

# THE KINETICS OF THE HYDROLYSIS OF UREA AND OF ARGININE

BY ROBERT C. WARNER

(From the Department of Chemistry, New York University College of Medicine,  
New York)

(Received for publication, September 13, 1941)

This investigation of the hydrolysis of urea and of arginine was undertaken in connection with a study of ammonia formation from proteins in alkaline solution.

## *Hydrolysis of Urea and Cyanate*

Fawsitt (1) and Werner (2) have studied the hydrolysis of urea by acids and by alkalies. They agreed in assuming that cyanate was an intermediate in the hydrolysis, but differed widely as to the details of the mechanism and as to experimental values for the velocity constants of the reactions involved.<sup>1</sup> The reverse reaction, the synthesis of urea from ammonia and cyanate, has been investigated in some detail by Walker and his coworkers (3) and more recently by Warner and Stitt (4) and by Svirbely and Warner (5). The latter workers have concluded, on the basis of the effect of ionic strength on the velocity, that the reaction is one between ammonium and cyanate ions.

Because of the unsatisfactory nature of the data on the kinetics of the hydrolysis of urea, we have reinvestigated the rates and mechanism of the reactions involved. We were chiefly interested in the hydrolysis by alkali, but the work was extended to include neutral and acid pH ranges.

<sup>1</sup> Werner states that the lack of agreement between his results and those of Fawsitt on the rate of hydrolysis of urea was due to the fact that his experiments were performed under a reflux, whereas Fawsitt's reaction mixtures were heated in sealed tubes. Contrary to Werner's contention, we have found no difference in the rate of hydrolysis in an alkaline reaction mixture, whether heated in sealed tubes or under a reflux.

*Methods*

Reaction mixtures were prepared by adding measured quantities of HCl, NaOH, or chosen buffer solutions to standard solutions of urea or of potassium cyanate. A series of samples of each reaction mixture was sealed in small Pyrex tubes which were heated in a boiling water bath (100°) or a boiling methyl alcohol bath (66°). Tubes were removed at various time intervals, chilled in ice water, and the contents analyzed by the methods described below.

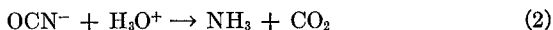
Ammonia was determined by the method of Conway and Byrne (6). The presence of cyanate in the reaction mixture does not interfere with this analysis. Ammonia plus cyanate was determined by acidifying an aliquot of the reaction mixture and allowing it to stand for 20 minutes, to convert the cyanate to ammonia and CO<sub>2</sub>. The ammonia was then determined in the Conway unit. Urea was determined by the method of Allen and Luck (7). It was found necessary to avoid large excesses of HCl, NaOH, or neutral salt to obtain complete precipitation of the xanthidrol urea.

Determinations of all the reactants were not made in all experiments.

*Results*

All of the nitrogen in the reaction mixtures was accounted for as urea, ammonia, and cyanate. Possible side reactions which could lead to the formation of cyanuric acid or cyamelide can thus be disregarded. Structural relationships suggest carbamate as an intermediate, and the work of Lewis and Burrows (8) indicates that it has some significance in the equilibria which would ultimately obtain in a neutral solution of urea at 100°. The rate of attainment of the carbamate-carbonate equilibrium is, however, so great at 100° at any pH that carbamate would not be expected to accumulate in the reaction mixtures (9, 10). The detailed analysis presented below indicates that carbamate cannot be formed in large enough amounts to be of quantitative significance in determination of the course of the hydrolysis in dilute solution.

Thus the following two reactions can tentatively be assumed.



This is a similar sequence of reactions to that postulated by Werner and Fawsitt in their studies on urea hydrolysis.

The reversible nature of Equation 1 in neutral solution has been well established (3-5). There is no evidence for the reversibility of Equation 2 under the conditions of these experiments.

On the basis of Equations 1 and 2, the following differential equations can be set up to describe the course of the hydrolysis

$$\frac{du}{dt} = -k_1u + k_2xy \quad (3)$$

$$\frac{dy}{dt} = k_1u - k_2xy + k_3x \quad (4)$$

$$\frac{dx}{dt} = k_1u - k_2xy - k_3x \quad (5)$$

where

$u$  = concentration of urea

$y$  = " " ammonia

$x$  = " " cyanate

$2u + y + x$  = total concentration of nitrogen =  $a$

$k_1$  = velocity constant of the forward component of Equation 1

$k_2$  = " " " " reverse reaction

$k_3$  = " " " Equation 2

These equations will hold at any pH, but the values of the constants may be expected to vary individually with the pH. In certain pH regions the equations can be modified to permit the ready derivation of the constants from the experimental data.

