that is, it increases the velocity of formation of urea, and hastens the attainment of equilibrium.

2. A 1% solution of enzyme will bring equilibrium in a 10 *N* ammonium carbonate-carbamate solution containing about equal amounts of each in about 10 hours at 55°, the optimum temperature for urease. With 0.1% solution of enzyme, the reaction goes many times more slowly, being about 1/3 completed in 98 hours. Without any enzyme, equilibrium would be attained at 55° only after about 600 days.

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[CONTRIBUTION FROM THE LABORATORIES OF THE OHIO STATE UNIVERSITY]

THE ACTION OF UREASE IN THE DECOMPOSITION OF UREA

BY EDWARD MACK AND DONALD S. VILLARS

Received October 5, 1922

A great deal of experimental work has been done and much speculation indulged in regarding the chemical reactions which occur when urea is transformed into ammonia and carbon dioxide. It seems well established that ammonium cyanate is one of the intermediate products. Thus Burrows and Fawsitt[1] give the reaction as

$$CO(NH_2)_2 \rightleftarrows NH_4^+ + OCN^-;\ NH_4^+ + OCN^- + 2H_2O \rightleftarrows 2NH_4^+ + CO_3^=.$$

Werner,[2] however, advances much more convincing arguments for the view that urea exists largely in the form, $H\text{–}N = C\begin{smallmatrix}NH_3\\|\\O\end{smallmatrix}$, and goes through the reactions,

$$H\text{—}N=C\begin{smallmatrix}NH_3\\|\\O\end{smallmatrix} \longrightarrow (H\text{—}N=\overset{+\,NH_3}{CO} + HO.CN) \longrightarrow NH_4CNO \longrightarrow (NH_4)_2\,CO_3.$$

The transformations of urea into ammonium cyanate, and of ammonium cyanate into urea, are the equivalent of the transformations of the two forms of cyanic acid. Ammonia and the keto form give urea. Ammonia and the enol form give ammonium cyanate.

We are interested here in the course of the reaction when the enzyme urease is mixed with the urea solution.

If the enzyme increases the velocity of formation of ammonium cyanate from urea, it should catalyze the reverse reaction. We have submitted this to test. Enough ammonium chloride and potassium cyanate were mixed with water to make a 0.1 *M* solution of each, and urease was added. The potassium chloride formed by double decomposition has only a slight retarding effect[3] on the urease. The experiment was made at 25° and portions of solution were occasionally withdrawn for determination of the

[1] Burrows and Fawsitt, *J. Chem. Soc.*, **105**, 609 (1914).

[2] Werner, *J. Chem. Soc.*, **113**, 83 (1918).

[3] Armstrong and Horton, *Proc. Roy. Soc.*, **85**, 109 (1912).

urea present, by the Fosse method, by which the urea was precipitated as dixanthyl urea.[4]

TABLE I

UREA FROM AMMONIUM CYANATE

Time Hours	Ppt. of dixanthyl urea 0% urease	0.1% urease
0.3	0.0024	0.0000
2.86	0.0101	0.0000
4.1	0.0160	0.0000
8.8	0.0287	0.0001
25.5	0.0699	0.0201
124.1	0.1967	0.0920

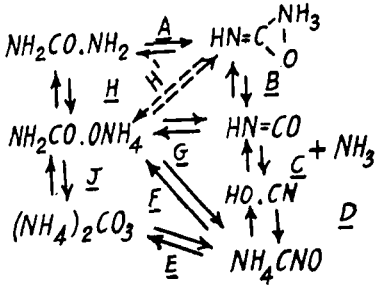
The formation of urea fares much better in the absence of urease than in its presence; in fact, the urease seems to cause the disappearance of any urea which is formed during the first 9 hours, after which, however, urea accumulates in the system, probably due to a slow poisoning of the urease by ammonium cyanate, which finally destroys the activity of the enzyme. Evidently, urea in its decomposition in the presence of urease does not follow the same path that it normally takes, in the absence of urease.

Bayliss and also Werner are inclined to think that the course actually followed is a direct hydrolysis of the urea.

$$NH_2CO.NH_2 \xrightarrow{H_2O} NH_2CO.ONH_4 \xrightarrow{H_2O} (NH_4)_2CO_3.$$

If we represent the chain of reactions diagrammatically, it is possible by making use of the data of various workers in this field, to eliminate finally all of the reactions from consideration except one, and to conclude, in this way, that some particular reaction state is catalyzed by urease.

Starting with urea in the carbamide form, it seems possible that it could be changed to ammonium carbonate by following six different paths according to the diagram, namely, *A B C D E*, *A B C D F J*, *A B G J*, *H G C D E*, *H F E*, and *H J*. Whatever particular stage is affected by



the enzyme, assuming that *only one stage is affected*, must itself be much the slowest reaction in its chain, for the speed of the whole process is

[4] Preceding paper, THIS JOURNAL, **45**, 501 (1923).

regulated by the speed of this one reaction. Furthermore, the velocity of transformation of urea into ammonium carbonate is proportional to the concentration of the urease present.

