The explanation of these phenomena will be more evident from the data to be presented at a later date in detail on the nature of the heme and porphyrin of the enzyme, which has been suggested in preliminary reports (Schultz *et al.*, 1961; Schultz *et al.*, 1964).

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Decomposition of Carbamylphosphate in Aqueous Solutions*

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Received April 6, 1964

In a detailed investigation of the mechanism of the decomposition of carbamylphosphate, the first-order rate constants for the release of orthophosphate from the monoanion and dianion (occurring predominately between *p*H 2–4 and 6–8, respectively) are similar but different. There is no significant effect of buffer concentration or ionic strength on the decomposition of either of these ionic species. This rules out a general acid or base catalysis and suggests that charge formation is not involved in the rate-determining step. There is also an acid-catalyzed reaction (below *p*H 2) and a base-catalyzed reaction (above *p*H 8). The rate of the base- (and acid- [M. Halmann, A. Lapidot, and D. Samuel, 1962, *J. Chem. Soc.*, 1944]) catalyzed decomposition, in contrast to the mono- and dianion decomposition, is increased significantly by raising the ionic strength of the solution. The pK_1 and pK_2 of carbamylphosphate have been found to be 1.1 and 4.9. A *p*H profile of ^{18}O incorporation into the orthophosphate formed during decomposition of carbamylphosphate in $H_2{}^{18}O$ showed maximal incorporation (92%) at *p*H 3.0, where the monoanion represents 97.5% of the ionic species present, with a symmetrical decrease on raising or lowering the *p*H from 3.0. These data show that P—O bond cleavage occurs with the monoanion, whereas C—O bond cleavage occurs with both the dianion and neutral species. Ammonia release is not first order between *p*H 4 and 6 because the cyanate formed from the decomposition of carbamylphosphate dianion is only slowly hydrolyzed to ammonia. For this reason ammonia release cannot be used to follow the rate of carbamylphosphate decomposition. When carbamylphosphate hydrolysis is carried out between *p*H 4 and 6 in the presence of azide, no increase in rate is observed although carbamylazide accumulates. Since the *p*H curve of carbamylazide formation reflects carbamylphosphate dianion concentration, it has been concluded that azide is trapping cyanate formed from the dianion and not carbamyl cation as suggested by Halman and co-workers (*vide supra*). Our data support different mechanisms for the hydrolysis of the monoanion and dianion of carbamylphosphate involving P—O and C—O bond cleavage, respectively, and suggest that these reactions are monomolecular and may be facilitated by six-membered ring structures.

The importance of carbamylphosphate as a required intermediate for the biosynthesis of arginine, urea, and the pyrimidine ring, as well as its participation in other enzymatic reactions (Jones, 1963) warrants a rather detailed knowledge of its stability in aqueous solutions. Initial studies of the nonenzymatic decomposition of carbamylphosphate in water (Jones and Lipmann, 1960) showed that the rate of the release of orthophos-

* This work was supported in part by grants from the National Science Foundation (GB-885) and from the National Institutes of Health (CA-03658). A preliminary report of this work was presented at the American Chemical Society meetings held in New York City in September, 1963.

† This work will be submitted for partial fulfillment of the requirements for the Ph.D. degree at Brandeis University. Predoctoral Fellow of the National Institutes of Health (1962–64).

‡ Contribution No. 303.

phate varied with *p*H, and that the products of the base- and acid-catalyzed reactions differed.

The acid-catalyzed hydrolysis leads to the formation of ammonia,[1] orthophosphate and, presumably, CO_2.

$$NH_2CO_2PO_3H_2 + H_3O^+ \longrightarrow NH_4^+ + CO_2 + H_3PO_4 \quad (1)$$

The base-catalyzed reaction, however, yields no free ammonia. Titration studies showed that the products of the base-catalyzed decomposition were cyanate and orthophosphate.

$$NH_2CO_2PO_3^{2-} + OH^- \longrightarrow NCO^- + HPO_4^{2-} + H_2O \quad (2)$$

However, between *p*H 2 and 8 the rate of orthophosphate formation was remarkably constant and, in particular, devoid of a marked change in the rate of hydrolysis on going from the monoanion to the dianion region, as has been found for other acylphosphates. In contrast, the rate of formation of ammonia (Jones and Lipmann, 1960) was found to be about 15% of the rate of phosphate release at *p*H 6 and to increase as the *p*H was decreased so that at *p*H 1 the rates of ammonia and orthophosphate release were equal. A constancy of the rate of orthophosphate release with an increase in the rate of ammonia release could be explained in one of two ways: (1) the slow step in carbamylphosphate decomposition (as measured by orthophosphate formation) leads to a "carbamyl product" which is invariant with *p*H but whose subsequent mode of decomposition (as measured by ammonia formation) does vary with *p*H; or (2) the rate of orthophosphate release is fortuitously similar for the monoanion and dianion and their decomposition is proceeding by different mechanisms. The first suggestion has been supported by the paper of Halmann *et al.* (1962); the data of the present paper support the second alternative. We shall attempt to relate the findings of the two studies.

Materials and Methods

Dilithium carbamylphosphate was prepared by the method described by Spector *et al.* (1957). Its purity, 90–92%, was determined by differential phosphate analysis. Solutions practically free of lithium phosphate can be obtained by centrifugation of cold concentrated (0.25 M) solutions of the dilithium carbamylphosphate.

Water enriched with ^{18}O (1.575% ^{18}O) was obtained from the Weizmann Institute, Rehovoth, Israel.

Sodium cyanate was prepared by a method previously described (Scattergood, 1946), recrystallized from water, and stored under vacuum over P_2O_5 to prevent decomposition to ammonia.

Carbamylazide was prepared by reaction of KCNO and NaN_3 (Hantzsch and Vagt, 1901). Potassium cyanate, 8.1 g, was added to 50 ml of a 2 M NaN_3 solution with mixing. This solution was adjusted to *p*H 5.0 with HCl and brought to a total volume of 100 ml. Ethyl ether (300 ml) was added and the stoppered flask was shaken for 24 hours at room temperature. The ether layer, which was separated and dried over Na_2SO_4, was evaporated under vacuum and the residue was recrystallized from ether, mp 96–98°, yield 5.1 g.

All reagents used in the rate studies, azide-trapping studies, and $H_2{}^{18}O$ experiments were of reagent grade unless otherwise stated.

[1] Throughout the paper we have used the term *ammonia* for both the neutral species of this compound and its cation, the ammonium ion.

The ornithine transcarbamylase, which had a specific activity of 27,000 units/mg protein, was prepared from *Streptococcus faecalis* (ATCC 8043) as previously described (Jones, 1962).

Kinetic Data.—For determination of the rates of decomposition of carbamylphosphate as a function of *p*H, each 20-ml reaction mixture contained 0.06 M buffer, 0.001 M dilithium carbamylphosphate, and enough KCl to bring the total ionic strength to 0.6. The buffered mixtures were incubated in a constant temperature bath at 37 or 25° for 15–20 minutes before the addition of carbamylphosphate. After the addition 1-ml samples were taken at various time intervals for a period of about 2 hours.