In acid solution (less than pH 5) the rapid hydrolysis of cyanate reduces  $x$  to so small a value that it is possible to neglect the  $k_2xy$  term completely. Levy<sup>2</sup> has made measurements on the hydrolysis of cyanate under these conditions at 25°. He found that  $k_3 = 3.2$  at pH 4 and that it increases in direct proportion to  $(H^+)$ , a double inflection in the curve being evident near the ionization constant of cyanic acid. The reaction is too fast to measure at higher temperatures and cyanate can thus be considered to be absent in our acid reaction mixtures. This sets an effective lower pH limit to the reversibility of Equation 1.

In alkaline solution (greater than pH 12) Equation 1 can also

<sup>2</sup> Levy, M., personal communication.

be considered to be irreversible. This was shown by experiments on the hydrolysis of cyanate in NaOH in which no urea was formed, the sole products being ammonia and carbonate. The effect of ionic strength on the velocity of the reverse of Equation 1 indicates that the reaction is one between  $\text{NH}_4^+$  and  $\text{OCN}^-$  (4). These two considerations make it probable that an upper limit to the reversibility of the reaction is set by the dissociation  $\text{NH}_4^+ = \text{NH}_3 + \text{H}^+$ .

Below pH 5 or above pH 12, therefore, Equation 3 reduces to the first order equation

$$\frac{du}{dt} = -k_1 u \quad (6)$$

$k_1$  can be calculated either from the rate of disappearance of urea or the rate of formation of ammonia plus cyanate. The results at 100° and 66° will be found in Tables I and II respectively. The constants are calculated in all cases in natural logarithms and reciprocal hours.

Data were also obtained on the hydrolysis of KOCN in alkaline solution. For this situation Equation 5 reduces to a first order equation with respect to  $x$ , and  $k_3$  can be calculated. The data obtained appear in Table III.

In alkaline solution where  $k_1$  and  $k_3$  are of similar magnitude, the concentration of cyanate at time intervals during the hydrolysis of urea can be calculated from the separately determined values of these constants. An effective test of the validity of Equations 1 and 2 under these conditions is the comparison of the calculated concentration of cyanate with the experimentally determined value. In alkaline solution, Equation 5 becomes  $dx/dt = k_1 u - k_3 x$ .

If this is combined with Equation 6 and integrated, the relation

$$x = \frac{\alpha k_1}{2(k_3 - k_1)} (e^{-k_1 t} - e^{-k_3 t}) \quad (7)$$

is obtained.

In Table IV,  $x$  has been calculated from Equation 7 for each of the time intervals in a urea hydrolysis and is entered for comparison with the experimental value. The agreement is well within the experimental error. This calculation has been made

TABLE I  
*Urea Hydrolysis at 100°*

$k_1$  was calculated from urea determinations by the xanthydrol method or from  $\text{NH}_3 + \text{cyanate}$  determinations by the Conway method, as indicated. The ionic strength was equal to the NaOH concentration, except in the cases noted.

Concen- tration NaOH	Method	$\mu$	pH	$k_1$	Log $k_1 + 2$	Initial urea concentration
<i>M</i>						<i>M</i>
4.04	Xanthydrol		14.50	0.745	1.872	0.0625
3.24	Conway		14.37	0.534	1.728	0.050
2.83	Xanthydrol		14.29	0.438	1.641	0.040
2.43	Conway		14.21	0.345	1.538	0.025
2.01	Xanthydrol	2.01	14.10	0.313	1.495	0.020
2.02	"	3.02	14.15	0.322	1.508	0.040
2.02	"	4.02	14.20	0.352	1.547	0.040
1.52	Conway		13.98	0.239	1.379	0.025
1.48	"		13.95	0.230	1.362	0.050
1.00	Xanthydrol		13.76	0.206	1.314	0.010
0.750	Conway		13.64	0.185	1.267	0.025
0.732	"		13.63	0.182	1.269	0.050
0.501	Xanthydrol		13.47	0.178	1.251	0.005
0.405	Conway		13.39	0.167	1.223	0.025
0.371	"		13.35	0.168	1.224	0.025
0.252	"		13.20	0.166	1.220	0.025
0.244	Xanthydrol		13.18	0.165	1.218	0.005
0.241	"		13.18	0.170	1.231	0.005
0.240	Conway	1.24	13.18	0.157	1.196	0.025
0.192	"		13.08	0.157	1.197	0.025
0.105	"		12.83	0.155	1.190	0.025
0.0974	Xanthydrol	0.25	12.78	0.154	1.186	0.005
0.0968	"	0.25	12.78	0.150	1.176	0.01
0.0513	"	0.25	12.51	0.150	1.175	0.005
0.0510	"	0.25	12.50	0.153	1.186	0.005
0.0208	Conway		12.19	0.143	1.159	0.025
Acetate	Xanthydrol	0.25	4.01	0.144	1.159	0.005
Citrate	"	0.25	2.39	0.146	1.165	0.01
HCl						
0.0634	Xanthydrol	0.25	1.40	0.142	1.153	0.005
0.105	"	0.25	1.09	0.126	1.102	0.005
0.258	"		0.71	0.120	1.078	0.005
0.510	"		0.41	0.0885	0.947	0.01
1.016	"		0.08	0.0603	0.780	0.02
1.76	"		-0.22	0.0386	0.587	0.04
2.97	"		-0.59	0.0221	0.344	0.0625

TABLE II  
*Urea Hydrolysis at 66°*

All values are recorded as in Table I.

