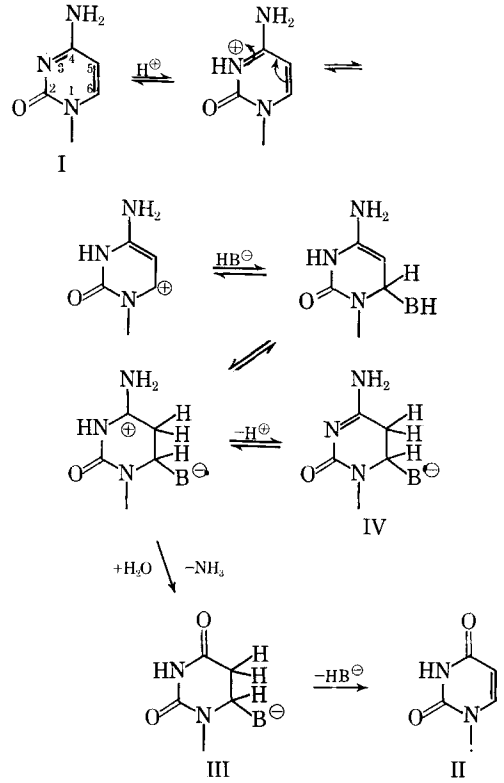
A Mechanism for the Hydrolytic Deamination of Cytosine Arabinoside in Aqueous Buffer

By ROBERT E. NOTARI*

The kinetics of hydrolytic deamination of cytosine arabinoside have been studied as a function of pH, temperature, and buffer composition. The reaction components were isolated, identified, and quantified. The sole catalytic buffer species in the phosphate system is $H_2PO_4^-$. The rate of reaction between $NaHSO_3$ and cytosine arabinoside has been studied and the resulting products characterized. A mechanism for the hydrolytic deamination of cytosine nucleosides, catalyzed by hydrogen ion and general acid-base, HB^-, is hypothesized. This mechanism is shown to be consistent with existing literature data as well as the kinetic and $NaHSO_3$ reaction data reported here.

THE ROLE which chemical mutagens play in the deamination of cytosine nucleosides (I) to uracil derivatives (II) has been the subject of several investigations directed toward elucidation of *in vivo* mechanisms of mutagenic activity (1–5). Recently, Shapiro and Klein (5) have proposed two kinetically equivalent mechanisms for the deamination of cytidine (or cytosine) in aqueous buffers of pH 3 to 6. Both mechanisms combine specific hydrogen-ion catalysis with general base catalysis by buffer anion, B^-. However, the catalytic interaction by buffer was not unequivocally defined. It was hypothesized that an anion, B^-, increased deamination rate either by (*a*) removal of a proton from water or (*b*) partial saturation of the 5,6-double bond. Isolation of the components in reaction mixtures revealed only cytidine and uridine (or cytosine and uracil) and did not, therefore, differentiate between the two alternate routes.

The present investigation lends support to the proposed hydrogen-ion catalysis and eliminates the possiblility that proton exchange between anion, B^-, and water is the mechanism for general base catalysis. Evidence presented here demonstrates that general acid-base catalysis results primarily from complete saturation of the 5,6-double bond by a nucleophile, HB^-, and that catalysis by other buffer species such as B^-, B^{2-}, or B^{3-} is either absent or relatively unimportant. The prime criteria for catalysis by a nucleophile such as HB^-, are shown to be (*a*) sufficient nucleophilicity to attack the 6-position following the initial protonation at the 3-position and (*b*) the ability to subsequently donate a proton resulting in the complete saturation of the 5,6-double bond (Scheme I).



$HB^\ominus$ = $HSO_3^\ominus$; $H_2PO_4^\ominus$; H_2 citrate$^\ominus$; H oxalate$^\ominus$; H succinate$^\ominus$

Scheme I

Received December 27, 1966, from the College of Pharmacy, Ohio State University, Columbus, OH 43210

Accepted for publication March 7, 1967.

This investigation was conducted while the author served as the 1966 Summer Professor, Product Research and Development, The Upjohn Co., Kalamazoo, Mich.

The author wishes to acknowledge the fine technical assistance of Mr. Carl Zipple, who perfected and performed the analyses. Appreciation for helpful comments on the manuscript is expressed to Drs. W. J. Wechter and R. C. Kelley.