Products formed during the hydrolysis of carbamylphosphate were measured as follows: (1) Orthophosphate formed was assayed by a modification of the Fiske-Subbarow method (Jones and Spector, 1960). The color formed was measured exactly 5 minutes after the start of the assay instead of the usual 20 minutes, because of the lability of carbamylphosphate in the presence of the reagents of the phosphate-assay mixture.[2] The determination of the color that was produced was made on a Klett-Summerson Model 800-3 photoelectric colorimeter with a 660 filter. The data for determination of first-order rate constants were treated by the method of least squares. (2) For determination of rates of NH_3 release either direct nesslerization or Conway distillation of NH_3 followed by nesslerization was used. (3) To convert carbamylphosphate and cyanate quantitatively to urea, 0.5-ml aliquots of the carbamylphosphate solution (*p*H 5.7, 0.60 M sodium succinate buffer) were added to 0.5 ml of 0.01 M KOH and incubated at 37° for 20 minutes. One ml of 1.0 M NH_4Cl, adjusted to *p*H 8.5, was added and the solution was placed for 5 minutes in a boiling-water bath. The Crokaert method was used for estimation of urea (Crokaert and Schram, 1958). (4) Carbamylphosphate was occasionally determined by its specific enzymatic conversion to citrulline by ornithine transcarbamylase in the presence of ornithine. Concentrations of enzyme used were such that conversion of carbamylphosphate to citrulline was complete in less than 1 minute. A 0.3-ml aliquot of the reaction mixture was incubated with 0.1 M Tris buffer, *p*H 8.5, 0.005 M ornithine, and enzyme at 37° for 5 minutes. A 0.2-ml aliquot of the 1 ml enzyme assay mixture was analyzed for citrulline (Crokaert and Schram, 1958).

$H_2{}^{18}O$ *Experiments.*—The methods for the incubation, isolation of phosphate, and the transfer of the oxygens of KH_2PO_4 to CO_2 were varied and are outlined. Initial experiments were carried out by addition of 44.0 mg of 90–92% pure carbamylphosphate to a solution of 1.0 ml of appropriate buffer and 10.0 ml of $H_2{}^{18}O$. The samples were normally incubated at 37° for 4–5 hours. Initial and final orthophosphate content was determined, as above, to estimate the amount of carbamylphosphate which had decomposed. The *p*H of the solution was determined at the end of the incubation with a Beckman Model G meter. The *p*H was then adjusted to 9 with NH_4OH and the solution was

[2] The Lowry and Lopez (1946) reagents for phosphate give no marked improvement in the stability of carbamylphosphate over the Fiske and Subbarow (1925) reagents, although readings made at 5, 10, and 15 minutes after addition of reducing reagent, with extrapolation to zero time, improve the value for orthophosphate content for either set of reagents. The Mokrasch (1961) method for determining the orthophosphate content of carbamylphosphate solutions improves the stability of carbamylphosphate, but is inapplicable in these studies because of its extreme sensitivity to salts.

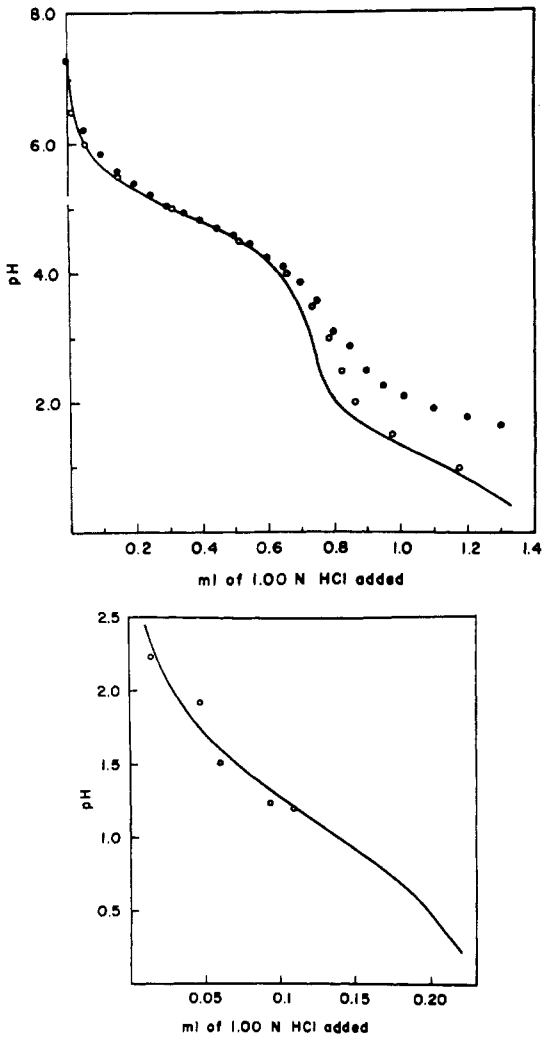


FIG. 1.–Titration curves for dilithium carbamylphosphate. (a) Estimation of pK_1 and pK_2 values. The closed circles represent observed *p*H values for the titration of 12.9 ml of 0.0564 M dilithium carbamylphosphate with standard acid. The open circles represent the titration curve corrected for HCl-LiCl buffer effects and the acid necessary to titrate a 3% initial orthophosphate contamination (see Materials and Methods). The solid line represents the theoretical titration curve for carbamylphosphate assuming $pK_1 = 1.1$ and $pK_2 = 4.9$. (b) Estimation of pK_1 value. The *p*H values found by treatment of 5.0 ml of 0.0499 M dilithium carbamylphosphate solutions with large single additions of acid were corrected for HCl-LiCl buffer effects, as described under Materials and Methods (open circles). The solid line represents the theoretical titration curve for carbamylphosphate ($pK_1 = 1.1$ and $pK_2 = 4.9$).

placed on a thoroughly washed Dowex-1 chloride column. The $H_2{}^{18}O$ was removed and the orthophosphate was eluted and precipitated as described by Jones and Spector (1960) except that the treatment of the phosphate eluate with charcoal was unnecessary. The KH_2PO_4 so obtained was dried and stored until assay over P_2O_5.

In some experiments a Radiometer Type III 1a automatic titrator was used to keep the carbamylphosphate solution at a constant *p*H. In a typical experiment, 44.0 mg of dilithium carbamylphosphate was added to 10 ml of water containing $H_2{}^{18}O$. One ml of dilute HCl solution was selected such that when it was added to the carbamylphosphate solution the initial *p*H was near that desired. Then a 1.1 M HCl solution, which had an ^{18}O content identical with the incubation mixture, was added automatically. After 4–5 hours the procedure described previously was followed for the isolation of the phosphate.

In experiments carried out at 100° the dilithium carbamylphosphate, $H_2{}^{18}O$, and buffer were placed in a pressure flask and incubated at 100° for 40 minutes. Initial and final phosphate concentrations were determined as before and the phosphate was isolated by barium precipitation as described by Halmann *et al.* (1962).

Three methods for the incorporation of oxygen from orthophosphate into CO_2 were used. The one used most regularly was that described by Williams and Hager (1958). A new method by Boyer *et al.* (1961) was also used and worked equally well. The third method is that described by Anbar and Guttmann (1959). The CO_2 formed was collected over liquid N_2 and analyzed in a Consolidated 21–103C mass spectrometer.

Azide Experiments.—In these experiments the total ammonia released was measured by direct nesslerization of aliquots of the incubation mixture. A typical experiment involved the incubation, for a period of approximately 24 hours at 37°, of a 0.001 M solution of carbamylphosphate, NaCNO, or carbamylazide with 0.06 M buffer and 1 M NaN_3, adjusted with HCl to the *p*H of the buffer when necessary. The initial ammonia was determined in each case and subtracted from the total ammonia found on complete decomposition. These differences were compared with the control in which carbamylphosphate was decomposed under similar conditions in the absence of azide. Those samples which were strongly acid were first neutralized with KOH to bring the *p*H to neutrality before nesslerization.

Titration Studies.—Concentrated dilithium carbamylphosphate solutions (0.25 M), nearly free of Li_3PO_4, were prepared as described above. These solutions usually contained no more than 3% inorganic phosphate as measured by differential phosphate analysis. The cold solutions were diluted just before titration to the desired concentration. After a short equilibration of the mixture in the water bath, the carbamylphosphate solutions were titrated with standard acid. A Radiometer No. 4 *p*H meter was used. In studies carried out at room temperature no water bath was used.

