbamate claimed by several investigators [Barendrecht, 1919; Mack and Villars, 1923; Kay, 1923] in a subsequent paper.

Cyanic Acid as the Intermediate Stage in the Urea/Urease System.

Before a compound can be considered as a possible intermediate stage in a catalysed reaction it must be shown to conform to the requirements of Ostwald's law of successive reactions [1902]. The intermediate reaction must take place more rapidly than the direct reaction under the same conditions. The concentration of such a compound will depend on its stability. As Hopkins has observed: "the degree to which a substance accumulates is itself no measure of its metabolic importance, no proof as to whether it is on some main-line of change, or a stage in a quantitatively unimportant chemical by-path" [Bayliss, 1917]. Cyanic acid in aqueous solution is unstable, and the degree to which it accumulates will depend on the difference between the rate of formation and the rate of removal. At the start of the reaction the cyanic acid gradually reaches a maximal concentration depending on the temperature and concentration of the enzyme, but independent of the concentration of substrate, provided that there is excess of substrate.

Working with low concentrations of enzyme preparation (0.1 to 0.2 %) and substrate (0.1 to 0.5 *M* urea) at temperatures of 5 to 20° the maximum concentration of cyanic acid is 3-4 mg. %. The figures obtained by the method of Mack and Villars, adopted in the present paper, are probably more accurate than those obtained by the use of collodion sacs described in the previous paper [Fearon, 1923], owing to the diffusion being retarded by the sac.

Towards the end of the zymolysis the rate of formation of cyanic acid begins to decrease owing to decrease of substrate and accumulation of ammonia in the system, which retards the hydrolysis of cyanic acid, as well as the dissociation of the substrate. Consequently, the removal of cyanic acid decreases in rate also towards the close of the reaction.

The Hydrolysis of Cyanic Acid.

In the liquid state below zero, and in aqueous solutions, cyanic acid is an equilibrium mixture of the two forms:

Liquid cyanic acid, below zero, contains about 60 % HOCN and 40 % HNCO. Rise of temperature leads to increase in HNCO, and fall of temperature increases the HOCN [Werner and Fearon, 1920]. Union of enol-cyanic acid with ammonia produces ammonium cyanate.

Union of keto-acid with ammonia produces urea.

When urea in solution undergoes dissociation, ammonia and cyanic acid in the keto-form are produced. Keto-cyanic acid is less stable at low temperatures than the enol-form of the acid, accordingly, some of it is transformed into enol-cyanic acid and some of it is hydrolysed to ammonia and carbon dioxide. The result may be represented as follows:

Owing to the existence of these two forms, the hydrolysis of cyanic acid in aqueous solution is complicated, and involves three distinct primary changes [Werner and Fearon, 1920]:

- (1) $\text{HOCN} \rightleftharpoons \text{HNCO} + \text{H}_2\text{O} = \text{CO}_2 + \text{NH}_3$.
- (2) $\text{HOCN} + \text{NH}_3 = \text{NH}_4 \cdot \text{OCN}$ (ammonium cyanate).
- (3) $\text{HN} : \text{CO} + \text{NH}_3 = \text{HN} \cdot \text{CONH}_3$ (urea).

At the outset of the reaction the keto-cyanic acid is hydrolysed with the production of CO_2 and NH_3 . In 15 minutes 15 % of a $N/4$ solution of cyanic acid at 0° was converted into CO_2 and NH_3 . As the ammonia gradually begins to accumulate in the solution it unites with the keto-acid to form urea, and the hydrolytic process gradually slows down.

In the presence of urease the hydrolysis of cyanic acid is considerably modified. First, since the urea molecule is decomposed at a comparatively low temperature (0° to 55°) most of the cyanic acid is liberated into solution in its less stable form and is more readily hydrolysed than if liberated from a normal cyanate. Then, urease is able to combine with ammonia, reducing its concentration in the liquid phase of the system. Also, the urea formed synthetically during the late stages of the hydrolysis of the cyanic acid is in turn broken down by the enzyme.

