

Equilibria for the Reaction of Amines with Formaldehyde and Protons in Aqueous Solution

A RE-EXAMINATION OF THE FORMOL TITRATION*

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SUMMARY

The existence of protonated hydroxymethylamines of secondary amines, $R_2\overset{+}{N}HCH_2OH$, in equilibrium with hydroxymethylamines and protonated amines, has been demonstrated by titration at equilibrium and by rapid titration of the protonated hydroxymethylamines. Analogous compounds, $R_2\overset{+}{N}CH_2OH$, are formed from formaldehyde and the sterically favorable tertiary amines: pyridine, *N*-methylimidazole, and triethylenediamine. The equilibrium constants for the formation of these cationic hydroxymethylamines are two to three orders of magnitude less favorable than for the formation of neutral hydroxymethylamines; conversely, the basicity of hydroxymethylamines is two to three orders of magnitude less than that of the parent amines. These differences are ascribed primarily to solvation and polar effects. Protonation of the dihydroxymethyl adducts of primary amines was not detected.

An analysis of the complex equilibria for the addition of protons and hydroxymethyl groups to imidazole suggests that protonation and hydroxymethylation do not occur on the same nitrogen atom.

The equilibrium constant

$$K_D = [R_2NCH_2NR_2]/[R_2NH]^2[H_2C(OH)_2]$$

for the formation of the methylenediamine adduct of morpholine has been shown to be $15,500 \pm 600$ by two titrimetric methods.

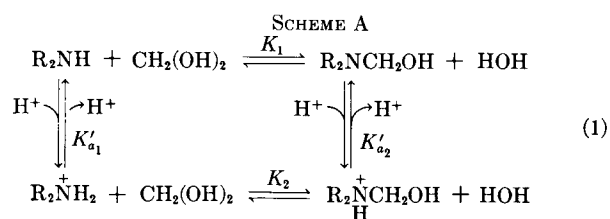
An attempt is made to evaluate the effects on the formol titration of changes in water concentration, formaldehyde polymerization, the methanol introduced with commercial formalin, and nonspecific medium effects in concentrated formaldehyde solutions. It is concluded that these factors either have little significant effect or largely cancel each other out.

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In the theoretical treatment of the formol titration, it has generally been assumed that formaldehyde combines with the free base form of primary or secondary amines to form hydroxymethylamines of negligible basicity and therefore causes a decrease in the pH of the solution (1, 2). Although this theory has been successful in describing of the effect of formaldehyde on the pH of dilute solutions that contain amines and formaldehyde, we were disturbed by the assumption that the hydroxymethylamine itself does not become protonated. The electron-withdrawing effect of the hydroxymethyl group is not much greater than that of hydrogen (3) and is certainly not so large as to eliminate detectable basicity of an amine. The pK_a of formaldehyde hydrate itself is 13.3 (4), which is only 2.4 units less than the pK_a of water of 15.7. Furthermore, protonated cyclic alkoxymethyleneamines (1,3-oxazines) are known, isolable compounds (5). The experiments which are described here were designed to examine the possibility that protonation of hydroxymethylamines may occur. It has been shown that cationic hydroxymethylamines may be formed from secondary and tertiary amines, but the formation of these compounds does not have a large effect on the formol titration as it is ordinarily performed.

The equilibria for the combination of secondary amines with formaldehyde and protons are given in Equation 1. In the classical treatment of the formol titration, only the equilibria K_1 and K'_{a1} , for the addition to the amine of formaldehyde and a proton,



respectively, are generally considered. We will be concerned with the equilibrium K'_{a2} , for protonation of the hydroxymethylamine, and K_2 for the addition of formaldehyde to a protonated amine. These two equilibria are not independent, and determination of one equilibrium constant fixes the value of the other.

Although a number of methylenediamines and their salts have been isolated (6), little is known regarding the extent to which they exist in aqueous solutions. The equilibrium constant for the formation of methylenedimorpholine has been determined

titrimetrically in relatively concentrated aqueous solutions of amine and formaldehyde.

The properties and equilibrium constants for the formation of protonated hydroxymethylamines and of methylenediamines are of significance in the interpretation of the formol titrations of amino acids, proteins (1, 2, 7), nucleic acids and their constituents (8–11), and of the condensation reactions of formaldehyde including the uncatalyzed and amine-catalyzed reaction with tetrahydrofolic acid (12), the Mannich reaction (13–15), and thiazolidine and oxazolidine formation (1).

Le Hénaff has independently carried out a comprehensive study of the equilibria for cationic hydroxymethylamine and methylenediamine formation by several techniques (16, 17) and Skell and de Luis have been able to demonstrate directly the existence of protonated hydroxymethylamines and cationic imines of formaldehyde in acid solution by nuclear magnetic resonance techniques.¹ The conclusions and results of these studies are generally in agreement with those reported here.

EXPERIMENTAL PROCEDURE

Reagent grade, 36.6 to 37.2% formaldehyde containing 10 to 11% methanol was obtained from Fisher. The concentration of diluted solutions was determined by iodometric titration (6). The concentrated formaldehyde was found to contain about 2×10^{-3} M formic acid by titration and to have a pH between 3 and 4. The formic acid was neutralized with sodium hydroxide; the sodium formate so formed does not affect formol titrations at pH values above 5. This formaldehyde was used in all experiments, unless noted otherwise. For experiments at pH values below 5, in which the sodium formate might act as a buffer, the formic acid was removed from the formaldehyde just prior to use by passage through a Dowex 1-hydroxide column. Methanol-free solutions of formaldehyde were obtained from paraformaldehyde and water, which were refluxed and distilled. These solutions showed no precipitate at concentrations up to 11 M.

Amines were redistilled or converted to the hydrochlorides and recrystallized before use. Reagent grade amino acids and inorganic reagents were generally used without further purification. Boiled, glass-distilled water was used throughout.