In Figure 1a the acid blank used to correct for HCl-LiCl buffer effects in determining pK_1 and pK_2 was obtained by titrating 12.9 ml of a 0.116 M LiCl solution with standard acid. The acid necessary to titrate the initial orthophosphate contamination was calculated over the entire *p*H range from the known pK_1 and pK_2 of orthophosphate. The sum of these two acid blanks was subtracted from the observed curve. In Figure 1b a single quantity of acid was added to 5 ml of 0.0499 M dilithium carbamylphosphate and the *p*H was noted. This procedure was used to reduce the time of titration and thereby avoid hydrolysis of carbamylphosphate. To correct for HCl-LiCl buffer effects the amount of standard acid required to bring 5.0 ml of a 0.0998 M LiCl solution to the *p*H obtained with the experimental carbamylphosphate solution was subtracted from the amount of acid which had been added to the experimental sample. Since the carbamylphosphate solution contained 3% orthophosphate, we have also subtracted the quantity of acid necessary to bring this amount of orthophosphate to the *p*H observed with the experimental carbamylphosphate solution.

RESULTS

A. Titration Experiments.—Titrations were carried out on 0.05 M carbamylphosphate solutions in which the

TABLE I
EXPERIMENTAL pK VALUES FOR CARBAMYLPHOSPHATE OBTAINED BY TITRATION[a]

pK	Temperature
1.0	r.t. (a)
1.0	r.t. (a)
1.0	22.5 (b)
1.2	25 (c)
1.2	25 (c)
5.0	r.t. (d)
4.9	25 (d)
4.9	25 (e)

[a] The determination of pK_1 at room temperature (a) 15 ml of 0.0497 M dilithium carbamylphosphate and 0.75 ml of 1.00 M HCl (pH of mixture = 4.0) were titrated with standard 1.00 M HCl. A correction for the HCl-LiCl buffer effects was made by titrating 15 ml of 0.0994 M LiCl solution with the standard acid. The acid required to bring the LiCl to any given pH was subtracted from the amount of acid required to bring the dilithium carbamylphosphate solution to the same pH value. A similar procedure was followed for those titrations done at 22.5° (b) and 25° (c). For pK_2 determination at room temperature and at 25° (d), the pH of a 0.049 M dilithium carbamylphosphate solution was taken after addition of 0.5 equivalent of standard acid. In addition, the pK_2 at 25° was also determined from a plot of log [salt]/[acid] versus pH (e). Room temperature was 22–25°. Operations were carried out as rapidly as possible, within approximately 10 minutes, to avoid hydrolysis of carbamylphosphate.

concentration of carbamyl- and orthophosphate were accurately determined immediately preceding the titration. The results of the titrations are shown in Table I and in Figure 1a,b. These data indicate pK_1 and pK_2 values of 1.1 and 4.9, respectively.

The observed titration curve (closed circles) shown in Figure 1a has been corrected (open circles) for both HCl-LiCl buffer effects as well as for an initial orthophosphate contamination. The theoretical curve (solid line) obtained by using 1.1 and 4.9 as the pK_1 and pK_2, respectively, for carbamylphosphate is also shown. The corrected experimental points in the pK_2 range agree well with the theoretical curve while those in the pK_1 range do not. The corrected curve can be shifted to approximate closely the theoretical curve if further corrections are made to compensate for the amount of carbamylphosphate that decomposes to orthophosphate during titration.[3] However, a more direct approach is to add a large known quantity of acid to give some pH in the region of pK_1 and then compare this value, after correction for HCl-LiCl buffer effects and initial orthophosphate contamination, with the theoretical value calculated using $pK_1 = 1.1$ and $pK_2 = 4.9$. The data presented in Figure 1b show that in this way pH values found after addition of known quantities of acid closely approximate the pH values theoretically expected and therefore the titration curve in Figure 1a (open circles) in the region of pK_1 is in error due to the hydrolysis of carbamylphosphate during the titration procedure. Previously determined values for the pK values of carbamylphosphate were 5.3 (Jones and Lipmann, 1960), and 5.23 and 2.7 (Halmann *et al.*, 1962). The pK_2 value reported here for carbamylphosphate is similar to the pK_2 value for acetylphosphate (Lipmann and Tuttle, 1944). The pK_1 value is similar to values documented for monoalkylphosphate esters (α-glycerophosphate, 1.37; fructose-6-phosphate, 0.97; glucose-1-phosphate, 1.10; Oesper, 1951). Although it seems that the pK_1 value should be the phosphate dissociation, it should be pointed out that the amido group might have a pK near this pH, since urea has a pK value of 0.18 and acetamide has a value of −0.51 (Hodgman, 1951).

[3] It should be noted that the hydrolysis of carbamylphosphate is not significant at the beginning of the titration (Fig. 1a) and therefore a correction for initial orthophosphate contamination alone accounts for the discrepancy between the observed and theoretical curves.

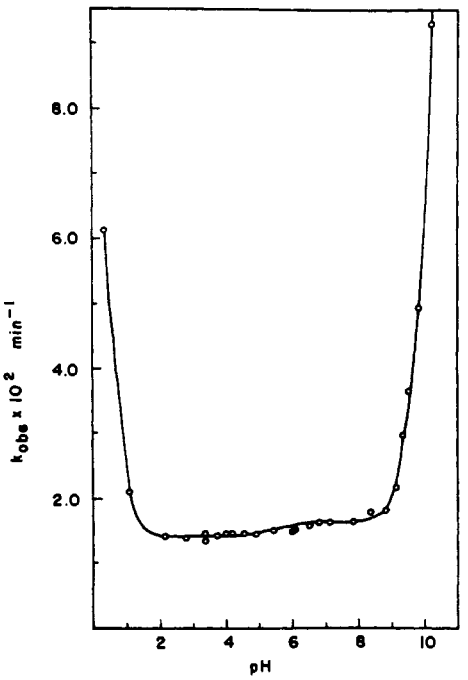


FIG. 2.—The observed first-order rate constants for the hydrolysis of 0.001 M carbamylphosphate solutions at 37° as a function of pH. Buffers were 0.06 M with KCl added to give an ionic strength of 0.6. The specific buffers used are listed in Table II.

TABLE II
THE OBSERVED RATE CONSTANTS FOR THE HYDROLYSIS OF 0.001 M CARBAMYLPHOSPHATE SOLUTION AT 37° IN 0.06 M BUFFER AND IONIC STRENGTH 0.6

Buffer	pH	$k_{obs} \times 10^2$ (min^{-1})	Buffer	pH	$k_{obs} \times 10^2$ (min^{-1})
HCl	0.35	6.14	Succinate	6.00	1.51
HCl	1.10	2.12	Maleate	6.01	1.49
HCl	2.18	1.41	Maleate	6.08	1.52
H_2SO_4	2.79	1.39	Maleate	6.55	1.60
H_2SO_4	2.79	1.39	Maleate	6.84	1.64
Formate	3.38	1.46	Imidazole	7.16	1.65
Formate	3.39	1.45	Tris	7.86	1.65
Formate	3.40	1.37	Tris	8.39	1.81
Formate	3.74	1.42	Borate	8.85	1.84
Succinate	4.00	1.46	Borate	9.16	2.17
Acetate	4.19	1.47	Borate	9.37	3.00
Acetate	4.52	1.46	Carbonate	9.53	3.66
Acetate	4.93	1.47	Carbonate	9.86	4.96
Succinate	5.48	1.53	Carbonate	10.21	9.31

B. Phosphate Release from Carbamylphosphate in Water.—Over the pH range from 0.35 to 10.2 the rates of phosphate release were followed as has been described. The first-order rate constants are presented in Figure 2 and Table II as a function of pH. There are acid- and base-catalyzed reactions below pH 2 and above pH 8.5, respectively. Between pH 2 and 8.5 the rate curve is very nearly flat; however, on close examination, there are two distinct flat regions. The monoanion is the major ionic species between pH 2 and 4 and the rate of its hydrolysis is 1.42×10^{-2} min^{-1}.