The name for cytosine arabinoside approved by USAN is cytarabine.

* Present address: 1627 Farr St., Scranton, PA 18504

EXPERIMENTAL

Quantitative Analysis of Cytosine Arabinoside and Uracil Arabinoside—Thin-layer plates, 20 × 20 cm., were coated with a 3:1 mixture of Silica Gel G and Silica Gel GF 254. After activating for 1 hr. at 105°, they were scored to form nine 1-cm. channels and washed with benzene–propanol (1:1). Reaction aliquots of 25 μl. were spotted, dried, and then developed with water-saturated butanol–propanol (3:1) for about 2.5 hr. Chromatograms were air-dried for 1 hr. and photographed under 254 mμ ultraviolet light.

The appropriate spot was then scraped from the plate and eluted with five 3-ml. aliquots of 0.01 *M* H_2SO_4 in ethanol. The pooled extract was brought to 50 ml. and its ultraviolet absorbance determined against a similarly prepared blank. A primary standard was run on each plate and the standard absorbance used to calculate the amount of the unknown. Recovery was generally 95% with excellent reproducibility. The wavelength of maximum absorption under these conditions is 285 mμ for cytosine arabinoside and 263 mμ for uracil arabinoside.

Kinetics of Cytosine Arabinoside Loss—Reactions were prepared with "Water for Injection" which had been boiled and cooled under nitrogen. The solutions were filtered under nitrogen through a Millipore filter (0.045) then dispensed into 5-ml. ampuls and sealed. Ampuls were placed in appropriate constant-temperature baths and analyzed as a function of time as described above. (See Tables I and II for experimental conditions.)

Preparation of Cytosine Arabinoside-Bisulfite Reaction Products—An aqueous solution containing 0.2 *M* cytosine arabinoside and 2.0 *M* $NaHSO_3$ was allowed to react for 85 hr. at 70°. The reaction was considered complete when a 254 mμ ultraviolet light no longer revealed a cytosine arabinoside spot on a thin-layer chromatogram. A similar solution was prepared with uracil arabinoside and treated in the same manner.

Characterization of the Components in Reaction Mixtures—Products resulting from the reaction of bisulfite with cytosine arabinoside or from the deamination of cytosine arabinoside were compared to standards on thin-layer chromatograms. The standards were: cytosine arabinoside, uracil arabinoside, cytosine, uracil, and D-arabinose. The following solvents were employed with silica gel support: *A*, butanol–propanol (3:1) saturated with water; *B*, 2-propanol–ammonia–water (7:1:2); *C*, chloroform–propanol (3:1); *D*, ethyl acetate–acetic acid–

TABLE I—EXPERIMENTAL CONDITIONS AND APPARENT FIRST-ORDER RATE CONSTANTS FOR THE DEAMINATION OF CYTOSINE ARABINOSIDE[a]

°C.	Observed pH	Buffer Compn. [$NaH_2PO_4 \cdot H_2O$]	Buffer Compn. [Na_2HPO_4]	Buffer Compn. [NaCl]	10^3k (hr.$^{-1}$)
70	6.15 ± 0.15	0.120	0.012	0.000	3.11
		0.048	0.0048	0.094	1.71
		0.024	0.0012	0.125	1.18
		0.000	0.000	0.153	0.705[b]
	6.90 ± 0.10	0.040	0.040	0.000	1.13
		0.029	0.029	0.043	0.872[c]
		0.016	0.016	0.092	0.619
		0.000	0.000	0.153	0.292[b]
	7.81 ± 0.05	0.0055	0.055	0.000	0.504
		0.0033	0.033	0.063	0.470
		0.0022	0.022	0.094	0.417
		0.000	0.000	0.153	0.379[b]
60	6.90 ± 0.10	0.029	0.029	0.043	0.390[c]
55	6.90 ± 0.10	0.029	0.029	0.043	0.273[c]
47	6.90 ± 0.10	0.029	0.029	0.043	0.153[c]

[a] The initial concentration of cytosine arabinoside was 4 × 10^{-2} *M*. All solutions contained the antimicrobial agent, benzyl alcohol (8.8 × 10^{-2} *M*) and had the ionic strength adjusted to 0.153. [b] Calculated from the intercept of the plot $k_{obsd.}$ *vs.* $NaH_2PO_4 \cdot H_2O$. (See Fig. 4.) [c] The heat of activation (ΔHa in Kcal./mole) for this system is 16.4. (See Fig. 5.)