All these factors will increase the amount of free keto-cyanic acid in the solution, which is the form in which the acid is most readily attacked by water.

The Mechanism of Urease Action.

As the present paper is chiefly concerned with the chemistry of the zymolysis of urea the interpretation of the data obtained from the study of the rates of zymolysis under varying conditions will not be discussed. The

most comprehensive account to date will be found in the work of Lovgren [1921], which includes an exhaustive bibliography of 212 references. The problem at issue can, however, be restated in more precise terms in view of the detection of cyanic acid in the urea/urease system. The urealytic property of the enzyme is due to its power of dissociating urea in solutions at ordinary temperatures, from 0° to 60° (above which the enzyme is rapidly destroyed).

The Adsorption Stage in the Urea/Urease System.

With the exception of Barendrecht [1919] and Yamasaki [1918], there is general agreement amongst the investigators of the kinetics of the urea/urease system that the first stage in the zymolysis is a union between the substrate and the enzyme. The precise nature of this union has not yet been determined. Armstrong, Benjamin and Horton [1913], Bayliss [1915, 1918] have come to the conclusion that urease acts by surface condensation or adsorption. Van Slyke and Cullen [1914] consider that the change is due to a chemical combination of enzyme and substrate, followed by a decomposition of the intermediate product according to the laws of chemical reactions deduced from mass action.

In support of the adsorption theory, Bayliss [1915] has described some simple and convincing experiments in which he shows that urease is able to act in a medium in which it is quite insoluble. Working with solutions of 4 % urea in 80 % to 89.3 % alcohol he found that urease was able to decompose from 7.8 % to 1.8 % of the substrate although the enzyme was quite insoluble in the medium.

The Action of Urease in Absolute Alcohol.

The determination of the degree of adsorption of urea by urease was attempted by extending the experiments of Bayliss to solutions of urea in absolute alcohol.

A preparation of soy-urease, freed from fat by ether extraction in a Soxhlet extractor, was extracted at 40° with absolute alcohol until the filtrate no longer gave an opalescence on dilution with water and addition of silver nitrate. 50 g. of this preparation, which was very active, were added to 50 cc. of a saturated solution of pure urea in absolute alcohol at 18°. The mixture was incubated in a closed flask at 18°, and frequently shaken. A similar flask containing a saturated alcoholic solution of urea was kept under the same conditions as a control. The urea in each solution was determined from time to time by drawing off 2 cc. samples and determining the urea gravimetrically after evaporation of the alcohol on a water-bath. Equilibrium was reached after ten days.

Table III.

	%
Urea in control solution	4.290
„ in presence of enzyme	3.475
„ removed from solution	0.815
„ adsorption	19.0 of urea present

On filtering off the enzyme, the filtrate was found to contain no ammonia, but on dilution with water and after addition of silver nitrate gave a white precipitate soluble in dilute nitric acid, and responding to the test for cyanate by hydrolysis and nesslerisation, as described in Part I of the present communication [1923]. This was not observed in the control solution of urea, or in suspensions of the enzyme in absolute alcohol. The residue from the experiment was still active, and appeared unchanged.

This experiment supports the theory that adsorption is the preliminary stage in the zymolysis of urea. It also indicates that adsorption alone is inadequate to explain the change. Only a very small fraction of the adsorbed urea can have been dissociated.

It may be possible to find an explanation in terms of general adsorption for cases of enzymes which accelerate reactions, such as the hydrolysis of esters, where increase of concentration of the reactants in the surface layer will bring about increase of reaction-rate in accordance with the law of mass action. This subject has been recently reviewed by Bayliss [1919]. But in the case of enzymes such as urease, which initiate, or appear to initiate, reactions, general adsorption is not sufficient to explain the zymolysis, since a saturated solution of urea is quite as stable as a weak solution, in the absence of infection. Increase of concentration in the surface layer is by itself insufficient to bring about decomposition.

Selective Adsorption in the Urea/Urease System.