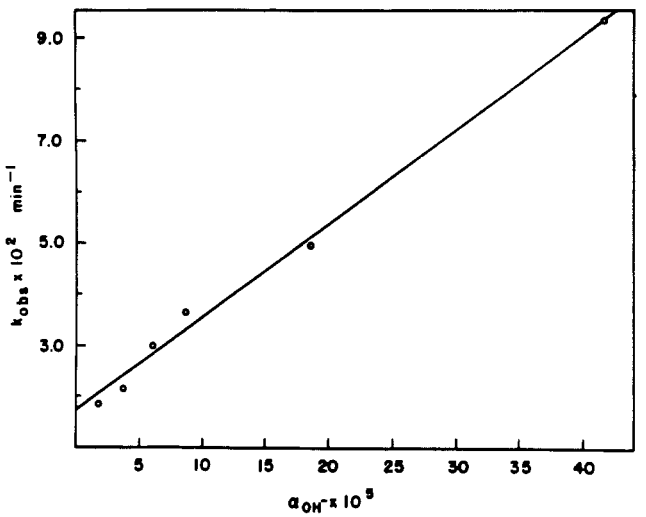


FIG. 3.—Base catalysis of orthophosphate elimination from 0.001 M carbamylphosphate solutions at 37° in 0.06 M buffer at 0.6 ionic strength. Buffers are listed in Table III.

The second flat portion of the curve is between *p*H 6.5 and 8.0, where the dianion exists alone, and the rate of its hydrolysis is 1.64×10^{-2} min^{-1}. This latter figure is supported by the intercept at the ordinate of Figure 3, which is a plot of the first-order rate constants for the base-catalyzed reaction. The intercept should represent the rate of the uncatalyzed release of phosphate from the dianion. This value is 1.70×10^{-2} min^{-1}, which agrees well with the 1.64×10^{-2} min^{-1} obtained from Figure 2.

TABLE III

TEMPERATURE DEPENDENCE OF BASE-CATALYZED ELIMINATION REACTION OF CARBAMYLPHOSPHATE[a]

Temperature (°C)	Buffer	*p*H	$a_{OH^-} \times 10^{5b}$ (M)	$k_{obs} \times 10^2$ min^{-1}
37	Borate	8.85	1.82	1.84
		9.16	3.72	2.17
		9.37	6.03	3.00
	Carbonate	9.53	8.71	3.66
		9.86	18.63	4.96
		10.21	41.70	9.31
25	Carbonate	9.50	31.6×10^{-6}	0.59
		10.18	151.2×10^{-6}	1.95

[a] At 37°: $k = 1.82 \times 10^{+2}$ M^{-1} min^{-1}. At 25°: $k = 1.14 \times 10^{+2}$ M^{-1} min^{-1}. All buffers were 0.06 M with sufficient KCl to give an ionic strength of 0.6. [b] a_{OH^-} was calculated from *p*H = $-\log a_{H^+}$ and $K_w = (a_{H^+})(a_{OH^-})$, where $K_w = 10^{-14.0}$ at 25° and $K_w = 10^{-13.59}$ at 37° (Edsall and Wyman, 1958).

The second-order rate constants for the base-catalyzed reaction at 37 and 25° in the *p*H range 8–10.2 were calculated from data shown in Figure 3 and Table III to be $1.82 \times 10^{+2}$ M^{-1} min^{-1} and $1.14 \times 10^{+2}$ M^{-1} min^{-1}.

There is no general acid or general base catalysis of the hydrolysis of the mono- and the dianion of carbamylphosphate (Halmann *et al.*, 1962, and Table IV) and only a very small increase in rate when the ionic strength of the solution is increased. However, there is a marked increase in the rate of the acid- (Halmann *et al.*, 1962) and base-catalyzed reactions with increased ionic strength. In base, this effect might well be attributed to a decrease in the electrostatic repulsion between the phosphate dianion and the incoming hydroxide ion, which probably aids the elimination reaction by removing a hydrogen from the amide nitrogen.

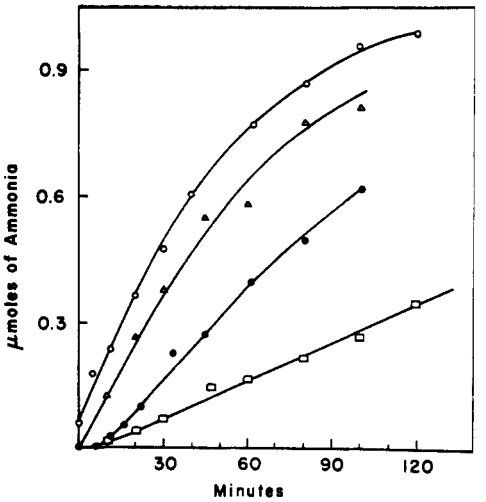


FIG. 4.—Time-dependent release of ammonia from 0.001 M carbamylphosphate solutions buffered at various *p*H values at 37° and ionic strength of 0.6. Open circles are for ammonia release at *p*H 2.28 (chloride buffer); open triangle, *p*H 3.52 (formate); solid circles, *p*H 5.4 (succinate); open squares, *p*H 6.0 (maleate). All buffers were 0.06 M. For all curves, except that at *p*H 2.28, the initial ammonia present in the carbamylphosphate solution was subtracted from the observed values.

TABLE IV

BUFFER AND IONIC STRENGTH EFFECTS ON THE HYDROLYSIS OF CARBAMYLPHOSPHATE AT 37°[a]

Buffer	Molarity	Ionic Strength	*p*H	$k_{obs} \times 10^2$ min^{-1}
Imidazole	0.06	0.6	7.16	1.65
	0.18	0.6	7.00	1.63
Succinate	0.06	0.6	4.00	1.46
	0.18	0.6	3.93	1.46
Imidazole	0.06	0.034	6.87	1.48
	0.06	0.6	7.16	1.65
	0.06	2.0	7.18	1.71
Formate	0.06	0.041	4.26	1.30
Acetate	0.06	0.6	4.19	1.47
Formate	0.06	2.0	4.14	1.58
Carbonate	0.06	0.084	9.75	2.38
	0.06	0.6	9.75	4.50

[a] Where necessary ionic strength was raised by addition of KCl.

C. Ammonia Release from Carbamylphosphate in Water.—The first-order rate constant for ammonia release at *p*H 2.28 is 1.41×10^{-2} min^{-1}, which is identical with the rate of phosphate release at this *p*H. Above *p*H 4 ammonia release does not occur with strict first-order kinetics. Figure 4 illustrates the fact that at *p*H 5.4 and 6.0 there is a lag of 5–10 minutes before the release of ammonia is linear with time. This lag suggests that the release of ammonia at *p*H 5 and 6 occurs, in part, from some other substance than carbamylphosphate. The most likely substance would be cyanate, the product of the base-catalyzed elimination of carbamylphosphate. We therefore tested whether cyanate accumulates under these conditions.