TABLE II—MATERIAL BALANCE BASED ON ANALYSIS OF CYTOSINE ARABINOSIDE (CA) AND URACIL ARABINOSIDE (UA) PRESENT AFTER 425 hr., pH 6.9, 0.06 *M* PHOSPHATE BUFFER, μ 0.15, AND VARYING $NaHSO_3$ CONCENTRATION

°C.	Sample[a]	Total Initial moles	% Total as CA	% Total as UA	% Total Recovered	% Unacct. for
70	*A*	0.0402	68.7	22.2	90.9	− 9.1
	B	0.0410	64.6	20.6	85.2	−14.8
	C	0.0407	63.1	20.8	83.9	−16.1
	D	0.0406	56.2	14.1	70.3	−29.7
	E	0.0405	34.3	0.0	34.3	−65.7
60	*A*	0.0402	84.8	9.5	94.3	− 5.7
55	*A*	0.0402	91.2	9.2	100.4	+ 0.4
47	*A*	0.0402	94.8	3.2	98.0	− 2.0
	B	0.0410	88.3	4.1	92.4	− 7.6
	C	0.0407	92.6	3.7	96.3	− 3.7
	D	0.0406	74.1	3.2	77.3	−22.7
	E	0.0405	51.4	0.0	51.4	−48.6

[a] [$NaHSO_3$]: *A*, 0.000; *B*, 0.004; *C*, 0.010; *D*, 0.020; *E*, 0.040.

ethanol (23:1:1); *E*, butanol–water (86:14). One additional system, *F*, employed aluminum oxide gel and 2-propanol–ammonia–water (7:1:2). Both supports contained phosphors.

RESULTS

Effect of $NaHSO_3$ on Kinetics of Cytosine Arabinoside Loss—The effect of $NaHSO_3$ on the rate of loss of cytosine arabinoside in aqueous phosphate buffer, pH 6.9, 70°, $\mu = 0.15$, is illustrated in Fig. 1. The relative order of the rates was the same at 40°. The 0.01 *M* $NaHSO_3$ solution contained the calculated amount of bisulfite necessary to react with the oxygen content in the headspace of a sealed ampul. Thus, there is an increased rate of cytosine arabinoside loss with increased [$NaHSO_3$] and a pronounced increase in rate when [$NaHSO_3$] exceeds that required for the atmospheric oxygen.

Rate of loss of cytosine arabinoside was found to be first order in the absence of $NaHSO_3$ (Fig. 2). Reactions were not first order in the presence of $NaHSO_3$. The change in order was attributed to $NaHSO_3$ reacting with cytosine arabinoside. (An earlier study demonstrated a loss of $NaHSO_3$ in excess of that consumed by the headspace oxygen.) Assuming a 1:1 reaction permits calculation of the time required for the oxygen and reactant to completely expend the $NaHSO_3$. It can be seen in Fig. 2 that first-order plots become both linear and parallel after the $NaHSO_3$ is depleted.

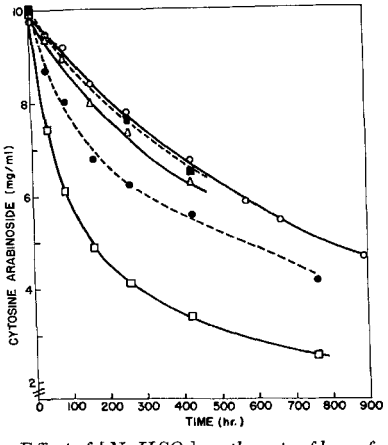


Fig. 1—Effect of [$NaHSO_3$] on the rate of loss of cytosine arabinoside in 0.06 M *phosphate buffer, pH 6.9, 70°, $\mu = 0.15$. Key:* ○, A (*0.000*); ■, B (*0.004*); △, C (*0.010*); ●, D (*0.020*); □, E (*0.040*).