The adsorbent properties of urease, apart from its urealytic power, do not appear to have been investigated hitherto. Urea in neutral solution is present as a closed-ring compound. In this form it is not attacked by nitrous acid and does not give a condensation derivative with xanthhydrol [Werner, 1923]. The action of acids or alkalis is to open up the ring and form salts with the amphoteric *free* urea:

When the neutral urea molecule undergoes dissociation it gives rise to an alkaline component, ammonia, and an acid component, keto-cyanic acid. Acids or alkalis accelerate the decomposition of urea in aqueous solutions above the dissociation point by combining with either the alkaline or the acid component.

The behaviour of urease towards ammonia and cyanic acid was investigated in order to see if, in addition to its property of adsorbing urea, it showed any evidence of selective adsorption, or greater preference for one constituent of the system. Preliminary observations were made on the nature of the electric charge borne by urease in colloidal suspension.

Solutions of soy-urease, decanted from 5 % suspensions, and dialysed for a couple of hours against distilled water to remove excess of electrolytes,

were subject to a potential difference of 100 volts in a conductivity tube (Hardy's modification of Whetham's apparatus), giving a fall in potential gradient of 1 volt per cc.

The colloid showed an electro-negative charge. After 20 minutes at 15° the particles had accumulated in the region of the anode.

Control experiments with urease suspensions boiled for 10 minutes showed an almost complete disappearance of charge.

In the absence of more precise information as to the nature of the enzyme found in the urease preparations conclusions from experiments on impure preparations must be tentative. When an active preparation of urease free from foreign protein is available the work will be repeated.

The behaviour of soy-urease towards ammonia was then investigated. If the ammonia group of the neutral urea is adsorbed by the electro-negatively charged surface leaving the unstable cyanic acid in the outer zone of the Helmholtz "double layer," the rapid decomposition of the substrate should continue until the surface is saturated with adsorbed ammonia.

It is well known that ammonia retards the action of urease, as first shown by Armstrong and Horton [1912], but the fact that urease is able to adsorb ammonia has not been previously demonstrated.

The Adsorption of Ammonia by Urease.

Samples of soy-urease freed from fat were shaken up with solutions of ammonium sulphate of varying strength. After standing for 24 hours at room temperature, the mixtures were centrifuged and the ammonia in the supernatant liquid determined by the colorimeter.

The mixtures consisted of 2 g. soy-urease and 50 cc. standard ammonium sulphate (Folin). The average temperature was 18°.

Table IV.

	Before adsorption. Ammonia N in mg. %	After adsorption. Ammonia N in mg. %	Percentage adsorption
A.	28	16.6	40.8
B.	14	6.5	53.5
C.	7	0.0	100

A control solution of urease (4 %) in water alone did not liberate any ammonia after 24 hours.

The supernatant liquid from a 4 % solution of soy-urease in water alone, after standing for 24 hours before being centrifuged, contained no ammonia.

For the purposes of comparison, the ammonia-adsorbing property of a sample of permutit was determined under the same conditions.

The mixture consisted of 2 g. of washed permutit and 50 cc. ammonium sulphate solution. Time, 24 hours. Temperature 18°.

	Before adsorption. Ammonia N in mg. %	After adsorption. Ammonia N in mg. %	Percentage adsorption
	28	6.6	76.4

Permutit does not adsorb urea in aqueous solution.

Urease is not destroyed by the action of ammonia or ammonium salts at ordinary temperatures. Suspensions of soy-urease in $N/10$ NH_4OH after three months at 12° to 14° regained urealytic activity on washing with 2% acetic acid and with water. Even solutions of urease incubated with excess of $10 N$ ammonium carbonate and carbamate for 10 hours at 55° , in accordance with the method of Mack and Villars [1923], for showing the synthesis of urea, on removal of the ammonia were active towards urea, although a considerable amount of the enzyme material had been destroyed by the action of the strong alkali.

The action of urease on cyanic acid is difficult to investigate, since it is not possible to obtain the unstable form of the acid in solution apart from interfering substances. Mack and Villars have studied the action of urease on $0.1 M$ solutions of ammonium cyanate (made by mixing equal parts of $0.1 M$ solutions of NH_4Cl and KCNO) at 25° .