Since there is no *sensitive* color test for cyanate, we have tested for its presence by two indirect methods. Cyanate can be estimated as the difference between the orthophosphate and the base-distillable ammonia formed from carbamylphosphate; or, more directly, one can take advantage of the fact that both cyanate and carbamylphosphate react quantitatively with

TABLE V

*p*H DEPENDENCE OF P—O BOND CLEAVAGE FOR THE HYDROLYSIS OF CARBAMYLPHOSPHATE IN $H_2{}^{18}O$ AT 37°[a]

Experiment	Buffer	Preparation of Isotopic CO_2[b]	*p*H	Atoms (% excess ^{18}O)[c]	P—O Bond Fission (%)
1	HCl	W-H	0.2	0.092	32.4
2	a.t.[d]	W-H	0.92	0.154	54.0
3	HCl	W-H	1.48	0.212	78.7
4	HCl	B	1.48	0.206	76.2
5	HCl[e]	W-H	1.50	0.207	72.3
6	HCl[f]	W-H	1.50	0.222	77.5
7	HCl	W-H	1.50	0.200	70.0
8	HCl	A-G	1.50	0.194	68.0
9	a.t.[d]	W-H	2.0	0.227	80.0
10	a.t.[d]	W-H	3.05	0.255	92.0
11	a.t.[d]	W-H	3.5	0.247	88.8
12	0.6 M acetate	W-H	4.4	0.172	60.2
13	0.21 M acetate	A-G	4.68	0.148[g]	47.0
14	a.t.[d]	W-H	5.2	0.119	43.0
15	a.t.[d]	W-H	6.05	0.056	21.0
16	0.13 M imidazole	W-H	6.8	0.071[h]	15.5
17	0.1 M imidazole	W-H	7.0	0.034	12.5
18	0.1 M imidazole	A-G	7.0	0.022	8.0
19	0.1 M imidazole	A-G	6.91	0.028	9.8
20	0.1 M imidazole	A-G	6.98	0.028	9.5
21	0.1 M imidazole	A-G	6.98	0.013	4.4
22	0.6 M formate[i]	W-H	3.41	0.207	72.3
23	HCl[i]	W-H	1.3	0.226	78.8
24	0.2 M acetate[i]	A-G	4.28	0.240	81.8

[a] Conditions are given under Materials and Methods. The error inherent in the procedure for isolation and analysis of the orthophosphate samples is approximately ±5%. [b] Initials indicate authors of method used for incorporation of ^{18}O from KH_2PO_4 into CO_2: W-H = Williams and Hager (1958); B = Boyer *et al.* (1961); and A-G = Anbar and Guttmann (1959). [c] Reaction solution contained water with 1.244 atom per cent ^{18}O unless otherwise stated. [d] a.t. indicates that an automatic titrator was used to maintain the indicated *p*H by addition of 1.10 M HCl in $H_2{}^{18}O$ of the same atom per cent (1.244) as the reaction solution. [e] Reaction mixture was adjusted to *p*H 1.0, N_2 was bubbled through for 1 hour, *p*H was readjusted to 8.8, and the sample was then placed on a Dowex-1 chloride column to collect the orthophosphate as described under Materials and Methods. [f] Reaction mixture adjusted to *p*H 8.8 with 1 N NaOH when $KHCO_3$, 10 times the number of moles of carbamylphosphate used, was added; the sample was placed on a Dowex-1 chloride column and KH_2PO_4 was isolated as described under Materials and Methods. [g] Reaction solution contained water with 1.325 atom per cent ^{18}O. [h] Reaction solution contained water with 2.39 atom per cent ^{18}O. [i] Hydrolysis performed at 98°.

excess ammonia between *p*H 8 and 9 to yield urea. The difference between urea (cyanate and carbamylphosphate) formed and carbamylphosphate remaining is then a measure of the cyanate present. Figure 5 shows that these two methods for measuring cyanate give identical results. In addition, the curve for the change in cyanate concentration with time is that expected if cyanate were formed as an intermediate between carbamylphosphate and ammonia (Frost and Pearson, 1961). The cyanate concentration reaches a maximum (at about 60 minutes) when its concentration is nearly equal to that of carbamylphosphate, and then cyanate decomposes with the rate less than the rate of carbamylphosphate decomposition.[4]

D. Incorporation of ^{18}O into Orthophosphate Released on Decomposition of Carbamylphosphate in $H_2{}^{18}O$.—It has been reported previously that the ^{18}O incorporation into inorganic phosphate during hydrolysis of carbamylphosphate in $H_2{}^{18}O$ at *p*H 1.0 was about 70% (Jones and Lipmann, 1960). This value could not be substantiated by Halmann *et al.* (1962). We have carried out a more detailed study over a wider *p*H range than first studied and obtained the data presented in Figure 6 and Table V. Figure 6 shows that the ^{18}O incorporation into orthophosphate decreases symmetrically as the *p*H is raised or lowered from 3. This is in good agreement with the change in concentration of the monoanion, indicated by the solid line.

Since these data differ from those of Halmann *et al.* (1962) we felt obliged to make sure that our techniques were not in error. For this reason we compared the Williams and Hager (1958) and Boyer *et al.* (1961) methods for analysis of the ^{18}O content of phosphate (samples 3 and 4, Table V) and the Williams and Hager (1958) and Anbar and Guttmann (1959) methods (samples 5, 6, and 8, Table V). In addition, samples 5 and 6 were handled as indicated in the legend of Table V, to eliminate the possibility that our separation of the phosphate over the Dowex column leads to the retention of $C^{18}O_2$ in the phosphate samples. Finally, as indicated in Table VI, we carried duplicate samples through our standard procedure (method 1), compared this with one of the procedures used by Halmann *et al.* (1962) (method 2), and observed no significant differ-

[4] At *p*H 6 the $t_{1/2}$ value for decomposition of cyanate to ammonia is 345 minutes at 37°, while the $t_{1/2}$ for carbamylphosphate is 45 minutes.

TABLE VI

COMPARISON OF TWO METHODS FOR ISOLATION AND ASSAY OF THE EXTENT OF ^{18}O INCORPORATION INTO ORTHOPHOSPHATE DURING HYDROLYSIS OF CARBAMYLPHOSPHATE IN $H_2{}^{18}O$

Method[a]	*p*H 1.5	*p*H 4.7	*p*H 7.0
1	75[b]	52[c]	14[d]
2	68	47	8

[a] In method 1, phosphate was isolated from reaction solution over Dowex-1 chloride and precipitated as KH_2PO_4. It was converted to CO_2 by the method of Williams and Hager (1958). The error inherent in the procedure is ±5%. In method 2, phosphate was isolated as $Ba_3(PO_4)_2$ and converted to CO_2 by the Anbar and Guttmann (1959) procedure. [b] Average of 4 values, *p*H 1.48–1.50, see Table V. [c] Taken from dashed line of Fig. 6. [d] Average of 2 values, *p*H 6.8 and 7.0, see Table V.

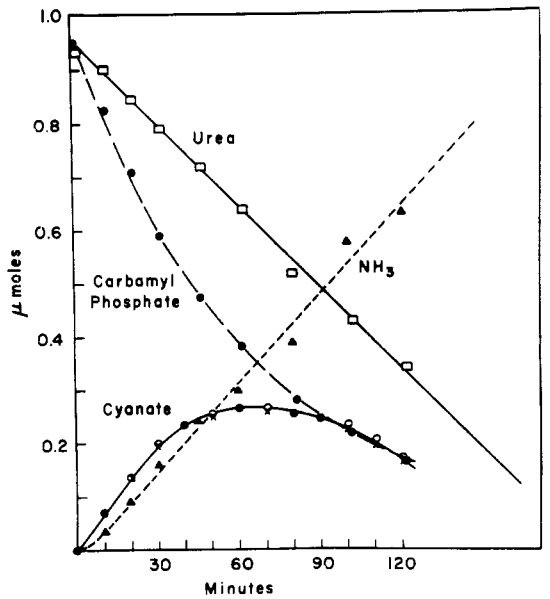


FIG. 5.—Time-dependent release of ammonia and cyanate from 0.001 M carbamylphosphate solution at *p*H 5.74. Carbamylphosphate determined enzymatically as citrulline, closed circles; ammonia, solid triangles; urea (sum of carbamylphosphate and cyanate), open squares; cyanate, determined as difference between carbamylphosphate decomposed and base-distillable ammonia, open circles; and determined by difference between urea and carbamylphosphate, crosses.