Concentration NaOH	Method	$\mu$	pH	$k_1$	Log $k_1 + 4$	Initial urea concentration
<i>M</i>						<i>M</i>
4.04	Xanthydrol	4.04	14.50	0.0386	2.587	0.0625
3.14	Conway	3.14	14.35	0.0228	2.358	0.025
1.57	"	1.57	13.98	0.00802	1.904	0.025
0.792	"	0.79	13.66	0.00363	1.560	0.025
0.315	"	0.31	13.28	0.00237	1.364	0.025
0.105	"	0.10	12.84	0.00209	1.321	0.025
0.0513	Xanthydrol	0.25	12.50	0.00205	1.311	0.005
0.0102	"	0.25	11.80	0.00189	1.277	0.005
HCl						
0.0105	Xanthydrol	0.25	2.10	0.00213	1.328	0.005
0.0530	"	0.25	1.39	0.00217	1.336	0.005
0.0981	"	0.25	1.13	0.00236	1.373	0.005
0.490	"	0.49	0.43	0.00154	1.187	0.01
1.47	"	1.47	-0.12	0.000934	0.970	0.04
1.69	"	1.69	-0.20	0.000743	0.871	0.04
3.14	"	3.14	-0.74	0.000316	0.500	0.0625

TABLE III  
*Hydrolysis of Potassium Cyanate*

The three values in phosphate buffers were indirectly obtained, as explained in the text.

Temperature	NaOH concentration	$k_3$
°C.	<i>M</i>	
100	1.48	0.170
	0.40	0.175
	0.40	0.177
	0.37	0.178
	0.100	0.179
	0.100	0.182
	0.0968	0.172
	0.25	0.0055
66	Phosphate buffer	
	<i>pH</i>	
	7.55	1.7
	6.95	3.2
100	6.30	13.0

for a number of other experiments at different hydroxyl ion concentrations with equally good results. It can thus be concluded that carbamate or other possible intermediates are not quantitatively significant in determining the course of urea hydrolysis under these conditions.

The treatment of the data obtained between pH 5 and 12 is more difficult. As was previously stated, no urea is formed during the hydrolysis of cyanate in NaOH of a concentration of 0.1 *N* or greater. As the pH is progressively decreased, urea begins to accumulate in the reaction mixtures. This formation of urea is apparent in 0.01 *N* NaOH and increases to a maximum at about

TABLE IV  
*Urea Hydrolysis at 100° in 0.0968 N NaOH*

Urea,  $\text{NH}_3$ , and  $\text{NH}_3$  + cyanate were determined. The observed cyanate was obtained by difference; calculated cyanate was obtained from Equation 7, with  $k_1 = 0.150$  and  $k_2 = 0.172$ . The total nitrogen concentration was calculated from the urea and  $\text{NH}_3$  + cyanate determinations.

Time	Urea	$\text{NH}_3$	$\text{NH}_3$ + cyanate	Observed cyanate	Calculated cyanate	Total nitrogen concentration
<i>hrs.</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>M</i>
0.00	0.0100	0.00	0.00	0.00	0.00	0.0200
1.00	0.00852	0.00153	0.00283	0.00130	0.00123	0.0199
2.00	0.00726	0.00324	0.00532	0.00218	0.00225	0.0199
3.00	0.00631	0.00457	0.00723	0.00266	0.00280	0.0198
4.30	0.00524	0.00605	0.00924	0.00319	0.00321	0.0197
5.75	0.00419	0.00802	0.01131	0.00329	0.00341	0.0197

pH 8. Below this pH the formation of urea again decreases as the rate of hydrolysis of cyanate becomes greater. Since the ionization constant of  $\text{NH}_4^+$  is about  $8.7 \times 10^{-10}$  at 100° (11), this is consistent with the interpretation that only  $\text{NH}_4^+$  participates in the reverse of Equation 1.

At pH values at which the ammonia is partially or completely present as the free base, a fraction of it will be in the small gas phase in the sealed tubes. For this reason and because of uncertainties in the ionization constant of  $\text{NH}_4^+$  at 100° no attempt has been made to account quantitatively for the data in the pH region 8 to 12.