Measurements of pH were made with a G-200B glass electrode and a Radiometer type 4b pH meter, standardized with Beckman buffers at pH 10.00, 7.00, and 4.00 and (when appropriate) with 0.1 M hydrochloric acid at pH 1.10. Solutions of amine-amine hydrochloride buffers and formaldehyde were equilibrated in a thermostat at $25.0^\circ \pm 0.1^\circ$. Determinations of pH were generally made within 1 min after removal from the water bath, during which time no significant temperature change took place. Solutions were mixed to give a final volume of 20 ml and readings were taken after 5 min and then at varying time intervals up to 48 hours. At pH values below 9.5, there was no change in the readings in this time interval, which shows that equilibrium was attained before the time of the first reading. Furthermore, the same pH values were obtained when the reaction mixtures were prepared by incubation of amine and formaldehyde, followed by the addition of acid, as when they were prepared from amine hydrochloride and formaldehyde and equilibrium was attained after the addition of alkali. At pH values greater than 9.5, there was a slow decrease in pH after the initial readings, possibly caused by carbon dioxide absorption (similar decreases in pH were

noted in solutions of the nonvolatile amine, proline, and in solutions which did not contain formaldehyde). The initial determinations of pH were used in these experiments.

In experiments with tertiary amines, it was necessary to determine the pH at alkaline pH values, at which the buffering capacity of hydrated formaldehyde, which has a pK_a of 13.3 (4), significantly affects the pH of the solution. In such experiments, the formaldehyde was first neutralized to the pH of the amine buffer. Under these conditions, the effect of buffering by formaldehyde hydrate is to decrease slightly the observed changes in pH which are caused by the reaction of formaldehyde with amine.

Calculations of equilibrium constants were made according to the usual procedures (1, 2), in terms of the concentrations of hydrated formaldehyde and taking the activity of water in the solvent as 1.0, unless noted otherwise. The concentration of free, hydrated formaldehyde, $[F_F]$, was obtained from the amount of formaldehyde added, corrected for the amount bound to amine. The concentrations of free and hydroxymethylated amine in dilute formaldehyde solutions are determined by the amounts of free and protonated amine added, the pH, and the ionization constant of the amine. Derivations of the equations that were used to calculate equilibrium constants in this work are reported elsewhere (1, 2).²

RESULTS

The results are based on measurements of the change in the pH of solutions of amine buffers to which increasing amounts of formaldehyde were added. Although other methods were sometimes used, equilibrium constants for the addition of formaldehyde to amines were generally calculated from plots of $pH_0 - pH_{obs} = \Delta pH$ (where pH_{obs} and pH_0 are the pH values in the presence and absence of formaldehyde, respectively³) against the logarithm of the concentration of hydrated formaldehyde (Fig. 1) (1, 2). In such plots, the observed decrease in the pH of an amine-amine hydrochloride buffer solution becomes larger with increasing formaldehyde concentration $[F_F]$, and a plot of ΔpH against $\log [F_F]$ approaches a limiting slope of 1.0 or 2.0, depending on whether the free amine combines with 1 or 2 moles of formaldehyde. The equilibrium constants for formation of the mono- and dihydroxymethyl adducts may be obtained by extrapolation of the lines of slope 1.0 and 2.0 to $\log [F_F] = 0$ or from nonlogarithmic plots of the data (1, 2). Since the titration curves of amines maintain their normal sigmoid shape in the presence of formaldehyde, the values of ΔpH correspond to $\Delta pK'$, the change in the apparent pK of the amine in the presence of formaldehyde (1).

Primary Amines—The results obtained with L-alanine are shown in the *lower curve* of Fig. 1 as an example. The limiting slope of the logarithmic plot is 2.0, as expected for the combination of primary amines with 2 moles of formaldehyde to form the dihydroxymethyl addition compounds. The equilibrium constants, L_1 , L_2 , and L_3 , obtained with dilute solutions of amine, and the maximum change in observed pH in the presence of 13 M formaldehyde are summarized in Table I for the primary amines examined.

² For further details, see R. G. Kallen's Ph.D. thesis, Brandeis University, 1965.

³ pH_{obs} corresponds to the pG , values of previous investigators (1, 2).

¹ P. Skell, personal communication.

$$L_1 = \frac{[\text{RNHCH}_2\text{OH}]}{[\text{RNH}_2][\text{CH}_2(\text{OH})_2]}$$

$$L_2 = \frac{[\text{RN}(\text{CH}_2\text{OH})_2]}{[\text{RNH}_2][\text{CH}_2(\text{OH})_2]^2}$$

$$L_3 = \frac{[\text{RN}(\text{CH}_2\text{OH})_3]}{[\text{RNHCH}_2\text{OH}][\text{CH}_2(\text{OH})_2]}$$

The association constants L_1 and L_2 were evaluated graphically from the intercept and slope, respectively, of plots of $(\Delta\text{pH} - 1)/[F_F]$, where $[F_F]$ is the concentration of free, hydrated formaldehyde. A theoretical curve, based on the calculated equilibrium constants, is drawn through the experimental points in Fig. 1 and shows satisfactory agreement with the data. The value of L_3 is determined by those of L_1 and L_2 . The plots of ΔpH against $\log [F_F]$ were found to approach and then follow a line of slope 2.0 up to formaldehyde concentrations of 13 M, except for *tert*-butylamine and cyclohexylamine which show small downward deviations at 9 and 13 M formaldehyde. Thus, there is no indication of the protonation of the mono- or dihydroxymethyl adducts of these amines under these experimental conditions. Le Hénaff has reported evidence for the existence of protonated hydroxymethyl adducts of glycine and methylamine under different experimental conditions (17) (*cf.* also Reference 18).