The process of transformation of urea into ammonium cyanate through the reactions *A B C D* is very slow. According to Burrows and Fawsitt[1] the reaction is so slow that at a temperature of 71.25° an original concentration of urea of 12.05 *M* is reduced to 11.27 only after 2760 minutes, and to 9.00 after 22,920 minutes. Of these four stages, *D*, which is simply the reaction of ammonia with cyanic acid, is very rapid. *A* and *C*, tautomeric transformations, are likewise probably quite fast. This leaves *B*, the decomposition of urea into ammonia and the keto form of cyanic acid, as the slow reaction whose speed regulates the speed of the process *A B C D*. However, *B* cannot be the stage catalyzed by the enzyme: (1) our own experiment just described, shows that the reverse reaction from cyanate to urea is *not* catalyzed; (2) Reactions *E* and *F J* are slower than *B*, as indicated by the work of Walker and Kay,[5] who have shown that at the end of the transformation of ammonium cyanate into urea, 4% was transformed into carbonate at 69°, and somewhat less at lower temperatures; and "special experiments showed that after the transformation of the cyanate had proceeded half way, no carbonate could be detected in the solution on adding calcium nitrate. During the period of half transformation of the cyanate, which is about 46 hours at 69°, no detectable amount of carbonate is formed." This means that *E* is much slower than *B*. Furthermore, Werner has shown that urease does not increase at all the velocity of hydrolysis of potassium cyanate. These arguments prove convincingly that urease does not catalyze any stage of the chain *A B C D*. Stage *E* must also be eliminated (in the chain *A B C D E*) because of *B*, which is much slower than measured velocities of transformation of urea into ammonium carbonate in the presence of urease. *J* is fast, almost instantaneous in acid solutions, quite slow in neutral and alkaline solutions. *F*, if it takes place at all, must be very slow, judging from the Walker and Kay experiment referred to above. *A B C D E* and *A B C D F J* are therefore, both, excluded as possible courses taken by the catalyzed reaction.

Now, *H* is known to be an extremely slow reaction, and since *E* is very slow, the two chains *H F E* and *H G C D E*, which both contain *H* and *E*, are impossible reactions; that is, no matter what stage in these two chains we imagine to be catalyzed by the urease, the speed of the process would still be controlled either by the speed of *H* or of *E*. The experimental fact is that the speed of the process is *controlled by the speed of the catalyzed reaction*. We are reduced then to the possibilities *A B G J* and *H J*.

It has been shown already that *B* is not the catalyzed reaction, and every

[5] Walker and Kay, *J. Chem. Soc.*, 71, 489 (1897).

other stage of *A B G J* is excluded because of the extreme slowness of *B*.

Before deciding whether it is Stage *H* or Stage *J* in the only possible path left, *H J*, we wish to describe a further experiment that we have made. Starting with a solution of urea (0.1 *M*) and 0.1% urease at 25° we have followed the rate of decomposition of the urea by the stage *H J*, by using the Fosse method, and at the same time we have studied the effect of the urease on the simultaneous formation of ammonium cyanate. Armstrong and Horton[3] state that urease prevents the formation of cyanate altogether; but our experiment shows that accompanying the transformation of the urea to ammonium carbonate through the carbamate stage, there is a formation of ammonium cyanate which probably proceeds at its normal rate unaffected by the enzyme.

Fifty-cc. portions of the solution were removed at intervals and treated with silver nitrate solution, which precipitated silver carbonate and, if any, cyanate. The precipitate was separated by filtration, washed and then digested with an excess of 2 *N* nitric acid on a water-bath. The acid drives off carbon dioxide and changes the silver cyanate into cyanic acid which forms ammonia and carbon dioxide in the acid medium: $HCNO + H_2O = NH_3 + CO_2$. The ammonia which was retained by the nitric acid was then determined colorimetrically by Nessler's reagent.

TABLE II

FORMATION OF AMMONIUM CYANATE

1	2	3	4	5	6	7
Time Minutes	Dixanthyl urea	*N* in 50 cc. G. × 10^4	*N* in 1000 cc. G. × 10^4	[*N*] × 10^4	Calc. [CNO] × 10^4 at equilibrium	Obs. [CNO] / Calc. [CNO]
5	0.3504	2.23	44.6	3.18	29.72	0.1071
10	0.3265	2.75	55.0	3.93	28.69	0.1368
20	0.2764	3.46	69.2	4.61	26.40	0.1871
80	0.0598	5.59	111.8	7.98	12.28	0.6499
165	0.0000	3.99	79.8	5.70	00.00	....

The fifth column gives the molar concentration of ammonia in terms of nitrogen. Col. 6 gives the molar concentration of cyanate that would be found if equilibrium were established between it and the urea. This value is calculated from the urea concentrations found from the second column, by using the equilibrium constant of Burrows and Fawsitt.[1] The constant is 0.000106 at 32°, but the change with temperature is so small that we may use this value also for 25°. It will be seen from the last column in the table that equilibrium was being approached, and must have been actually reached at sometime between 80 and 165 minutes; but owing to the final complete decomposition of the urea by the urease present, the reaction by which ammonium cyanate was formed from urea

was *reversed* and the concentration of cyanate began to fall off, as shown by the last number in the fourth column.