Below pH 7.5 the ammonia is completely ionized and the course of the reaction should be described by Equations 3, 4, and 5. Unfortunately, these equations cannot be integrated as they stand, and a numerical method of solving them must be resorted to. The method adopted was one outlined by Levy and Baggott (12). It is not suited to the estimation of the constants,  $k_1$ ,  $k_2$ , and  $k_3$ . Consequently these must be obtained independently.

The results of calculations on an experiment at pH 6.95 are shown in Fig. 1. The data, which include hydrolyses of both

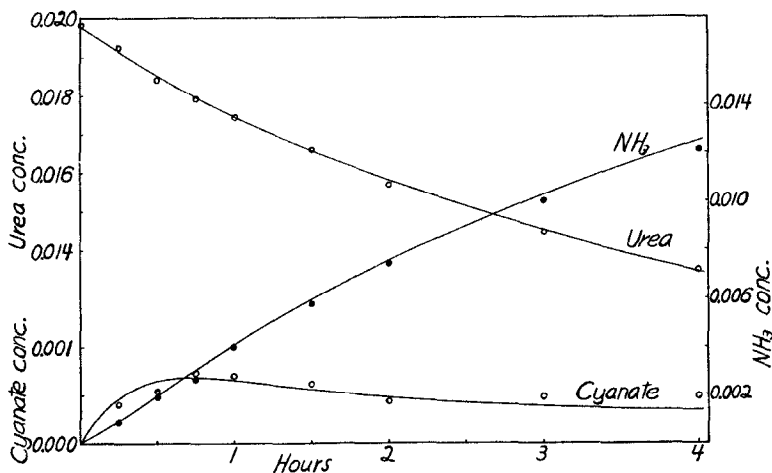


FIG. 1. Hydrolysis of urea in a phosphate buffer, pH 6.95, ionic strength 0.25, at 100°. Concentrations are expressed as moles per liter. See the text for explanation of the curves.

urea and cyanate, are recorded in Table V. The values of the constants were estimated as follows:

From the plot of  $\log k_1$  against pH in Fig. 2, it can be seen that a plausible extrapolation through the neutral region is a straight line parallel to the pH axis. This makes  $k_1 = 0.147$  between pH 2 and 12. Approximate confirmation of this can be obtained by calculating first order constants from the urea data in this experiment for time intervals of less than 1 hour.

A value for  $k_2$  can be obtained from the work of Warner (4, 5). His measurements include determinations of  $k_2$  as a function of ionic strength in the temperature range 30–80°.  $k_2$  was cal-

culated to be 240 at 100° and  $\mu = 0.25$  from the value at 70° and the critical increment over the temperature range studied.

An approximation to  $k_3$  can be obtained from the experiment on the hydrolysis of potassium cyanate. From the data in Table V it can be seen that the concentration of urea formed is

TABLE V

*Hydrolysis of Urea and Cyanate in Phosphate Buffer at pH 6.95 at 100°*

All values are recorded as in the preceding tables.

Ionic strength = 0.25.

Urea hydrolysis					
Time	Urea	NH <sub>3</sub>	NH <sub>3</sub> + cyanate	Cyanate	Total nitrogen concentration
<i>hrs.</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>M</i>
0.00	0.01982	0.00	0.00	0.00	0.0396
0.25	0.01925	0.00088	0.00128	0.00040	0.0398
0.50	0.01840	0.00195	0.00249	0.00054	0.0394
0.75	0.01788	0.00264	0.00338	0.00074	0.0391
1.00	0.01745	0.00400	0.00470	0.00070	0.0396
1.50	0.01662	0.00580	0.00642	0.00062	0.0397
2.00	0.01570	0.00750	0.00794	0.00044	0.0393
3.00	0.01444	0.01003	0.01051	0.00048	0.0394
4.00	0.01345	0.01213	0.01261	0.00048	0.0395

Cyanate hydrolysis				
Time	NH <sub>3</sub>	Urea	$\frac{k_3}{k_2}$	$k_3$
<i>hrs.</i>	<i>M</i>	<i>M</i>		
0.167	0.00229	0.00030	0.01	2.4
0.333	0.00364	0.00062	0.013	3.1
0.667	0.00499	0.00123	0.014	3.4
1.00	0.00540	0.00148	0.014	3.4
1.50	0.00588	0.00151	0.015	3.8

sufficiently small for time intervals up to 1 hour so that its subsequent hydrolysis ( $k_{1u}$  term) can be neglected as a first approximation.

Equation 4 is divided by Equation 5, the  $k_{1u}$  term being neglected, and integrated. After substitution of  $2u + y = a - x$ , the relation

$$u + y = \frac{k_3}{k_2} \ln \frac{k_3/k_2}{k_3/k_2 - y} \quad (8)$$

is obtained.

With the experimental values for  $u$  and  $y$ ,  $k_3/k_2$  was calculated for each of the time intervals, solution being made by approximation. The values obtained are entered in Table V together with those of  $k_3$  calculated by assuming  $k_2 = 240$ .