TABLE VII

PER CENT TOTAL CARBAMYL NITROGEN RELEASED AS AMMONIA FROM 0.001 M CARBAMYLPHOSPHATE, SODIUM CYANATE, OR CARBAMYLAZIDE SOLUTIONS INCUBATED WITH 1.0 M AZIDE FOR 24 HOURS AT 37° AS A FUNCTION OF *p*H[a]

	Carbamyl Nitrogen (%) Released as Ammonia from:		
*p*H	Carbamyl-phosphate	Cyanate	Carbamyl-azide
1.0	99	32[b]	0
2.68	95		
	98		
2.82		0	
3.33	92	3[c]	3[c]
4.27	63	4[c]	3[c]
5.25	44.7	20	17.5
6.26	83.5	77.5	75

[a] Buffers used were: *p*H 1, NaCl-HCl (sodium from the sodium azide added); 2.68 and 2.82, Na_2SO_4-$NaHSO_4$; 3.3 and 4.27, formate buffer; 5.25 and 6.26, succinate buffer. [b] *p*H 1.33. [c] These values are so close to zero that their significance is questionable.

ence between the two procedures, althouth there is a tendency for method 2 figures to be about 5% lower than those of method 1.

E. Effect of Azide on the Products of Carbamylphosphate Decomposition.—The rate of phosphate release from carbamylphosphate at 37° is not affected by azide (Halmann *et al.*, 1962). We have confirmed this observation and find that the rate of carbamylphosphate decomposition at *p*H 5.3 is 1.47×10^{-2} min^{-1} at 37° in the presence of 0.5 M azide. However, the rate and amount of ammonia released at *p*H 5.3 are markedly decreased by the presence of azide.

Cyanate has been shown to react with azide to yield carbamylazide (Hantzsch and Vagt, 1901). Since cyanate formation occurs (see section *C*) at *p*H 5.7, it seemed that carbamylazide could be formed when carbamylphosphate decomposes at *p*H 5.3 in 0.5 M

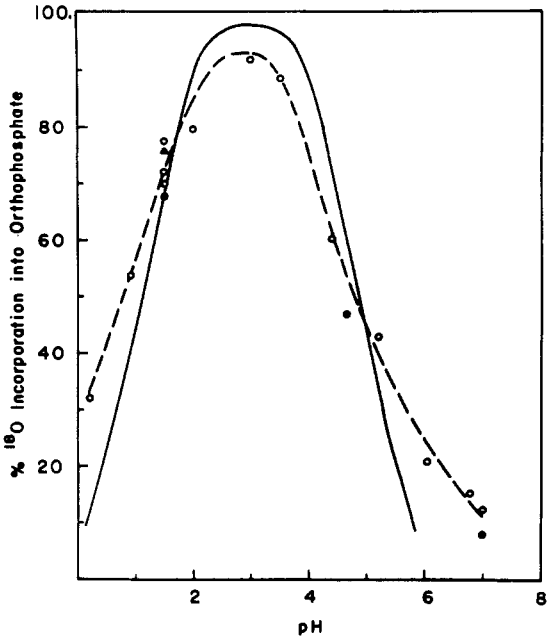


FIG. 6.—Dependence on *p*H of ^{18}O incorporation into orthophosphate during the hydrolysis of carbamylphosphate in $H_2{}^{18}O$ at 37°. The dashed line is a best fit to the experimental values indicated by three types of symbols to designate the method used for analysis of ^{18}O content of the isolated KH_2PO_4 or $Ba_3(PO_4)_2$. The open circles indicate use of the Williams-Hager (1958) method; the solid circles indicate the Anbar-Guttmann (1959) method; and the triangle indicates the Boyer *et al.* (1961) method. The error inherent in the procedure for isolation and analysis of the orthophosphate samples is approximately ±5%. The solid line is a theoretical line for P—O bond cleavage of carbamylphosphate monoanion (pK_1 = 1.1 and pK_2 = 4.9).

azide, thereby reducing the amount and rate of ammonia release. When equimolar concentrations (2 M) of sodium cyanate and sodium azide are incubated together at room temperature for 24 hours (at *p*H 5), the yield of carbamylazide is about 60% of theory. This low yield has been attributed to the fact that there is an equilibrium between product and reactants (Hantzsch and Vagt, 1901). However, if the concentration of azide is high so that it is in large excess over the cyanate present, the conversion of cyanate to carbamylazide is nearly complete (see Table VII). The reaction appears to be very rapid, for, after incubation of 0.001 M cyanate with 0.5 M azide at *p*H 5.1 for 5 minutes at room temperature, no ammonia is formed when the solution is acidified below *p*H 1. Using a high concentration (1 M) of azide to trap the cyanate produced on the decomposition of carbamylphosphate, we have studied the amount of ammonia formed from carbamylphosphate after 24 hours at 37° (Table VII, column 2). As controls, cyanate and carbamylazide solutions were also incubated with azide (Table VII, columns 3 and 4). Carbamylazide is stable from *p*H 1 to 4.27, but its stability decreases so that at *p*H 5.25 and 6.26 it is 18 and 75%, respectively, decomposed. The azide appears to trap cyanate quantitatively from *p*H 6.26 to 2.8, since the ammonia formed from cyanate is nearly identical to that formed from carbamylazide. Although most of the cyanate (70%) is trapped at *p*H 1, it is obvious that azide trapping is now no longer quantitative.

These two controls indicate, therefore, that from *p*H 2.7 to 4.3 cyanate formed on the decomposition of carbamylphosphate would be quantitatively trapped as carbamylazide, which is stable and does not liberate

ammonia. Therefore the ammonia released from carbamylphosphate under these conditions could not have been derived from cyanate. In addition, at *p*H 1 and 5.25 cyanate trapping and carbamylazide stability would fix 70 and 80%, respectively, of the cyanate as carbamylazide. At *p*H 1 (Table VII and Fig. 7) all the nitrogen of carbamylphosphate is released as ammonia so that no cyanate is formed at this *p*H. This will be discussed later. Figure 7 shows that at and above *p*H 2.7 the *p*H profile of the per cent of ammonia released agrees very well with the *p*H profile of P—O bond cleavage except for the point at *p*H 6.2 where carbamylazide is unstable. At *p*H 6.26 the ammonia release is that expected if one sums the amounts of ammonia derived from the carbamic acid and the now unstable carbamylazide derived from cyanate. The ammonia formed between *p*H 2.7 and 5.25 is therefore derived from carbamate formed during the P—O bond cleavage of the carbamylphosphate monoanion.

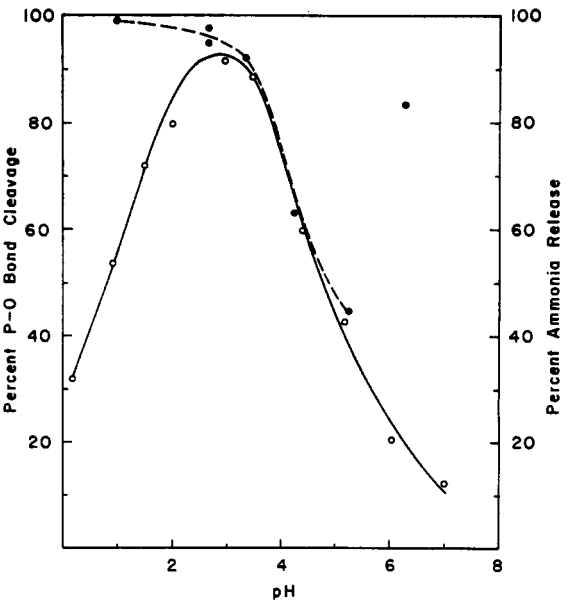


FIG. 7.—A comparison of the per cent total carbamylnitrogen released as ammonia from carbamylphosphate in the presence of 1 M azide and the *p*H dependence of the percentage P—O bond cleavage at 37°. Open circles and solid line represent per cent P—O bond cleavage found on decomposition of carbamylphosphate in $H_2{}^{18}O$ (Table V). Closed circles and dashed line represent per cent of total carbamylnitrogen released as ammonia.