Plots of ΔpH against $\log [F_F]$ for methylamine showed slopes of 1.0 and gave much larger apparent values of L_1 . However, these values are dependent on the concentration of amine and are only apparent constants, because this relatively unhindered

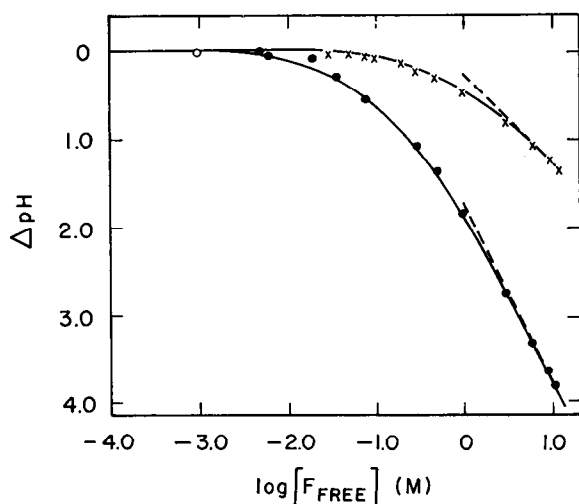


FIG. 1. The change in the pH of L-alanine (●—●) and N-methyl-DL-valine (×—×) buffers as a function of the logarithm of the formaldehyde concentration at 25°. The L-alanine was 0.05 M, 50% free base, ionic strength 0.075 M, and the solid, theoretical line is based on the equation

$$\Delta\text{pH} = \log (1 + 24[F_F] + 55[F_F]^2)$$

The N-methyl-DL-valine was 0.024 M, 10% free base, ionic strength 0.005 M, and the solid line is based on

$$\Delta\text{pH} = \log (1 + 2.1[F_F])$$

The dashed lines have slopes of 2.0 and 1.0, respectively.

TABLE I

Equilibrium constants for adduct formation between formaldehyde and primary amines at 25° and water activity 1.0

Compound	pK'	L_1	L_2	L_3	ΔpH^a
		M^{-1}	M^{-2}	M^{-1}	
Cyclohexylamine ^{b,d}	10.70	55	110	2.0	4.66
L-Alanine ^{e,f}	9.86	21	36	1.7	3.80
DL-Leucine ^{e,g,h}	9.78	20	31	1.6	3.58
DL-Valine ^{b,c,i}	9.60	13	5.2	0.40	2.85
<i>tert</i> -Butylamine ^{b,c,e}	10.74	5.9	1.6	0.27	3.08

^a The maximum observed ΔpH , in *ca.* 13 M formaldehyde.

^b 0.05 M.

^c Ionic strength, 0.025 to 0.075 M.

^d 10% free base.

^e 50% free base.

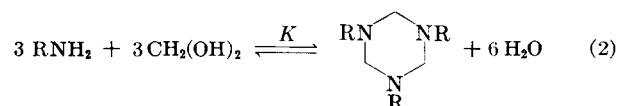
^f 0.005 M, ionic strength, 0.005 M; these values are more reliable than those based on the data shown in Fig. 1 which were obtained at a higher concentration of alanine at which trimerization may be significant.

^g Ionic strength, 0.015 M.

^h 0.01 M.

ⁱ 90% free base.

amine undergoes trimerization in the presence of formaldehyde (1, 17) (Equation 2).



Secondary Amines—The results obtained with secondary amines fall into two groups. N-Methyl-DL-valine is an example of Group A, which also includes the relatively bulky amines diethylamine and N-methylcyclohexylamine. As shown in the upper line of Fig. 1, the plot of ΔpH against $\log [F_F]$ for N-methyl-DL-valine approaches and then follows a line of slope 1.0 up to a formaldehyde concentration of 13 M; similar results were obtained for the other amines of this group. The equilibrium constants, K_1 , are relatively small, in the range 2 to 40 M^{-1} (Table II). The maximum ΔpH observed for these amines in 13 M formaldehyde is between 1.4 and 3.0.

$$K_1 = \frac{[\text{R}_2\text{NCH}_2\text{OH}]}{[\text{R}_2\text{NH}][\text{CH}_2(\text{OH})_2]}$$

$$K'_{a1} = \frac{[\text{R}_2\text{NH}][\text{H}^+]}{[\text{R}_2\text{NH}_2^+]}$$

$$K_2 = \frac{\frac{[\text{R}_2\text{NCH}_2\text{OH}]}{\text{H}}}{[\text{R}_2\text{NH}_2^+][\text{CH}_2(\text{OH})_2]}$$

$$K'_{a2} = \frac{[\text{R}_2\text{NCH}_2\text{OH}][\text{H}^+]}{[\text{R}_2\text{NCH}_2\text{OH}^+]}$$

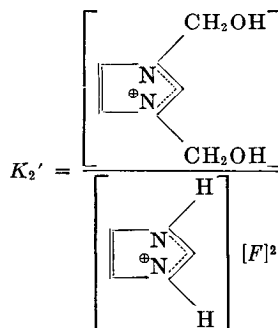
The amines in Group B, which includes piperidine, dimethylamine, morpholine, pyrrolidine, sarcosine, and DL-proline, are characterized by a relatively high affinity for formaldehyde, with values of K_1 in the range 76 to 1600 M^{-1} (Table II). Plots of ΔpH against $\log [F_F]$ exhibit a leveling off at high formaldehyde concentrations with these amines; the results for morpholine are

TABLE II

Equilibrium constants for adduct formation between formaldehyde and secondary amines at 25° and water activity 1.0

Compound	pK'	K ₁	K ₂	ΔpH _{max}	ΔpH _{obs} ^a
A. Diethylamine ^{b-d}	11.02	40			3.00
N-Methylcyclohexylamine ^{b,d,e}	11.09	18			2.75
N-Methyl-DL-valine ^{e-g}	9.93	2.1			1.40
B. Piperidine ^{b,d,e}	11.16	1600	1.5	3.0	
Dimethylamine ^{b,d,e}	10.78	1150	1.7	2.8	
Morpholine ^{b-d}	8.56	800	0.9	3.0	
Pyrrolidine ^{d,f,g}	11.16	690	1.4	2.0	
Sarcosine ^{b-d}	10.07	80	0.22	2.6	
DL-Proline ^{b-d}	10.68	76	0.5	2.2	
Imidazole ^{b-d}	7.05	15	3.2 ^h	0.7	

$$K_1 = \frac{[\text{R}_2\text{NCH}_2\text{OH}]}{[\text{R}_2\text{NH}][\text{F}]} \quad K_2 = \frac{\frac{[\text{R}_2\text{N}^+\text{CH}_2\text{OH}]}{\text{H}}}{[\text{R}_2\text{NH}_2^+][\text{F}]}$$



in which F = formaldehyde hydrate.