With these values for the constants, the equations were solved numerically to give  $u$ ,  $x$ , and  $y$  at various time intervals. The

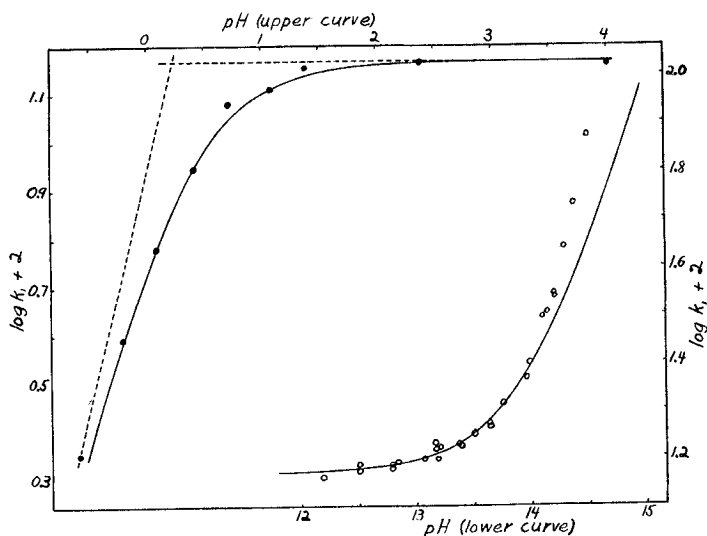


FIG. 2. Hydrolysis of urea as a function of pH at 100°. The dash line curves are the asymptotes approached by the solid line curves. Their intersection indicates the value of pK.

effect of varying  $k_3$  within the limits shown in Table V was tried and  $k_3 = 3.2$  was found to give the best fit with the data. The values obtained were plotted to give the smooth curves in Fig. 1. The experimental determinations for the urea hydrolysis are shown as the discrete points in the graph (Fig. 1).

The deviations of the experimental points for  $\text{NH}_3$  and urea from the calculated curves may be due to the small changes in the pH of the reaction mixtures as the hydrolysis proceeded, or they might be accounted for by further small adjustments in the

values for  $k_3$ . The cyanate determinations are less accurate than the others because of the "difference" method employed in the determination. Equally good agreement was obtained in an experiment at pH 7.55 with the same values for  $k_1$  and  $k_2$  and with  $k_3 = 1.7$ .

The mechanism assumed in Equations 1 and 2 will thus adequately account for the reaction under all conditions of pH.

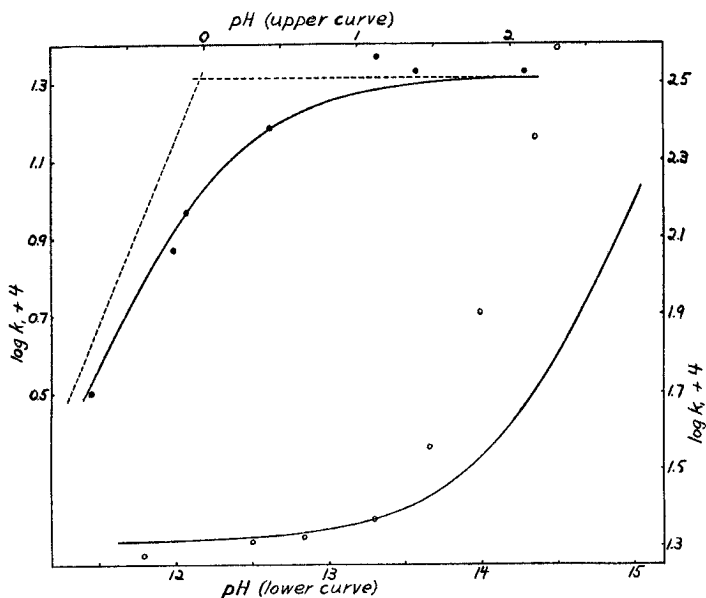


FIG. 3. Hydrolysis of urea as a function of pH at 66°. See legend to Fig. 2 for explanation of the dash line.

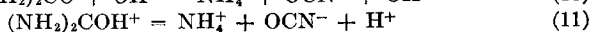
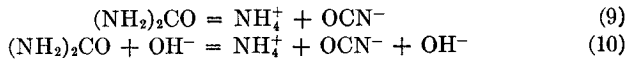
### Reaction Mechanism

Figs. 2 and 3 show a plot of  $\log k_1$  against pH at 100° and 66° respectively. pH was calculated from the concentration of HCl or NaOH and the activity coefficient data of Randall and Young (13) for HCl, and of Harned (14) for NaOH. It was assumed that  $pK_w = 13.93$ . These calculations give the pH at 25°. No attempt has been made to calculate the pH at the experimental temperatures. Any temperature correction for pH will change the shape of the curves only in so far as the activity coefficients change with the temperature. However, there would be a con-

siderable displacement of the alkaline end of the curve on the pH axis if such correction were made.