Faurholt[6] has investigated the velocity with which ammonium carbamate is transformed into carbonate (Stage *J*). In very weak acid solutions, such as of acetic acid, the transformation is almost instantaneous, 1–2 seconds, the equilibrium mixture containing about 4 times as much carbonate as carbamate. Urease works best at a P_H value of about 7.3 but is quite active in slightly acid solutions. We cannot see, therefore, that the conclusion can be avoided that it is the transformation of urea into ammonium carbamate which is catalyzed by the urease. Bayliss, moreover, has shown that *urease does not affect the J* stage. Yamasaki[7] has also shown that the concentration of carbamate builds up to a maximum during the course of the transformation of urea into ammonium carbonate. The conclusion that it is Stage *H*, or Stage *H'* which is catalyzed by the enzyme, agrees perfectly with the effect which we have described in the preceding paper, of the formation of urea from a mixture of ammonium carbamate and carbonate by the reversible action of urease.

It is well known that the speed of the transformation of urea into ammonium carbonate in the presence of urease is retarded greatly by the presence of hydroxyl ion; for example, the reaction velocity falls off as the ammonium carbonate accumulates in the system, unless there is a buffer mixture present to maintain a practically uniform hydroxyl ion concentration. This retardation is attributed in the literature to the effect of hydroxyl ion on the enzyme. While this may be the true explanation, it should be pointed out that, if we accept the figures of Faurholt, the hydrolysis of ammonium carbamate to carbonate becomes so slow in slightly alkaline solutions, that it is the rate of this reaction (*J*) which regulates the rate of the transformation of the urea in alkaline solution and accounts satisfactorily for the retardation.

Urease is highly specific in its action, and very little if any action on methyl or ethyl urea, either symmetrical or asymmetrical, has been clearly established. Werner advances this as still another argument in favor of the structural formula $HN=C\begin{matrix}NH_2\\|\\O\end{matrix}$, as characteristic of urea. It seems likely that the reaction *H'* rather than *H* is the one catalyzed by the enzyme.

Having narrowed down the possibilities to a single reaction, an attempt at explanation of the mechanism of the enzyme catalysis in this case might seem to be in order. It can hardly be doubted that the effect is one involving the surface of the enzyme particles. One is tempted by the considerable success attending the recent effort made by Kruyt and van

[6] Faurholt, *Z. anorg. allgem. Chem.*, **120**, 85 (1921).

[7] Yamasaki, *Science Rep. Tohoku Imp. Univ.*, **9**, 98 (1920).

Duin[8] to apply the Langmuir-Harkins theory of oriented molecules to the explanation of the catalytic effects of suspended carbon particles on certain chemical reactions, to make use of the same ideas in the case of urea and urease. However, the experiments of Onodera,[9] carried on in Bayliss' laboratory, showing that urease probably has a *co-enzyme*, made up, moreover, of two parts, one dialyzable, the other not, indicate that what at first has seemed a simple reaction, must in reality be rather complicated. There is not yet available enough information regarding this reaction to let us decide what particular bonds of the urea molecule are opened by the enzyme urease.

Summary

1. Experimental data have been presented to show that the transformation of ammonium cyanate to urea is not catalyzed by urease.

2. When urea is hydrolyzed, in the presence of urease, forming ammonium carbamate, which changes into ammonium carbonate, there is a simultaneous formation of ammonium cyanate from the urea.

3. By a process of elimination it is proved that the particular reaction stage catalyzed by urease is the transformation of urea into ammonium carbamate.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE COLLEGE OF LIBERAL ARTS, NORTHWESTERN UNIVERSITY]

ARSENATED BENZOPHENONE AND ITS DERIVATIVES. II[1]

BY W. LEE LEWIS AND H. C. CHEETHAM

Received October 23, 1922

The first paper[2] of this series dealt with the condensation of dichloro-*p*-arsinobenzoyl chloride with aromatic hydrocarbons and phenyl ethers in the presence of anhydrous aluminum chloride. The present paper is a further extension of the application of Friedel and Crafts' reaction, heretofore little used in the preparation of arsenicals, and takes up analogous condensations with dichloro-*o*-arsinobenzoyl chloride. An arsenated ketone, namely *p*-arsono-acetophenone was also prepared from amino-acetophenone, which necessitated a study of available methods of obtaining amino-acetophenone. The ultimate goal of this research was the preparation of a type of arsenical in which the benzoyl group with and

[8] Kruyt and van Duin, *Rec. trav. chim.*, **40**, 249 (1921).

[9] Onodera, *Biochem. J.*, **9**, 575 (1915).

[1] This work was done under a grant from the United States Interdepartmental Social Hygiene Board, Washington, D. C. Certain of the water-soluble compounds have been submitted to Dr. A. S. Loevenhart of the University of Wisconsin for pharmacological study.

[2] Lewis and Cheetham, THIS JOURNAL, **43**, 2117 (1921).