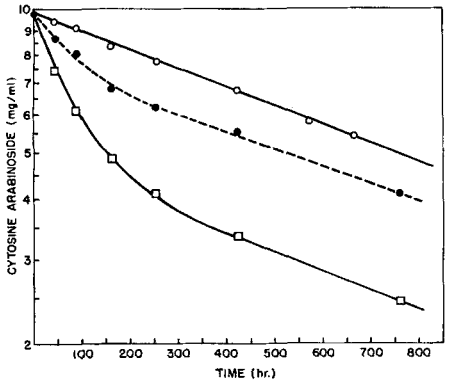


Fig. 2—Effect of [$NaHSO_3$] on the first-order plots for cytosine arabinoside loss in 0.06 M *phosphate buffer, pH 6.9, 70°, $\mu = 0.15$. Key:* ○, A (*0.000*); ●, D (*0.020*); □, E (*0.040*).

A material balance based on the concentration of cytosine arabinoside and uracil arabinoside in several reaction mixtures at 70°, 425 hr., gives further support to the existence of an additional bisulfite reaction product (Table II). It is obvious, from these results, that another reaction product must exist and that its yield increases with increased $NaHSO_3$ concentration. For example, 66% of the initial cytosine arabinoside was converted to the alternate product in the presence of 0.04 *M* $NaHSO_3$ at 70°. In the absence of bisulfite all of the material was accounted for within the limit of experimental error; no other product was formed.

Thin-layer chromatograms, which were viewed under a 254 mμ ultraviolet light, revealed only two spots: cytosine arabinoside and uracil arabinoside (R_f values 0.42 and 0.70 using solvent system *A*). The preservative, benzyl alcohol, appeared as a spot near the solvent front and will be ignored for the purpose of this discussion. Charring with H_2SO_4 and heat revealed a third spot just above the origin. An aqueous solution containing 0.2 *M* cytosine arabinoside and 2.0 *M* $NaHSO_3$ produced no cytosine arabinoside or uracil arabinoside spot after 85 hr. at 70°. Charring with H_2SO_4 and heat again revealed a spot just above the origin.

Bisulfite is known to react with α,β-unsaturated ketones by a 1,4-addition to yield a sulfonate which reverses to starting material upon addition of NaOH (6). Addition of NaOH to the above 85-hr. bisulfite reaction gave the following chromatographic results in the solvents listed: (*a*) no uracil spot, *B,C,D,E;* (*b*) no arabinose spot, *A,B;* (*c*) definite uracil arabinoside spot, *A,B,C,E,F;* and (*d*) definite but minor cytosine arabinoside spot, *A,E,F*. It was assumed that no cytosine was present since the absence of uracil and arabinose was demonstrated.

It was concluded that the major bisulfite reaction product was the sulfonate of uracil arabinoside (III) and the minor reaction product was the sulfonate of cytosine arabinoside (IV). This conclusion is consistent with the following experimental observations: (*a*) the product of the bisulfite reaction has a low R_f value and is not visible under a 254 mμ ultraviolet light; (*b*) the rate of cytosine arabinoside loss is not first order in the presence of $NaHSO_3$; (*c*) there is an increased yield of reaction product other than II with increasing $NaHSO_3$ concentration; (*d*) there is a complete loss of I without formation of II in the presence of a tenfold excess of $NaHSO_3$; and (*e*) treatment of the bisulfite reaction product(s) with NaOH yields primarily II and traces of I.

The reaction between uracil arabinoside and $NaHSO_3$ did not result in an appreciable decrease in the size of the uracil arabinoside spot size during the 85-hr. reaction period. The reaction, thus, appeared to be slower than the corresponding cytosine arabinoside reaction. It seems unlikely, in light of this evidence, that the product III is formed by this route in the cytosine arabinoside reactions.

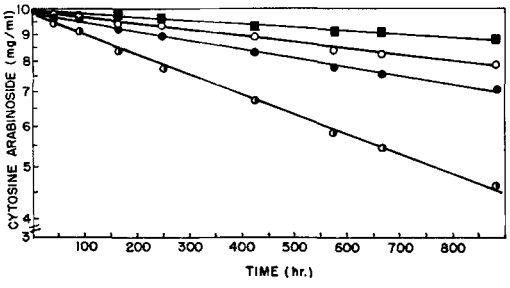


Fig. 3—First-order plots for deamination of cytosine arabinoside in 0.06 M phosphate buffer, pH 6.9, μ = 0.16. Key: ■, 47°; ○, 55°; ●, 60°; ◑, 70°.