The curve shows that between pH 2 and 12  $k_1$  is independent of  $(H^+)$ . Below pH 2 and above pH 12, there are inflections in the curve, each of which indicates inverse proportionality of  $k_1$  to  $(H^+)$ . The inflection in acid solution is in the region of the ionization constant of urea. This constant ( $K_w/K_b$ ) was determined by Walker and Wood (15) to be about 0.7 at 25°. The inflection in alkaline solution does not correspond to any known ionization of urea.

The log  $k_1$ -pH curve is consistent with an uncatalyzed reaction (or a water catalysis) between pH 2 and 12. Superimposed on this is a hydroxyl ion catalysis which becomes rate-controlling above pH 13. The drop in log  $k_1$  below pH 2 can be accounted for by assuming a rate of hydrolysis of the urea ion which is negligible in comparison with that of the neutral molecule. These assumptions can be expressed in the following equations.



From these equations, the following expression can be formulated for the observed rate of reactions,  $k_1$ , in terms of the intrinsic rates,  $a$ ,  $b$ , and  $c$ , of Equations 9, 10, and 11, respectively.

$$k_1[(U) + (U^+)] = a(U) + b(U)oh + c(U^+) \quad (12)$$

where  $(U)$  = concentration of the neutral urea molecule

and  $(U^+) =$  " " " " positive urea ion

$oh$  and  $h$  = the activities of hydroxyl and hydrogen ions respectively

$(U)$  and  $(U^+)$  are related by the equation

$$K = \frac{(U)h}{(U^+)} \quad (13)$$

If we combine Equations 12 and 13 and neglect the  $c(U^+)$  term

$$k_1 = a \frac{K}{h + K} + b \frac{K}{h + K} \frac{K_w}{h} \quad (14)$$

In the region where  $b$  is rate-controlling,  $K/(h + K) = 1$  and Equation 14 can be written

$$k_1 = a \frac{K}{h + K} + b \frac{K_w}{h} \quad (15)$$

The constants  $a$  and  $b$  can be derived from the experimental data. Between pH 2 and 12,  $a$  is the rate-controlling constant. In this region  $K/(h + K) = 1$  and  $k_1 = a$ . At 100° the average value for  $k_1$  is 0.147. With this value for  $a$ ,  $b = 0.0826$  was found to give the best fit with the data in alkaline solution. Similarly  $K = 0.58$  was derived from the data in acid solution. The solid lines in Fig. 2 are plotted from the equation

$$k_1 = 0.147 \frac{K}{h + K} + 0.0826 \frac{K_w}{h} \quad (16)$$

where  $K = 0.58$ .

The experimental points show marked deviations from this equation only in strongly alkaline solution in which the observed rate rises more rapidly than would be predicted from the equation. No other mechanism could be found which would give a more satisfactory account of this part of the curve.

The data at 66° were treated similarly and the equation

$$k_1 = 0.00205 \frac{K}{h + K} + 0.00115 \frac{K_w}{h} \quad (17)$$

where  $K = 1.07$  was derived. The solid lines in Fig. 3 are plotted from this equation. The deviations in alkaline solution are more marked in this case than at 100°.

There are several alternate formulations of the reaction mechanism which would result in a dependence of  $k_1$  on pH, formally equivalent to that described by Equation 14. Assumptions such as a reaction between  $U^+$  and  $OH^-$  in acid solution, or the involvement of a hypothetical urea anion in alkaline solution, cannot be excluded. The mechanism suggested above seems to be the most plausible.

#### *Influence of pH on $k_3$*

Table III contains the observed values for  $k_3$  in alkaline solution, together with the values at pH 6.95 and 7.55, which were derived as previously described, and a value at pH 6.30 obtained by the same method.

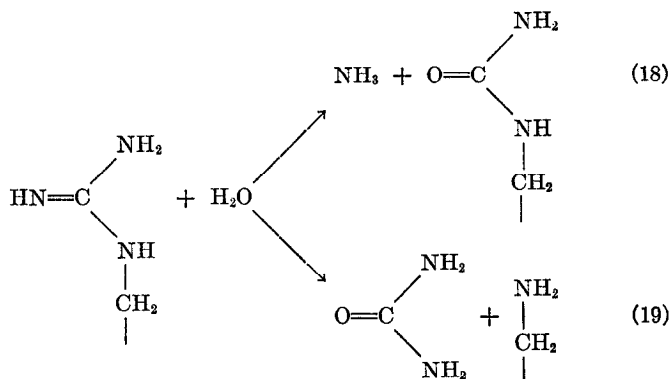
The three constants obtained in buffer solutions show an approximate proportionality to  $(H^+)$ . In NaOH solution,  $k_3$  is independent of pH. These observations, together with those of Levy,<sup>2</sup> at 25° indicate that a hydrogen ion catalysis of the reaction is rate-controlling below about pH 8 and that a water catalysis becomes rate-controlling above this pH. These assumptions lead to an equation of the type  $k_3 = k'h + k''$ , where  $k'$  and  $k''$  are the intrinsic constants for the hydrogen ion and the water catalysis respectively.