^a ΔpH_{obs} is a linear function of log[F_F] for these amines and this value represents the observed shift in pH at ca. 13 M formaldehyde, the highest concentration examined.

^b 0.05 M.

^c 50% free base.

^d Ionic strength, 0.025 to 0.075 M.

^e 10% free base.

^f 0.022 to 0.025 M.

^g Ionic strength 0.002 to 0.005 M.

^h K₂ includes a statistical factor (see "Results"); K'₂ = 2.3.

shown in Fig. 2. This leveling off is more apparent if the data are plotted nonlogarithmically according to the usual method (1, 2), as shown for the amino acid proline in Fig. 3. If hydroxymethylamine formation were the only reaction occurring, such a plot should be linear (*dashed line*). The leveling off of these plots at high values of ΔpH and log [F_F] is evidence for protonation of the hydroxymethyl adducts of these amines (Equation 1). In Fig. 3, the initial slope of the line is K₁, and the line levels off at the value of K₁/K₂ = K'_{a2}/K'_{a1}, in which K₂ is the equilibrium constant for the addition of formaldehyde to the protonated amine and K'_{a2} and K'_{a1} are the acid dissociation constants of the protonated hydroxymethylamine and amine, respectively (Equation 1).

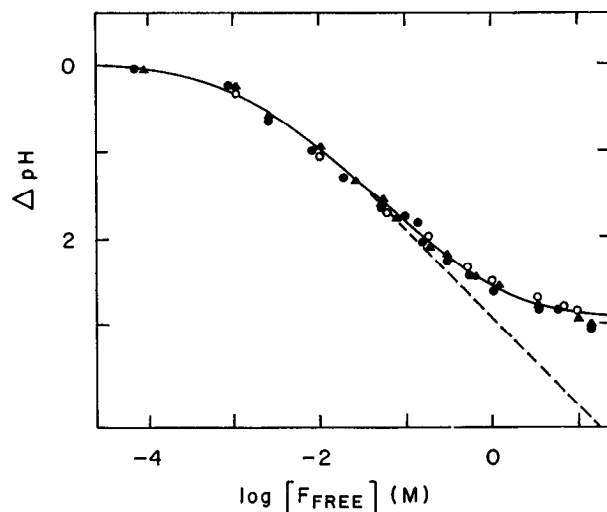


Fig. 2. The change in the pH of 0.05 M morpholine buffers, 50% free base, as a function of the logarithm of the free formaldehyde concentration at 25°. ●, ionic strength 0.05 M; ○, ionic strength 1.0 M; ▲, experiment carried out with methanol-free formaldehyde. The *solid*, theoretical line is drawn from the equation

$$\Delta\text{pH} = \log(1 + 800[F_F]) - \log(1 + 0.9[F_F])$$

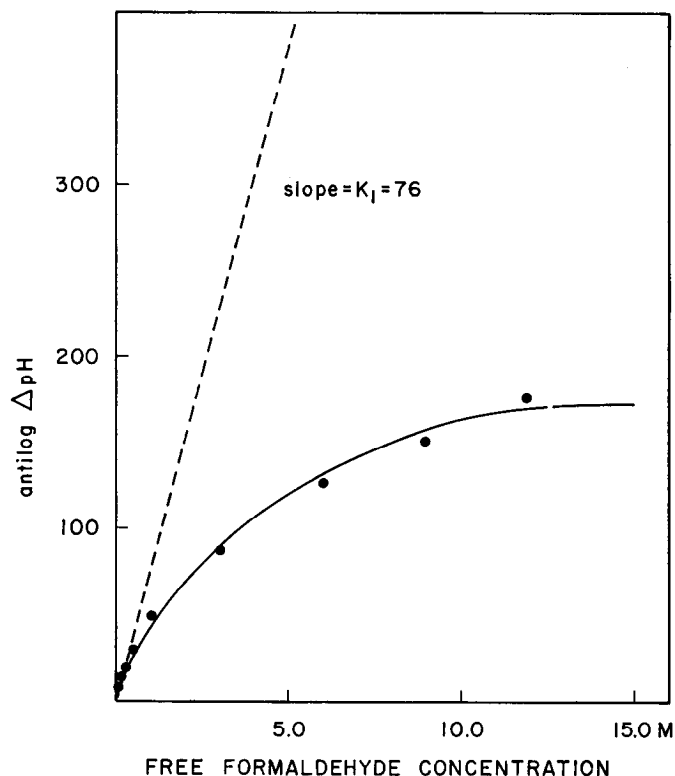


Fig. 3. Dependence of the change in hydrogen ion activity of 0.05 M DL-proline buffers, 50% free base, on the concentration of formaldehyde at 25°, ionic strength 0.025 M. The *solid*, theoretical line is based on the equation

$$\text{antilog } \Delta\text{pH} = (1 + 76[F_F]) / (1 + 0.5[F_F])$$

The *dashed line* is the theoretical line based on the assumption that protonation of the hydroxymethyl adduct does not occur.

tion 1). The logarithmic plots follow Equation 3 and the inter-

$$\Delta\text{pH} = \log \frac{(1 + K_1 [F_F])}{(1 + K_2 [F_F])} \quad (3)$$

relationship of the equilibrium constants is given in Equation 4.

$$\text{antilog } \Delta\text{pH}_{\text{max}} = K_1/K_2 = K'_{a2}/K'_{a1} \quad (4)$$

The value of K_1 may be determined in the usual manner from an extrapolation of the linear portion of the plot (slope = 1.0) to its intersection with the $\log [F_F] = 0$ axis, and the value of K_2 is then determined from the above relationships. The *solid lines* in the figures are calculated lines, based on the equilibrium constants given in Table II, and show satisfactory agreement with the experimental data up to approximately 10 M formaldehyde.