DISCUSSION

Our studies of the hydrolysis of carbamylphosphate were in progress at the time Halmann *et al.* (1962) published their work on this subject. We continued and extended our investigation because some of our data were not in agreement with Halmann *et al.*, and these data led us to different conclusions about the mechanisms involved, particularly with respect to the decomposition of carbamylphosphate monoanion and dianion.

Halmann *et al.* (1962), on the basis of their data, proposed the following $S_{N}1$ mechanism for decomposition of the neutral, monoanionic, and dianionic species of carbamylphosphate shown in equation (3).

$$\begin{aligned} &NH_2CO{-}OPO_3H_2 && PO_4H_2^- \xrightarrow[\text{fast}]{H_3O^+} NH_4^+ + CO_2 + H_3PO_4 \\ &NH_2CO{-}OPO_3H^- \xrightarrow[\text{slow}]{} NH_2CO^+ + PO_4H^{2-} && \xrightarrow[\text{fast}]{H_2O} NH_4^+ + HCO_3^- + H_2PO_4^- \\ &NH_2CO{-}OPO_3^{2-} && PO_4^{3-} \xrightarrow[\text{fast}]{OH^-} NCO^- + H_2O + HPO_4^{2-} \end{aligned} \qquad (3)$$

The essential feature of this mechanism is that the rate-determining step is a dissociation to an invariant carbamyl product (the carbamyl cation) and a phosphate anion. The particular ionic nature of the phosphate-leaving group was presumed by Halmann *et al.* not to influence the rate of the decomposition. After the formation of the carbamyl cation, the second, fast reaction occurs with water, hydronium ion, or hydroxide ion to produce the stable carbamyl end products.

Four categories of data were cited to support the proposed mechanism. (1) The equality of the rates of solvolysis of the neutral, monoanionic, and dianionic species of carbamylphosphate and the fact that this common rate was not influenced by added reagents appeared to Halmann *et al.* (1962) to be compatible with the formation of a common intermediate in the rate-determining step regardless of the state of charge of the phosphate group of carbamylphosphate.[5] (2) The lack of general acid and base catalysis in the hydrolysis tended to eliminate an $S_{N}2$ reaction. (3) The hydrolysis in $H_2{}^{18}O$ from *p*H 14 to 1 N perchloric acid showed predominantly C—O bond cleavage.[6] (4) Carbamylazide was isolated in appreciable quantity at *p*H 4 during the hydrolysis of carbamylphosphate in azide solution. Azide did not change the rate of hydrolysis and therefore it was considered to be an agent capable of trapping the carbamyl cation.

We have confirmed the finding of Halmann *et al.* (1962) that there is no general acid or base catalysis of the decomposition of carbamylphosphate, indicating that an $S_{N}2$ reaction is unlikely. In azide solutions we also observe carbamylazide formation with no change in the rate of carbamylphosphate decomposition. Much of our other data, however, are not in agreement with those of these authors. Our assessment of the data of both laboratories led us to conclude that there is not a unitary mechanism for the uncatalyzed decomposition of carbamylphosphate, but rather that there are two distinct mechanisms that are not independent of the degree of dissociation of the phosphate group of carbamylphosphate but depend on it.

A major disagreement in experimental results between the two laboratories concerns the *pK* values of carbamylphosphate. Halmann *et al.* (1962) give values of 2.7 for pK_1 and 5.23 for pK_2 derived from titration studies not documented in their paper. An earlier estimate of the pK_2 (Jones and Lipmann, 1960) was 5.3, and the titration curve presented at that time indicated that the pK_1 was less than 2. The presented data (Table I and Fig. 1) show that all the previous values require revision; values of 1.1 for pK_1 and 4.9 for pK_2 give the best fit to the experimental data when appropriate corrections are made for the purity of the carbamylphosphate and for the LiCl and HCl present

[5] Ammonia release was studied for the neutral and monoanion species, while cyanate release, determined titrimetrically was studied for the dianion.

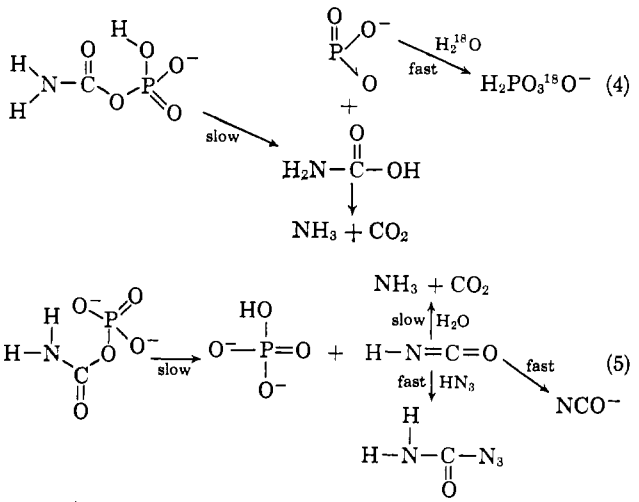
[6] In the range from *p*H 5 to 1 N perchloric acid, approximately 20% P—O bond cleavage was observed. Therefore Halmann *et al.* (1962) concluded that the neutral and monoanion of carbamylphosphate have a mixed hydrolysis: 80% C—O bond cleavage, and 20% P—O bond cleavage.

in the solution. The revision of the pK_2, although significant, is relatively small; however, the change from 2.7 to 1.1 for pK_1 means that the neutral species of carbamylphosphate exists in high concentration only at *p*H values where the decomposition of carbamylphosphate is catalyzed by acid (see Fig. 2). Therefore the hydrolysis of the neutral species is not easily studied. In addition, the *p*H where the monoanion is in maximum concentration, accounting for 97.5% of the total ionic species, is 3, not 4 as assumed by Halmann *et al.* (1962).

The conclusion that separation mechanisms are involved in the decomposition of the mono- and dianion of carbamylphosphate is supported principally by (1) the distinct and different rate constants[7] for the decomposition of carbamylphosphate monoanion and dianion and (2) the fact that the monoanion decomposes with P—O bond cleavage while the dianion (and neutral species) decomposes with C—O bond cleavage.[8] The $H_2{}^{18}O$ studies have been confirmed by studies on the solvolysis of carbamylphosphate in methanol-water solutions where significant quantities (55% methylphosphate at 0.89 mole fraction methanol) of methyl phosphate are found at *p*H values where the monoanion is the predominant species, while the neutral and dianion hydrolysis appear to produce no methylphosphate.

We are proposing on the basis of the evidence presented here as well as some of the evidence presented earlier by Halmann *et al.* (1962) that: (1) The decomposition of carbamylphosphate monoanion (reaction 4) proceeds by a monomolecular elimination of carbamic acid to form monomeric metaphosphate which reacts rapidly with water to yield orthophosphate. Monomeric metaphosphate has been proposed as an intermediate in the hydrolysis of some acylphosphates and alkylphosphates (Di Sabato and Jencks, 1961; Butcher and Westheimer, 1955; Kumamoto and Westheimer, 1955; Vernon, 1957; Bunton *et al.*, 1958). (2) The decomposition of carbamylphosphate dianion very likely proceeds by a monomolecular elimination of cyanic acid to yield phosphate dianion (reaction 5).

It is possible that the decomposition of the monoanion (reaction 4) proceeds, as is the case for other acylphosphates (Di Sabato and Jencks, 1961; Jencks, 1962), by a concerted proton transfer from phosphate to the leaving carbamic acid which then rapidly decomposes to ammonia and carbon dioxide. The mechanism is analogous to that proposed for the monoanion of acetylphosphate (Di Sabato and Jencks, 1961), where similar and more extensive data support this mechanism. This mechanism is also consistent with the observation of ^{18}O incorporation into orthophosphate from $H_2{}^{18}O$, indicating P–O bond cleavage. With the decomposition of acetylphosphate monoanion, ^{18}O incorporation also occurs into orthophosphate to the extent of 89% at *p*H 3.8 (Park and Koshland, 1958). In addition, the rate of hydrolysis of acetylphosphate monoanion (1.1×10^{-2} min^{-1} at 39°; Koshland, 1952) is remarkably similar to that of carbamylphosphate monoanion (1.42×10^{-2} min^{-1} at 37°).