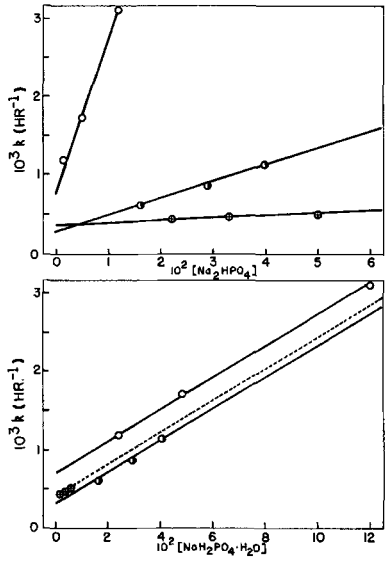


Fig. 4—Apparent first-order rate constants for cytosine arabinoside deamination at 70°, μ = 0.15, as a function of (top) disodium phosphate concentration and (bottom) sodium phosphate concentration. Key: ○, pH 6.2; ◑, pH 6.9; ⊕, pH 7.8.

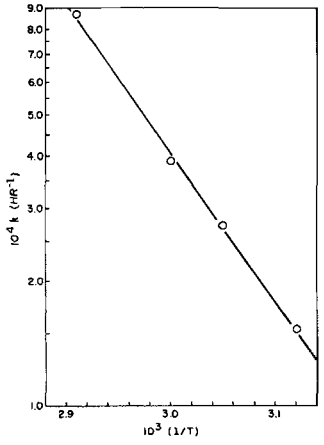


Fig. 5—Arrhenius plot of first-order rate constants for hydrolytic deamination of cytosine arabinoside in 0.06 M phosphate buffer, pH 6.9, μ = 0.15.

Kinetics of Cytosine Arabinoside Deamination in Buffer Solutions—Good first-order plots were obtained for deamination of cytosine arabinoside to uracil arabinoside in aqueous phosphate buffer solutions lacking $NaHSO_3$. Typical examples are shown in Fig. 3. Apparent first-order rate constants, calculated from the slopes of such plots, are tabulated in Table I.

The dependency of the observed first-order rate constants on the concentration of the buffer components is demonstrated in Fig. 4. The parallelism which is present in the plot of *k versus* $NaH_2PO_4 \cdot H_2O$ (Fig. 4, bottom) and absent in the corresponding plot for Na_2HPO_4 (Fig. 4, top) clearly demonstrates that the sole catalytic species in this buffer is $H_2PO_4^-$.

The intercept values, or the rate constants in the absence of buffer, are also reported in Table I. The observed increase in rate in going from pH 6.9 to 6.2 may be interpreted as specific hydrogen-ion catalysis in agreement with that previously reported (5). The failure of HPO_4^{2-} to catalyze the reaction demonstrates that removal of a proton from H_2O by base is not the mechanism by which general base catalysis is occurring. The very small difference between pH 6.9 and 7.8 suggests that specific hydroxyl-ion catalysis does not contribute to an appreciable extent in this pH region.

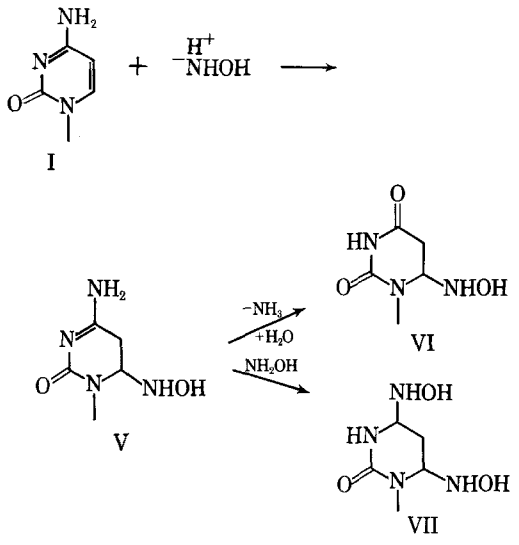
An Arrhenius plot of the apparent first-order rate constants for the deamination of cytosine arabinoside in 0.029 $NaH_2PO_4 \cdot H_2O$, 0.029 Na_2HPO_4, pH 6.9, $\mu = 0.15$ is given in Fig. 5. The value of ΔHa, in the presence of catalytic species, is 16.4 Kcal./mole.