The association constants, K_2 , for the reaction of the protonated amines with formaldehyde are between 0.2 and 1.7; *i.e.* two to slightly more than three orders of magnitude less than the association constants for the reaction of the corresponding free amines with formaldehyde (Table II). This indicates that the pK'_{a2} values of the protonated hydroxymethylamines are about 2 to 3 units below the pK'_{a1} values of the protonated amine and that the acid-strengthening effect of a single hydroxymethyl group is 2 to 3 pH units.

The pK' values of protonated hydroxymethyl derivatives of piperidine and morpholine were also determined directly by first forming the hydroxymethylamine in alkaline solution, then rapidly acidifying the solution to form the protonated hydroxymethylamine, which is kinetically stable in sufficiently acid solution, diluting, and finally adding measured amounts of base to

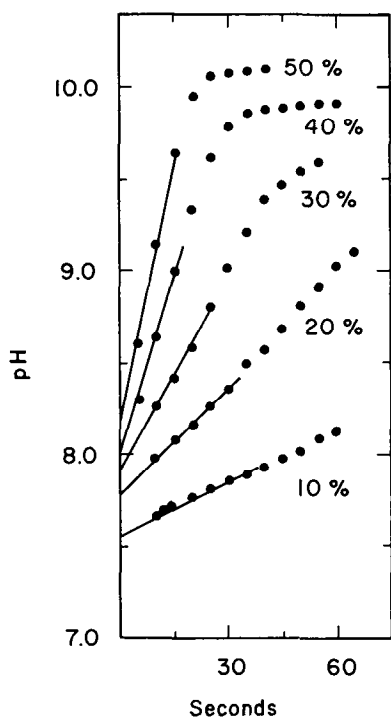


Fig. 4. The change in pH with time of partially neutralized 0.011 M hydroxymethylpiperidine buffers at 1°, caused by hydrolysis. Protonated hydroxymethylpiperidine was prepared by the addition of hydroxymethylpiperidine (0.1 M piperidine and 0.125 M formaldehyde) to excess hydrochloric acid (8 volumes, 0.0114 M). The pH was determined at intervals after partial neutralization of portions of this solution with standard sodium hydroxide, as indicated in the figure.

give partial neutralization. Rapid hydrolysis of the hydroxymethylamine follows, but by extrapolation of the observed pH readings at 1° to zero time the pK' of the protonated hydroxymethylamine may be determined (Fig. 4). The pK' values of protonated hydroxymethylpiperidine at 10, 20, 30, 40, and 50% free base were found to be 8.51, 8.38, 8.46, 8.23, and 8.20, or approximately 2.4 to 2.7 pH units below that of protonated piperidine at this temperature. A similar experiment with morpholine gave pK' values of 5.79, 5.89, 5.86, 5.82, and 5.84 for protonated hydroxymethylmorpholine, which had been neutralized 10, 20, 30, 40, and 50% to the free base, respectively. These results confirm the pK' values of 8.56 and 5.89 for the conjugate acids of hydroxymethylpiperidine and hydroxymethylmorpholine, respectively, which were obtained by formol titrations at 1°.

The equilibria for the reaction of formaldehyde with free and protonated morpholine show little sensitivity to the experimental conditions. As shown in Fig. 2, essentially the same results are obtained if the experiments are carried out with formaldehyde which was prepared from paraformaldehyde, *i.e.* in the absence of methanol. There is no significant effect of varying the ionic strength between 0.05 and 1.0 M with potassium chloride. Although the absolute values of the pH at a given formaldehyde concentration are increased about 0.3 pH unit at the higher ionic strength, a similar increase is observed at all formaldehyde concentrations and there is no effect on the ΔpH , *i.e.* potassium chloride has the same effect on the protonation of the free amine and the hydroxymethylamine. Identical results were obtained in experiments with morpholine which were carried out over a 10-fold range of buffer concentrations, at 0.01, 0.05, and 0.10 M, which shows that the equilibrium constants are independent of the amine concentration in this concentration range and that formation of methylenedimorpholine is not significant under these experimental conditions. Identical results were also obtained when the solutions were allowed to stand for 10 to 30 min and for 24 and 48 hours, which shows that equilibrium is attained rapidly under these experimental conditions. The plot of ΔpH against $\log [F_F]$ was the same with morpholine buffers that were 90% and 50% in the form of the free base, which indicates that the equilibria are not significantly affected by the ratio of free to protonated amine.

The values of L_1 and L_2 reported here for alanine and leucine agree within 10% with previous results (1, 19-21). The corresponding constants for proline and sarcosine differ by about 2-fold and 4-fold, respectively, from most previous reports (19, 20, 22). The values of K_1 and K_2 for morpholine of 800 and 0.9 M⁻¹, respectively, may be compared to the corresponding values of 1100 ± 200 and 1.9 to 2.1 M⁻¹ obtained by Le Hénaff at 20° (17) and 900 M⁻¹ for K_1 by Skell.¹ There is better agreement between our values for K_1 and K_2 of 1150 and 1.7 M⁻¹ and Le Hénaff's of 1500 ± 500 and 1.6 to 2.0 M⁻¹, respectively, for dimethylamine (17), but a discrepancy compared to Skell's values¹ of 1100 and 11 M⁻¹. It is evident that these equilibrium constants are not of high precision; the variability among the results of different laboratories has been commented on previously and no satisfactory explanation offered (1). The perturbing effects of concentrated formaldehyde solutions which are discussed below undoubtedly decrease the precision of the measurements reported here, most of which were carried out in relatively concentrated solutions in order to obtain an unequivocal demonstration of the existence of cationic hydroxymethylamines.

Tertiary Amines—Buffers prepared from tertiary amines may show a decrease (Group A), no change (Group B), or an increase (Group C) in pH in the presence of concentrated formaldehyde (Table III). The changes in pH are considerably smaller than those observed with primary or secondary amines and correspond to less than a 10-fold change in hydrogen ion activity.