There are insufficient data to derive accurate values for the constants in the equation.

### *Hydrolysis of Arginine*

Arginine is known to be stable in acid solution and survives long periods of boiling with strong acid during the hydrolysis of proteins. Concentrated alkali hydrolyzes arginine with the production of 2 equivalents of ammonia. This fact is the basis of the Van Slyke procedure for the estimation of arginine (16).

The course of the reaction has been indicated by the work of Hellerman and Stock (17) who followed the hydrolysis in 0.1 N NaOH at 37°. They measured ammonia and urea and found a constant proportion between the two. Arginine disappeared according to a first order equation. They interpreted these results to mean that either of the C—N bonds in the guanidine part of the molecule could be hydrolyzed. The hydrolysis to form urea occurred at the greater rate. The other products of the reaction must have been ornithine and citrulline. We have



extended this analysis to include the effects of pH and temperature. The methods used were the same as in the urea experiments.

From Hellerman and Stock, it is assumed that arginine can be hydrolyzed in two ways, each reaction following a first order course with respect to arginine. These reactions can be represented by Equations 18 and 19.

Urea, of course, will be further hydrolyzed, but this reaction is sufficiently slow that it can be neglected at temperatures of 66° and below, up to about 50 per cent completion of the arginine hydrolysis. The citrulline will also be hydrolyzed but at a lower rate than urea, as is shown below. It can likewise be neglected.

Equations 18 and 19 can be described as follows:

$$\frac{du}{dt} = k_u z \quad (20)$$

$$\frac{dy}{dt} = k_y z \quad (21)$$

$$\frac{dz}{dt} = -kz \quad (22)$$

where

$z$  = concentration of arginine

$u$  = " " urea

$y$  = " " ammonia

$a$  = original arginine concentration =  $z + u + y$

Equation 22 integrates to give

$$kt = \ln \frac{a}{z} = \ln \frac{a}{a - (u + y)} \quad (23)$$

from which  $k$  can be calculated. If Equation 20 is divided by Equation 21 and integrated,  $u/y = k_u/k_y = m$ .

If Equations 20 and 21 are added and  $(a - z)$  substituted for  $(u + y)$ , it is evident that  $k = k_u + k_y$ . The system can thus be described by the two constants  $k$  and  $m$  and the others can be derived, if desired.

The results obtained at 66° and 35° are assembled in Table VI.

The reaction mixtures were made up from standard NaOH and arginine monohydrochloride. The NaOH concentration given in Table VI was calculated by allowing 1 equivalent for

TABLE VI  
Hydrolysis of Arginine by NaOH at 66° and 35°

Temperature	NaOH	$\mu$	Calculated pH	$k$	Log $k + 2$	Average $m$	Variation in $m$ during experiment	Initial arginine concentration
°C.	$M$							$M$
66	0.100	0.150	12.66	0.0584	0.766	5.65	5.1-6.3	0.05
	0.127	0.177	12.77	0.0665	0.823	5.75	5.0-6.9	0.05
	0.393	0.443	13.32	0.145	1.161	5.57	5.1-6.6	0.05
	1.058	1.108	13.77	0.194	1.288	5.25	4.7-6.8	0.05
	2.35	2.40	14.18	0.205	1.312	5.16	4.8-5.9	0.05
	4.03	4.09	14.49	0.209	1.321	5.5	5.0-6.1	0.05
					Log $k + 3$			
35	0.185	0.224	12.99	0.00455	0.667	6.43	6.3-6.6	0.039
	0.936	0.975	13.71	0.00886	0.948	6.18	5.3-6.8	0.039
	4.00	4.08	14.49	0.00944	0.975	5.46	4.1-6.6	0.078