DISCUSSION

Scheme I illustrates the proposed mechanism for either the hydrogen-ion, general acid-base catalysis of cytosine arabinoside (I) deamination or for 1,4-addition by HSO_3^-. The major bisulfite addition product is the sulfonate of uracil arabinoside (III), although some of the cytosine sulfonate (IV) is definitely present. It was concluded from thin-layer chromatographic and material balance data (Table II) that the product resulting from the deamination of cytosine arabinoside in the presence of general acid-base other than HSO_3^- (Scheme I) was uracil arabinoside (II).

There is precedence for both the 1,4-addition by HSO_3^- (6) and also the 5,6-addition to pyrimidine nucleosides. The reaction of NH_2OH with cytosine derivatives has been investigated by several workers (1–3). The reaction pathway involves initial conjugate addition in which NH_2OH saturates the 5,6-double bond to form V which can either deaminate to VI or exchange with a second molecule of NH_2OH at the 4-position (VII). The first addition was illustrated as attack by H^+–NHOH by Schuster (1). (Scheme II.)

The compound V was not isolated since saturation at the 5,6-position results in rapid nucleophilic exchange at C-4 to form VI or VII. This predisposition to deamination which results from saturation of the 5,6-position has been observed on several occasions. The nucleophilic exchange reaction which occurs during hydrolytic deamination has been found to proceed very slowly for cytosine as compared to the corresponding 5,6-dihydro derivatives (3). The deamination of cytidylic acid hydrate was shown to



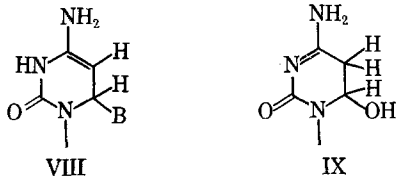
Scheme II

be rapid even at room temperature (4). In addition to this, the rate constants for the deamination of this 5,6-saturated species were independent of both the type and concentration of the buffer. The heat of activation for this deamination is the same as that reported here for the general acid-base catalyzed deamination, 16 Kcal./mole. In the absence of general acid-base species, HB^-, the value for ΔHa is 22 Kcal./mole (7).

An hypothesis explaining the observed increase in deamination rates of the 5,6-saturated cytosines is offered in steps 1–4, Scheme I. Furthermore, it is asserted that this represents the primary mechanism by which specific hydrogen-ion, general acid-base catalysis is occurring. Initial protonation at N-3 followed by saturation of the 5,6-position results in an electron deficiency at C-4 thus facilitating nucleophilic attack by water. Partial saturation to form VIII, as represented by Shapiro and Klein (5), would not result in electron deficiency at C-4.

Accordingly, $H_2PO_4^-$ is an effective catalytic species while HPO_4^{2-}, which will not supply a proton, is not (Fig. 4). This fact also excludes the suggestion that a possible catalytic mechanism is through abstraction of a proton from water by the base (5). If this were the case HPO_4^{2-} would be expected to exhibit a greater catalytic effect than $H_2PO_4^-$.

Catalysis by saturation of the 5,6-double bond is also consistent with the fact that cytidylic acid hydrate (IX) is not subject to buffer effects and has the same ΔHa for deamination as the $H_2PO_4^-$ catalyzed reaction reported here.



There is at present insufficient data available to allow the calculation of catalytic constants for buffer species other than the phosphate system which is defined in this paper. Studies are currently in progress, by the author, to quantitatively evaluate the catalytic effects of other buffers. However, the hypothesis that cytosine deamination is subject to general acid-base catalysis by HB^- can be shown to be consistent with the existing data of several workers. For example, the observed first-order rate constant for cytosine arabinoside deamination in the presence of citrate ion, pH 7.4, 70°, was found to be 3.77×10^{-4}/hr. (7). This agrees with the intercept value of the plot, *k versus* phosphate buffer concentration, pH 7.8, 70°, which is 3.79×10^{-4}/hr. (Fig. 4; Table I). Furthermore, the value of ΔHa for cytosine arabinoside deamination in the presence of $citrate^{3-}$ was shown to be 22 Kcal./mole (7). These data are consistent with the prediction that $citrate^{3-}$ does not meet the criteria for general acid-base catalysis as outlined here.