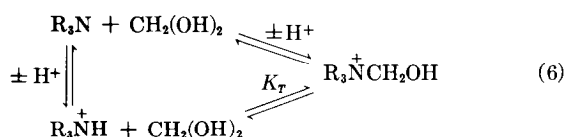
The changes in the pH of buffers prepared from amines of Group A are negligible at low and moderate formaldehyde concentrations and increase monotonically with increasing formaldehyde concentration to the maximum observed ΔpH values in 13 M formaldehyde. This is attributed to a nonspecific solvent effect on the activity coefficients of the amine buffer, on the system used for pH measurement, or both. Methanol and ethylene glycol are similar in structure to formaldehyde hydrate (methylene glycol) and concentrated solutions of these substances cause changes in the observed pH values of triethylamine buffers which are very similar to those observed in the presence of concentrated formaldehyde (Table IV). The addition of such relatively nonpolar materials to water would be expected to increase the activity coefficient of the charged, protonated amine and to decrease the activity coefficient of the uncharged amine, and, therefore, to decrease the pH of the solution (Equation 5).

$$\text{pH} = \text{pK} + \log \frac{C_{\text{R}_3\text{N}}}{C_{\text{R}_3\text{NH}^+}} + \log \frac{\gamma_{\text{R}_3\text{N}}}{\gamma_{\text{R}_3\text{NH}^+}} \quad (5)$$

The magnitude of this solvent effect would be expected to be different for different amines. For example, a smaller effect would be expected for *N,N*-diethylglycine, which exists as an anion in the basic form and as a dipolar ion in the acidic form, and is also not unexpected for *N*-methylmorpholine, which contains a second uncharged, but strongly solvated oxygen atom. Buffers prepared from these two amines (Group B) show very little change in pH in the presence of concentrated formaldehyde.⁴

Group C consists of the cyclic amines, *N*-methylimidazole,

amines exhibit an increase in pH in the presence of moderate concentrations of formaldehyde (0.1 to 1.0 M), followed by a decrease in pH at high concentrations (>3.0 M). The results observed with pyridine are shown in Fig. 5 and those with *N*-methylimidazole in the upper curve of Fig. 6; similar results were obtained with triethylenediamine, but the amount of the pH increase, although significant, is too small to permit a quantitative treatment with this compound. The decrease in pH at high formaldehyde concentrations is attributed to an activity coefficient or electrode effect, similar to that observed with other tertiary amines. The increase in pH at lower concentrations of formaldehyde is difficult to account for by any such nonspecific effect and is attributed to a reaction of the amine with formaldehyde to form the cationic addition compound, $\text{R}_3\text{N}^+\text{CH}_2\text{OH}$



⁴ It has been reported that the addition of formaldehyde to dimethylglycine causes a decrease in pH (19). However, the formaldehyde solution used in these experiments had a pH of 4.8 and may have contained formic acid, in spite of purification by distillation over calcium carbonate.

TABLE III

Tertiary amines: maximum shift observed in pH in 0 to 13 M formaldehyde and equilibrium constants for adduct formation between formaldehyde and protonated amines at 25° and water activity 1.0

Compound	pK' _a	$\Delta\text{pH}_{\text{max}}$	K_T^a
A. Triethylamine ^{b-d}	10.75	0.30	
<i>N,N'</i> -Dimethylpiperazine ^{e-g}	4.15	0.34	
	8.26	0.40	
<i>N,N</i> -Dimethylcyclohexylamine ^{c,e,f}	10.59	0.40	
<i>N</i> -Methylpiperidine ^{c,h,i}	10.22	0.60	
<i>N</i> -Methylpyrrolidine ^{c,h,i}	10.43	0.97	
B. <i>N,N</i> -Diethylglycine ^{b-d}	10.65	0.09	
<i>N</i> -Methylmorpholine ^{c,i,k}	7.51	0.09	
C. <i>N</i> -Methylimidazole ^{c,i,l}	7.15	-0.67	1.6
Pyridine ^{c,f,i}	5.27	-0.32	0.5
Triethylenediamine ^{c,f,i}	8.81	-0.12	m

^a $K_T = [\text{R}_3\text{NCH}_2\text{OH}]/[\text{R}_3\text{NH}^+][F_F]$.

^b 0.2 M.

^c 10% free base.

^d Ionic strength, 0.2 M.

^e Ionic strength, 0.025 to 0.075 M.

^f 0.05 M.

^g Measured at 40% free base for pK' 8.26 and 90% monocation for pK' 4.15.

^h 0.3 M.

ⁱ Ionic strength, 0.3 M.

^j 50% free base.

^k 0.022 to 0.025 M.

^l 0.1 M.

^m Too small to be measured accurately.

Effect of partial replacement of water by nonaqueous solvents on apparent pH of triethylamine buffer solutions^a

Methanol	pH	ΔpH	Ethylene glycol	pH	ΔpH
M			M		
0	9.70	0	0	9.70	0
1.6	9.68	0.02	0.1	9.67	0.03
6.2	9.53	0.27	4.5	9.47	0.23
12.4	9.15	0.55	8.9	9.25	0.45
18.5	8.68	1.02	13.4	8.84	0.86

^a Temperature, 25°; 0.05 M buffer; ionic strength, 0.05 M.