They find that urea is formed more readily from such solutions in the absence of urease than in its presence, which is as might be expected, because the concentration of ammonia in their experiments is not sufficient to inhibit completely the action of the enzyme.

Urease does not increase the rate of formation of ammonium cyanate from urea, and consequently need not necessarily catalyse the reverse reaction. The action of urease is to liberate the unstable form of cyanic acid from urea; this is rapidly hydrolysed until the progress of the reaction has resulted in the accumulation of ammonia sufficient both to retard the enzyme by forming an adsorption compound to the exclusion of the substrate and to retard the hydrolysis of the keto-cyanic acid. Ammonium cyanate can only appear at a late stage in the urea/urease system.

The Significance of Ammonia Adsorption in the Urea/Urease System.

Ammonia adsorption may in itself be sufficient to account for the dissociation of adsorbed urea, but it must be remembered that several other factors are concerned. The concentration of adsorbed substrate is difficult to determine, and until it is known, the ammonia concentration on the enzyme surface can only be inferred from kinetic data.

Urea adsorption by urease is a rapid process in aqueous solution, as was found by addition of soy-urease to solutions of urea just above freezing-point, and rapid separation again of the enzyme by alcoholic precipitation and centrifugalisation. The residue was then taken up with water, and incubated. The adsorbed urea underwent decomposition, thus affording a rough method of determining the extent and rate of adsorption.

Substrate, 10 cc. of 2% urea. Temperature 0° .

Enzyme, 0.1 g. urease *Dunning*, in fine powder.

The mixture was shaken for 30 seconds, and then treated with 80 cc. absolute alcohol, which completely precipitated the enzyme. The mixture

was then rapidly separated by centrifugalisation, and the residue was incubated at 36°.

Ammonia formed = 19 mg. urea, or 9.5 % of substrate present.

By an extension of this method the rate of adsorption might be determined. The conclusions from qualitative experiments on the mechanism of enzyme action must be restricted owing to lack of knowledge as to the degree of purity of the various preparations, and the necessity for distinguishing between accidental and essential properties of the ferment.

Until urease is obtainable in a form of biochemical purity, corresponding to the *amylase* prepared by Sherman, an extension of the work outlined in the present paper does not appear to be profitable.

In addition to the effect of selective adsorption of ammonia it must be noted that adsorbed urea is probably subjected to a considerable mechanical pressure in the surface layer.

Williams [1920], working with charcoal, has calculated the surface pressure to be of the order of 50,000 atmospheres. Many of the instances of irreversible adsorption may be ascribed to compression effects, such as the coagulation of adsorbed proteins at ordinary temperatures, a change which in the absence of the adsorbent requires pressures of 17,000 to 20,000 atmospheres [Bridgman 1914].

The dissociation of urea gives rise to the very compressible base ammonia, hence it is to be expected, although it has not yet been demonstrated, that high pressures alone should bring about the dissociation of urea. Lewis and Burrows [1912] have found that the decomposition of urea in solution by heat is always accompanied by a diminution in volume. It has also been found that in the Haber ammonia process increase of pressure displaces the equilibrium so that more is formed of the component occupying the lesser volume, namely ammonia [Haber, 1914].

A Theory of the Mode of Action of Urease.

From qualitative data it is possible to construct a theory of the mode of action of urease, which will explain, amongst other things, the specific activity of the enzyme.

Urea is adsorbed from solution by urease and then dissociated into ammonia and cyanic acid in its unstable form. The ammonia is adsorbed by the enzyme, the cyanic acid remains in solution.

In aqueous solutions the cyanic acid is rapidly hydrolysed into ammonia and carbonic acid. The resulting ammonium acid carbonate is able to combine with another molecule of ammonia, and so will tend to reduce the concentration of adsorbed ammonia, thus freeing the enzyme.

For this reason, acids in low concentration accelerate the reaction by combining with the ammonia. Alkalies in low concentration retard the zymolysis by neutralising the carbonic acid, by fixing the cyanic acid as a much more stable cyanate, and also by being adsorbed themselves.