A re-examination of the data of Shapiro and Klein (5) also lends support to the mechanism proposed in Scheme I. Their observed first-order rate constants, $k_{obsd.}$, are listed in Table III. The concentrations of each individual buffer species were calculated and the results are also given. A plot of $[H_{n-1}B^-]$ *versus* $k_{obsd.}$ for the citrate buffer data under good first-order conditions ($[H_2\ citrate^-]/[cytidine] \geq 4$) was found to be linear with an intercept value of 1.26×10^{-2} (hr.$^{-1}$). This value for the deamination of cytidine in the absence of buffer effects is comparable to the first-order rate constants reported for the formate, acetate, or lactate buffers under the same experimental conditions (Table III). It was also reported that cytidine deaminated only 2% after 21 hr. of refluxing in either water or 0.5 *M* sodium acetate at pH 7.2. It can be concluded that these buffers do not contain any major catalytic species for cytosine deamination.

Table III—Concentrations of Each Buffer Species in 0.5 *M* Solutions at pH 4, the Corresponding First-Order Rate Constants for Cytidine Deamination at 97° ($k_{obsd.}$ in hr.$^{-1}$),[a] and the Portion of $k_{obsd.}$ Attributed to Catalysis by Buffer ($k_{cat.}[cat.]$)[b]

Buffer	HnB	$Hn_{-1}B^-$	$Hn_{-2}B^{2-}$	$10^2 k_{obsd.}$	$10^2 k_{cat.}$ [cat.]
Citrate	0.046	0.385	0.069	3.4	2.1
Oxalate	0.000	0.305	0.195	2.8	1.5
Succinate	0.293	0.202	0.005	2.0	0.7
Formate	0.198	0.306	...	1.4	0.1
Lactate	0.288	0.288	...	1.3	0.0
Acetate	0.432	0.068	...	1.3	0.0

[a] $k_{obsd.}$ = the observed first-order rate constants as reported by Shapiro and Klein (5). [b] These values are calculated from the equation $k_{cat.}[cat.] = k_{obsd.} - 1.26 \times 10^{-2}$ (hr.$^{-1}$), where 1.26×10^{-2} is the value for the intercept of the plot $k_{obsd.}$ *vs.* $[H_2\ citrate^-]$.

A re-examination of the data of Shapiro and Klein (5) also lends support to the mechanism proposed in Scheme I. Their observed first-order rate constants, $k_{obsd.}$, are listed in Table III. The concentrations of each individual buffer species were calculated and the results are also given. A plot of $[H_{n-1}B^-]$ *versus* $k_{obsd.}$ for the citrate buffer data under good first-order conditions ($[H_2\ citrate^-]/[cytidine] \geq 4$) was found to be linear with an intercept value of 1.26×10^{-2} (hr.$^{-1}$). This value for the deamination of cytidine in the absence of buffer effects is comparable to the first-order rate constants reported for the formate, acetate, or lactate buffers under the same experimental conditions (Table III). It was also reported that cytidine deaminated only 2% after 21 hr. of refluxing in either water or 0.5 *M* sodium acetate at pH 7.2. It can be concluded that these buffers do not contain any major catalytic species for cytosine deamination.

Conversely, it is evident from Table III that the citrate buffer system does in fact catalyze cytidine deamination. Since it has been demonstrated that $citrate^{3-}$ is not catalytic, it follows that one of the protonated species must be the catalyst. As a further test of catalysis by HB^-, the derivative of $[H_2\ citrate^-]$ with respect to $[H^+]$ was set equal to zero and the expression solved for the pH of maximum $H_2\ citrate^-$ concentration. Results indicate that the pH of maximum HB^- concentration in a citrate buffer of fixed total concentration would be 3.9. This is consistent with the data which show a rate maximum between 3.5 and 4.0 for this system (5).