(Equation 6). The net effect of such a reaction is to decrease the concentration of the acidic form of the amine buffer, and, therefore, to increase the pH. This reaction is chemically analogous to the reaction of protonated secondary amines with formaldehyde to form the protonated hydroxymethyl addition compound, $\text{R}_2\text{NH}^+\text{CH}_2\text{OH}$ (Equation 1). The changes in the pH of an amine buffer caused by this reaction are described by Equation 7,

$$\Delta\text{pH} = -\log (1 + K_T[F_F]) \quad (7)$$

in which $K_T = \frac{[\text{R}_3\text{NCH}_2\text{OH}]}{[\text{R}_3\text{NH}^+][\text{CH}_2(\text{OH})_2]}$. The

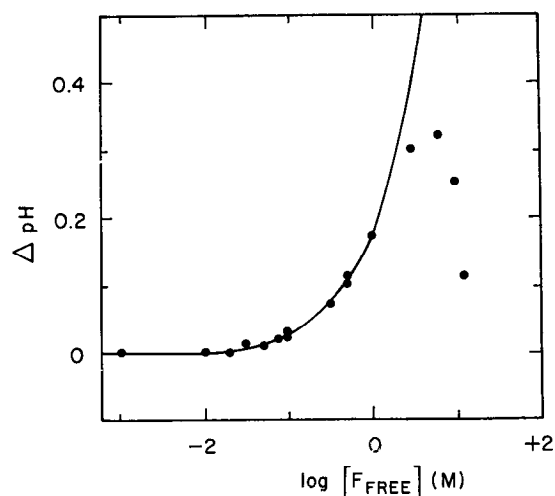


FIG. 5. The change in the pH of 0.05 M pyridine buffers, 50% free base, as a function of the logarithm of the formaldehyde concentration at 25°, ionic strength 0.025 M. The solid line is based on the equation

$$\Delta\text{pH} = -\log(1 + 0.5[F_F])$$

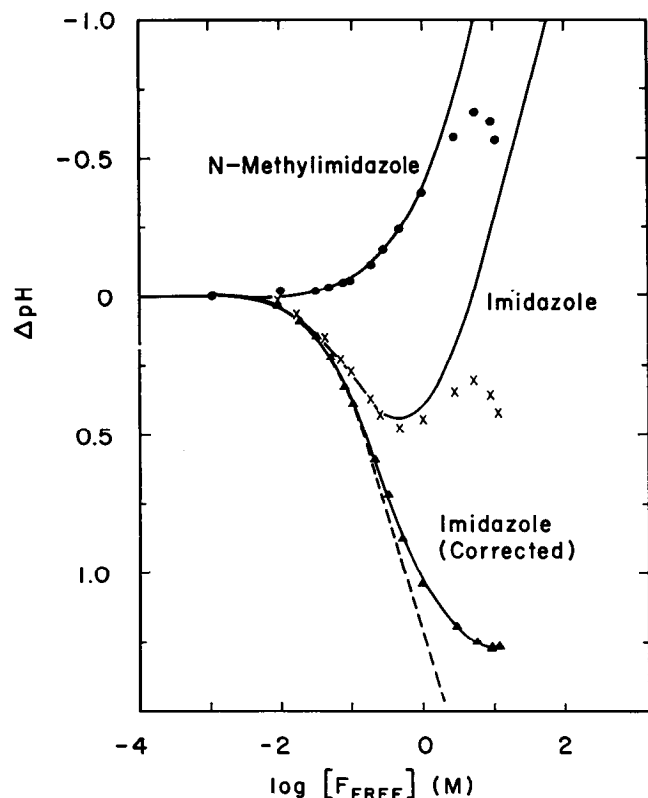


FIG. 6. The change in the pH of imidazole and *N*-methylimidazole buffers as a function of the logarithm of the formaldehyde concentration at 25°. The *N*-methylimidazole was 0.1 M, 50% free base, ionic strength 0.05 M, and the solid line is based on the equation

$$\Delta\text{pH} = -\log(1 + 1.6[F_F]).$$

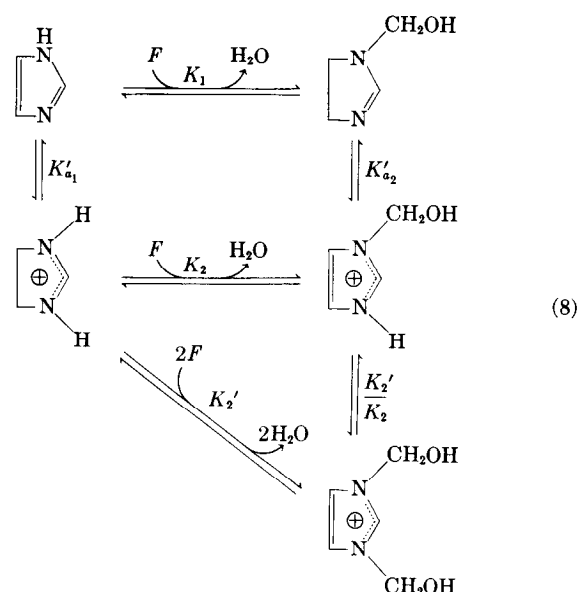
The imidazole was 0.05 M, 50% free base, ionic strength 0.025 M and the solid line is based on the equation

$$\Delta\text{pH} = \log(1 + 15[F_F]) - \log(1 + 3.2[F_F] + 2.3[F_F]^2)$$

The lower solid line is a corrected curve for imidazole (see text); the lower dashed line is the theoretical line if no protonation of the hydroxymethyl adduct occurred.

equilibrium constants calculated from Equation 7 for the reactions of the amines of Group C are given in Table III and were used to calculate the solid lines shown in Figs. 5 and 6. The theoretical lines agree satisfactorily with the observed changes in pH at moderate concentrations of formaldehyde and then deviate from the experimental values as the activity coefficient and electrode effects of concentrated formaldehyde become significant. The values of the equilibrium constants for these tertiary amines are similar to those for the reaction of protonated secondary amines with formaldehyde (K_2 , Table II).⁵