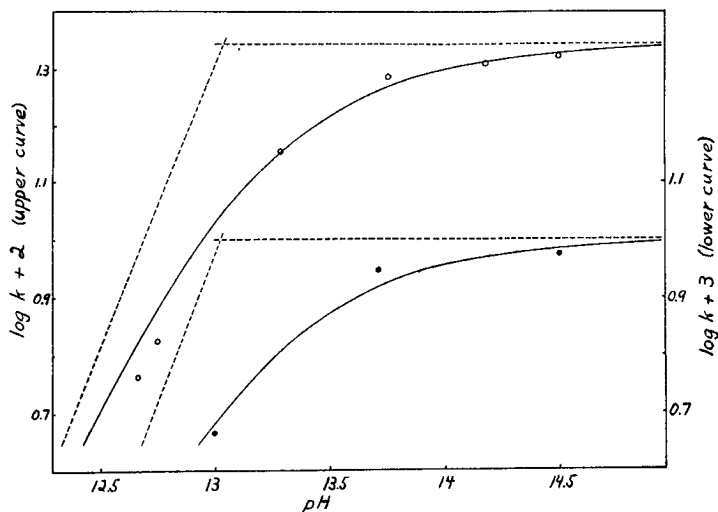


FIG. 4. Hydrolysis of arginine as a function of pH at 100° (upper curve, left-hand ordinates) and at 66° (lower curve, right-hand ordinates). See legend to Fig. 2 for explanation of the dash line.

neutralization of the hydrochloride. The pH of reaction mixtures was calculated approximately by assuming  $pK'_3$  for arginine =

12.5 and by using the data of Harned (14) for the activity coefficient of NaOH. No attempt was made to correct the pH for the change in temperature.

There was some variation in  $m$  as the hydrolysis proceeded, and the limits in this variation in each experiment are shown in Table VI. There are no consistent changes in  $m$  with pH. Consequently, consideration of the effect of pH on the reaction can be limited to  $k$ .

A plot of  $\log k$  against pH is shown in Fig. 4. The inflection in the curve occurs at about pH 13, which is somewhat higher than  $\text{pK}'_3$  for arginine. However, it seems likely that this ionization is responsible for the inflection. No definite decision can be made between alternative possible mechanisms. In view of the stability of arginine at lower pH, the most direct description

TABLE VII  
*Hydrolysis of Citrulline by NaOH at 100° and 66°*

Temperature	NaOH concentration	$k_4$
°C.	$M$	
100	0.124	0.0143
	1.12	0.0180
	3.18	0.0646
66	1.40	0.0011
	3.18	0.0028

of the  $\log k$ -pH curve results from the following assumptions: (a) the rate of hydrolysis of the un-ionized guanidine group is independent of the pH; (b) the rate of hydrolysis of the charged guanidine group is negligible in comparison to (a).

The assumptions can be expressed in Equation 24

$$k = k' \frac{K}{h + K} \quad (24)$$

where  $k'$  is the intrinsic rate constant for the hydrolysis of the guanidine group and  $K$  is the ionization constant for this group.

The best fit with the data is obtained at 66° with  $k' = 0.221$  and  $K = 9.34 \times 10^{-14}$ , and at 35° with  $k' = 0.010$  and the same value for  $K$ . The solid curves in Fig. 4 are plots of Equation 24 with the above constants.

*Hydrolysis of Citrulline*

A few experiments on the hydrolysis of citrulline at 100° and 66° were performed in order to give complete account of the ammonia formed during the hydrolysis of arginine. Citrulline was prepared from arginine by the method of Fox (18). The experiments were carried out as previously.

First order constants for the formation of ammonia in three experiments at 100° and two at 66° were derived from Equation 25,

$$k_4 t = \ln \frac{a}{(\text{citrulline})} = \ln \frac{a}{a - (\text{NH}_3)} \quad (25)$$

At the longer time intervals in all of the experiments, there was some tendency for a decrease in rate below that required for a first order reaction. No urea could be detected in any of the samples. No analyses for cyanate were made, although it seems possible from the structure of citrulline that some would be formed. The data are recorded in Table VII.

The rate of hydrolysis shows some increase at the higher NaOH concentration. The increase is not in direct proportion to the hydroxyl ion concentration. This indicates that there may be an inflection in the log *k*-pH curve that would be evident on a more extended investigation of the reaction.

The low rate of hydrolysis of citrulline provides the reason for the drastic treatment of arginine required by the Van Slyke method of analysis (16).

The author is indebted to Professor R. K. Cannan for his advice and criticism during the course of this work and to Dr. M. Levy for permission to quote from unpublished data.

## SUMMARY

1. Data are presented on the rate of hydrolysis of urea, cyanate, arginine, and citrulline under various conditions of pH and temperature.

2. Urea is hydrolyzed to ammonia and cyanate. This reaction is reversible at pH values at which ammonia is ionized. The reversibility is limited in acid solution by the rapid hydrolysis of cyanate.

3. Cyanate is hydrolyzed to ammonia and carbon dioxide. The

rate of hydrolysis is independent of pH in alkaline solution, but increases rapidly below about pH 9.

4. The assumption of cyanate formation as the only intermediate step in urea hydrolysis quantitatively accounts for the course of the reaction at all pH values.

5. Arginine is hydrolyzed by two simultaneous reactions: one producing ammonia and citrulline; the other, urea and ornithine. Each reaction is of the first order with respect to arginine concentration.

6. The change in the rate of hydrolysis of the above substances with respect to pH is discussed.

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