Attempts to correlate the catalytic effects of HB^- with that predicted from the Brönsted relation (8) are complicated by the dual nature of the catalyst which is defined here as participating both as an acid and a base. The values for the predicted catalytic constants, k_A (or k_B), in terms of G_A and α(or G_B and β) can be compared for each type of buffer species since the Brönsted relation is well obeyed within a group of similar catalytic species such as H_nB, $H_{n-1}B^-$, B^{2-}, *etc.* The value of the experimental catalytic constant for a potentially catalytic species, X, can also be calculated from $k_X = k_{cat.}$ [cat.]/(concn. X). (See Table III.) The assumption in this latter calculation is that one catalytic species predominates in each buffer as has been demonstrated for the phosphate system. The data were treated in this manner and the results, while not conclusive, are consistent with the present hypothesis. The catalytic constants as calculated from the data were compared to the catalytic constants as calculated from the Brönsted relation using the statistical correction and assigning a value of 0.5 to α or β. The only good correlation evident was that between k_{Hn-1B^-} and the values of k_A (in terms of G_A) for the species $H_{n-1}B^-$. This result is consistent with the choice of HB^- as the primary catalytic species. The fact that the correlation exists for k_A in this comparison and not for k_B implies that the catalytic species requires a donatable proton. The formate, lactate, and acetate ions, which exhibit no significant buffer effect as seen in Table III, also have no value for k_A since the anions cannot act as proton donors.

It can be concluded from the above discussion that the mechanism offered in Scheme I is consistent with the experimental data presented here as well as the related data of the other workers cited above.

SUMMARY

Consideration of the 1,4-addition products formed from the reaction between bisulfite and cytosine arabinoside and consideration of the effects of phosphate, oxalate, succinate, lactate, formate, and acetate buffers has demonstrated that cytosine deamination will be significantly catalyzed by a nucleophile bearing a donatable proton in the presence of hydrogen ions. The primary mechanism of hydrolytic deamination of cytosine nucleosides in aqueous catalytic buffer systems, as illustrated in Scheme I, is as follows: (*a*) protonation of N-3, (*b*) nucleophilic attack at C-6, (*c*) saturation at C-5 by proton addition, and (*d*) nucleophilic displacement at C-4 by H_2O with loss of NH_3.

REFERENCES

(1) Schuster, H., *J. Mol. Biol.*, **3**, 447(1961).
(2) Brown, D. M., and Schell, P., *ibid.*, **3**, 709(1961).
(3) Brown, D. M., and Phillips, J. H., *ibid.*, **11**, 663(1965).
(4) Johns, H. E., LeBlanc, J. C., and Freeman, K. B., *ibid.*, **13**, 849(1965).
(5) Shapiro, R., and Klein, R. S., *Biochemistry*, **5**, 2358 (1966).
(6) Schroeter, L. C., "Sulfur Dioxide," 1st ed., Pergamon Press Inc., New York, N. Y., 1966, p. 110.
(7) Lamb, D. J., and Smith, R. W., unpublished data.
(8) Bell, R. P., "Acid-Base Catalysis," Oxford University Press, London, England, 1941, p. 82.

Effect of Some Nonionic Surfactants on the Rate of Release of Drugs from Suppositories

By J. M. PLAXCO, JR., C. B. FREE, JR.*, and C. R. ROWLAND†

Twenty-eight nonionic surfactants were added to theobroma oil base suppositories. The amount of aminophylline, ephedrine alkaloid, and ephedrine hydrochloride released from these bases was determined by dialyzing the released drug through a cellophane membrane and determining the optical absorbance at the appropriate wavelength. Data obtained indicate that surfactants with HLB values of less than 11 had little effect on the rate of release. Surfactants with HLB values over 11 increased significantly, in most cases, the amount of drug dialyzed. Besides HLB values, other properties of surfactants such as chemical structure, composition, and melting point also affect the rate of release.

THE USE OF suppositories as a form of medication dates back to the time of Hippocrates. Many unsatisfactory agents were tried as bases, but it was not until the discovery and development of theobroma oil as a base in the 18th century that they received much attention. In more recent days much attention has been directed toward modifying and improving bases of theobroma oil, glycerinated gelatin, and polyethylene glycol polymers, and in developing new bases. Until fairly recently, as long as the suppository retained its shape on the shelf, melted or dissolved in the rectum, and showed no visible evidence of incompatibility it was considered satisfactory. Recently it has been recognized

Received November 14, 1966, from the School of Pharmacy, University of South Carolina, Columbia, SC 29208

Accepted for publication March 9, 1967.

Presented to the Basic Pharmaceutics Section, A.PH.A. Academy of Pharmaceutical Sciences, Dallas meeting, April 1966.

This investigation was supported in part by grant GM-11801-01 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

* Present address: College of Pharmacy, University of Florida, Gainesville, FL 32601

† Present address: College of Pharmacy, Ohio State University, Columbus, OH 43210