There is no direct evidence to rule out a direct attack of water on the carbamylphosphate monoanion. However, the small entropy of activation (−4.3 entropy units) and the relatively small decrease in the rate of solvolysis in D_2O (k_H/k_D = 1.1 at *p*H 3.4)[9] observed by Halmann *et al.* (1962), as well as the absence of significant salt effects, are compatible with the monomolecular mechanism suggested above.

It is possible that the decomposition of the dianion of carbamylphosphate is also facilitated by a proton transfer, in this case, from the amide nitrogen to the phosphate group to eliminate cyanate and to form orthophosphate dianion. The small negative entropy of −4.2 eu (Halmann *et al.*, 1962) is consistent with the proposed restriction of rotation of the ring structure, and the lack of a deuterium isotope effect in the reaction is not inconsistent with this type of mechanism (Jencks and Carriuolo, 1960). This mechanism is also consistent with the absence of ^{18}O incorporation into orthophosphate from $H_2{}^{18}O$ and the absence of salt effects on the elimination reaction.

Depending on the *p*H of the solution, the cyanic acid formed is (1) ionized to yield the stable cyanate ion, or (2) hydrolyzed to yield carbamic acid, which in turn decomposes to ammonia and carbon dioxide. Our data have shown that ammonia release cannot be used to follow carbamylphosphate decomposition above *p*H 3, since the ammonia released above this *p*H is formed from the decomposition of both carbamic acid and cyanic acid and the rate of the latter is very *p*H dependent above *p*H 4. The formation of carbamylazide observed by Halmann *et al.* (1962) and interpreted by

[7] For the determination of the rate constants we have measured orthophosphate release, a procedure criticized by Halmann *et al.* (1962) since the decomposition of carbamylphosphate is catalyzed by molybdate. It is true that care must be exercised with the phosphate analysis; however, the rate obtained by phosphate analysis for the monoanion is validated by identical values with ammonia release at *p*H 2.3. The dianion rate is confirmed by the intercept on the ordinate of a plot of first-order rate constants for the base-catalyzed hydrolysis against the activity of hydroxide ion.

[8] We have no explanation for the discrepancy in the ^{18}O-incorporation results of the two laboratories, but have carried out experiments to show that when we use the same isolation and assay procedure as Halmann *et al.* (1962) we still observe only P—O bond cleavage for the monoanion and C—O bond cleavage for the dianion and neutral species.

[9] Halmann *et al.* (1962) give a k_H/k_D value of 1.34 for the monoanion. This value comes from studies on the rate of ammonia release between *p*H 3.9 and 4.6, where, from the pK values assumed by these authors, the monoanion was thought to be the predominant species. Our pK values indicate that at *p*H 3.9 the carbamylphosphate present is 91% monoanion and 9% dianion; at *p*H 4.6 it is 66% monoanion and 34% dianion. In addition, the dianion yields cyanate which subsequently decomposes to ammonia by a reaction with H_2O or D_2O. We feel, therefore, that the step showing a rate change in D_2O is the cyanate hydrolysis to ammonia. Confirming this interpretation are the data of Halmann *et al.* (1962) at *p*H 2 and 3.4 where the monoanion represents 89 and 96.5%, respectively, of the ionic species of carbamylphosphate and the k_H/k_D ratio is 1.05 and 1.11, respectively.

them to be due to a trapping of carbamyl cation appears from our data to be a reaction between cyanic acid and azide or hydrazoic acid. This conclusion is based on *p*H profiles of (1) carbamylazide stability, (2) the ability of azide to trap cyanate and cyanic acid, and (3) the extent of carbamylazide formation from carbamylphosphate. Except where carbamylazide is unstable (*p*H 6.2), the *p*H curve for the extent of carbamylazide formation from carbamylphosphate reflects quantitatively the amount of carbamylphosphate dianion present and follows precisely the curve for the change from C—O bond cleavage for the dianion to P—O cleavage for the monoanion.

Preliminary data (Lipmann and Jones, 1960) on the synthesis of carbamylphosphate from cyanate and orthophosphate indicated that the reaction (reaction 6) is (1) an equilibrium reaction ($K_e = 3$) and (2) a reaction between cyanic acid and orthophosphate dianion (or cyanate and orthophosphate monoanion).

$$HNCO + HOPO_3^{2-} \rightleftharpoons NH_2CO_2PO_3^{2-} \quad (6)$$

One might ask, therefore, why dianion hydrolysis under the present conditions goes to completion. It is (1) because at low concentrations of carbamylphosphate (10^{-3} M) the equilibrium concentration of carbamylphosphate cannot be greater than 10^{-5} M, a quantity not measurable in the assay system used, (2) because of the limited stability of cyanic acid between *p*H 4 and 6, and (3) because of the insignificant synthesis of carbamylphosphate from cyanate ion and orthophosphate dianion.

We have not yet attempted to study the rate of hydrolysis of the neutral species. The difficulties will be considerable since with a pK_1 value of 1.1, meticulous differentiation between the acid-catalyzed reaction and that of the neutral species will be required. The $H_2{}^{18}O$ studies show that hydrolysis of the neutral species occurs with C—O bond cleavage. The azide experiments at *p*H 1 rule out cyanate as a product since carbamylazide was not formed even though cyanic acid is trapped to the extent of 70% at this *p*H in 1 M azide. It is not possible with the limited data available to suggest either carbamic acid or the carbamyl cation as the carbamyl product of the decomposition of the neutral species.[10]

The unusual feature of the decomposition of carbamylphosphate is that the decomposition of the dianion occurs with C—O bond cleavage rather than P—O bond cleavage (Jencks, 1962). This different mode of decomposition is made possible by the ease of removal of a hydrogen from the amide group (not present in other acylphosphates) leading to the elimination of cyanate. Theoretically, either the monoanion or dianion could carry out the abstraction of the amide hydrogen, since they each have at least one ionized oxygen in the phosphate group. The fact that only the dianion abstracts a hydrogen from the amide group suggests that the ionized oxygen of the monoanion phosphate group is not sufficiently basic to do so.

It has been observed that substitution of electron-withdrawing groups for one of the acetylhydrogen atoms of acetylphosphate greatly increased the ability of the acetate to depart (Marcus and Elliot, 1958; Jencks, 1962). Such substitution increased the ratio of the rate of the dianion-to-monoanion hydrolysis by a factor of 20. It might be of interest to see if there is an effect of substitution with either electron-withdrawing or electron-donating groups for a single hydrogen of the amide group of carbamylphosphate on the *rate of decomposition* and the *position of bond cleavage* of the monoanion and dianion.

[10] Carbamic acid formation would require the participation of water, but carbamyl cation formation would occur by a monomolecular decomposition. Either product would probably yield ammonia nearly instantaneously even in the presence of azide, for azide trapping of the carbamyl cation at *p*H 2 or below would probably be very inefficient due to the *p*H-dependent reduction of azide-ion concentration and also because low yields have been observed for trapping of dimethyl carbamyl cation as dimethyl carbamylazide in water solution (Hall and Lueck, 1963).

Acknowledgments

The authors thank Miss Gloria F. Deutsch for the preparation of the ornithine transcarbamylase. We are extremely grateful to the Harvard University Chemistry Department and to Dr. G. O. Dudek for their cooperation and aid in the use of the mass spectrometer. Dr. William P. Jencks has offered constructive suggestions and criticism of our results and we are very grateful for his advice. Dr. William Allison carried out some initial studies on the decomposition of carbamylphosphate in $H_2{}^{18}O$ (Jones and Lipmann, 1960).

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