Carbonic acid being a weak acid, and its salts with ammonia being largely dissociated, a stage is reached in the urea/urease system when the enzyme is approaching saturation with ammonia, and the reaction rate steadily falls, depending on the diffusion of the end-products into the solvent. If at this stage the H-ion concentration of the solution be increased to its optimum range (p_H 6.8–7.0) by buffers or by aeration with carbon dioxide the reaction rate returns to its maximum, provided the substrate be in excess. Under such conditions the rate of the zymolysis approximates to linear [Armstrong and Horton, 1912]. Here, what is being measured is the rate of hydrolysis of free cyanic acid at an optimum H-ion concentration.

Acids in high concentration stop the zymolysis of urea completely; the action is probably due to the precipitation of the enzyme, either directly or associated with the globulin of the urease preparation.

In higher concentrations of acids some of the urease preparation goes into solution once more, but has no action on urea. This may be due to a reversal of the electric charge, but until the purification of urease has been accomplished the subject cannot be thoroughly investigated.

An additional, but probably less important, effect of acids is their action on the substrate itself, opening up the cyclic form of urea to produce the salt form which is not dissociated into cyanic acid and ammonia.

Reversibility of Action of Urease.

Since ammonia and carbon dioxide are not the primary products of the action of urease on urea, its synthesis from these substances is not an inevitable consequence of the general theory of enzyme action. Mack and Villars [1923] and Kay [1923] have undoubtedly obtained urea from the action of strong solutions of ammonium carbamate and carbonate on urease. However, it has not yet been demonstrated that this urea arises from an enzyme dehydration of the carbamate, and it appears unlikely, since Werner has shown that the classical Basarov synthesis [1868] of urea by the action of heat on ammonium carbamate involves the intermediate formation of cyanic acid, as first suggested by Mixter [1882].

The results of an extended investigation following the lines of Mack and Villars will be described in a subsequent communication.

Summary of the Dissociation Theory of Urease Action.

(1) At the beginning of the reaction the urea/urease system consists of the non-electrolyte urea in true solution, and the enzyme urease in suspension as a negatively-charged colloid:

(2) *Stage of Adsorption* (occurs in alcoholic and aqueous solutions). Urea is withdrawn from solution, and concentrated on the enzyme surface:

(3) *Stage of Dissociation.* Adsorbed urea is dissociated into keto-cyanic acid and ammonia, which is held by the enzyme:

(4) *Stage of Hydrolysis* (occurs in aqueous solutions only). The keto-cyanic acid is hydrolysed by the solvent, with the formation of ammonia and carbonic acid:

(5) The carbonic acid and acid carbonate combine with some of the adsorbed ammonia, and liberate the enzyme:

(6) The liberated urease combines with more urea, and so the cycle continues, until brought to rest by exhaustion of substrate or by accumulation of ammonia in sufficient concentration to inhibit stage (5). Stage (5), the breakdown of the adsorption compound, is the true characteristic of the urea/urease system (and probably all catalytic actions in heterogeneous systems). In the absence of stage (5) there can be no continuous zymolysis; the chemical changes would be linear, not cyclical.

CONCLUSIONS.

1. No evidence has been obtained of the occurrence of carbamic acid in the zymolysis of urea. Cyanic acid and ammonia appear to be the intermediate products of the action of urease on urea. Urease acts by dissociating its substrate.

2. Soy-urease is able to adsorb urea from alcoholic solution.

3. Soy-urease in aqueous solution carries an electro-negative charge, and is able to adsorb ammonia from aqueous solution.

4. A theory of the mechanism of urease is presented in outline. Urease combines with urea by adsorption. The adsorbed urea is dissociated into ammonia, which combines with the enzyme, and cyanic acid, which is hydrolysed by the solvent. The enzyme is then liberated from the ammonia compound.

5. It is suggested that urea dissociation is brought about by selective adsorption and also possibly by the pressure in the surface layer.

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