Imidazole—The changes in the pH of imidazole buffers in the presence of formaldehyde are the most complex of those for any of the amines examined (Fig. 6, ×). With increasing formaldehyde concentration there is observed a decrease, then an increase, and finally a second decrease in the observed pH. This complex behavior was analyzed with the aid of the data obtained for *N*-methylimidazole (Fig. 6, ●) and the scheme of Equation 8. It was assumed that the equilibrium constant for the addition



of formaldehyde to the conjugate acid of imidazole, K_2 , is the same as that for the analogous addition of formaldehyde to *N*-methylimidazole cation (allowing for the presence of two indistinguishable sites in the imidazolium cation by the insertion of a statistical factor of 2) and that activity coefficient and electrode effects of concentrated formaldehyde solutions are similar for the two reactions. The insertion of this statistical factor of 2 has only a small effect on the analysis of the data for imidazole. Thus, the increase and then the decrease in pH which are observed at high formaldehyde concentrations (Fig. 6) may be accounted for in the same way as the similar changes in pH with *N*-methylimidazole buffers. The experimental data for imidazole were corrected for (a) the reaction of imidazolium cation with formaldehyde, based on K_T for *N*-methylimidazole and the statistical factor of 2, and (b) the solvent effect observed with *N*-methylimidazole, *i.e.* the difference between the theoretical line and the experimental points at high formaldehyde concentrations with this compound. The corrected imidazole curve,

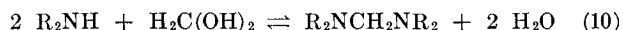
⁵ P. Le Hénaff has kindly informed us that he has obtained a value of $K_T = 4.3$ at 25° for the corresponding reaction of hexamethylene tetramine with formaldehyde.

shown as the *triangles* in Fig. 6, resembles the curves obtained with other secondary amines, although the values of ΔpH are smaller. From this curve, the values of K_1 , for the addition of formaldehyde to imidazole, and of K'_2/K_2 , for the addition of a second hydroxymethyl group to 1-hydroxymethylimidazole cation, may be calculated (Table II). The two lower *solid lines* of Fig. 6 have been calculated with these values of the equilibrium constants and show satisfactory agreement with the ex-

$$\Delta\text{pH} = \log(1 + K_1[F_F]) - \log(1 + K_2[F_F] + K'_2[F_F]^2) \quad (9)$$

perimental data. Equation 9 is the complete equation for this system.

Methylenediamine Formation—Two methods were used to demonstrate the formation of the corresponding methylenediamine from morpholine and formaldehyde (Equation 10) and



to determine the equilibrium constant for the reaction. In the first method, a solution of known pH was prepared from morpholine, morpholine hydrochloride, and formaldehyde, and to this solution were added increasing amounts of a solution which was prepared from exactly equimolar morpholine and formaldehyde. The concentrations of the reactants were such that the amount of free formaldehyde in solution was negligible compared to that of the other reactants. Under these conditions, there should be no change in pH if methylenediamine formation does not occur, because the pH is determined by the ratio $[\text{R}_2\text{NH}]/[\text{R}_2\text{NH}_2^+]$ and, from the known value of K_1 , all of the added amine would react with the added formaldehyde to form hydroxymethylamine. Formation of methylenediamine from the added hydroxymethylamine will cause a decrease in pH, because it will decrease the concentration of free amine and the $[\text{R}_2\text{NH}]/[\text{R}_2\text{NH}_2^+]$ ratio. Such a decrease is, in fact, observed on the addition of hydroxymethylmorpholine to morpholine buffers to which had been added two different concentrations of formaldehyde initially; this is evidence for the formation of methylenedimorpholine in these solutions (Fig. 7). It was shown that the maximal changes

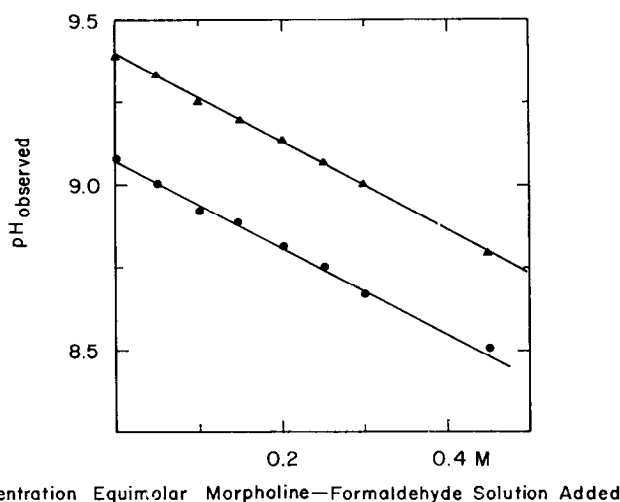


FIG. 7. The effect on the pH of 0.6 M morpholine buffers, 80% free base, of the addition of exactly equimolar amounts of formaldehyde and morpholine at 25° and ionic strength 1.0 M. ▲—▲, 0.05 M formaldehyde added initially; ●—●, 0.15 M formaldehyde added initially.

TABLE V
Calculated equilibrium constant (K_D) for methylenedimorpholine formation at 25°, ionic strength, 1.0M^a

pH	pH - pK'	Total formaldehyde [F _T]	Total base [N _T]	Free base ^b [N _F]	Hydroxymethylmorpholine ^c [N _H]	Methylenedimorpholine ^c [N _D]	[N _B]/[N _H]	K_D
		M	M	M	M	M		
9.07	0.19	0.30	0.726	0.192	0.066	0.234	8.08	14,750
9.00	0.12	0.35	0.776	0.164	0.088	0.262	6.95	14,500
8.94	0.06	0.25	0.576	0.142	0.066	0.184	6.58	15,750
8.85	-0.03	0.30	0.626	0.116	0.090	0.210	5.67	16,100
8.79	-0.09	0.50	0.926	0.101	0.175	0.375	4.72	14,740
8.78	-0.10	0.35	0.676	0.099	0.124	0.227	4.64	14,700
8.70	-0.18	0.40	0.726	0.082	0.156	0.244	4.13	15,250
8.62	-0.26	0.45	0.776	0.068	0.192	0.258	3.69	15,800

^a Original morpholine buffer, 0.6 M; 80% free base.

^b Calculated from $\text{pH} = \text{pK}'_a + \log[\text{base}]/[\text{acid}]$; $\text{pK}'_a = 8.88$.

^c N_H and N_D were calculated, assuming F_F to be negligible, from the simultaneous equations:

$$N_T - N_F = N_H + 2N_D \quad (1)$$

$$F_T = N_H + N_D \quad (2)$$

$K_D = \frac{[\text{N-CH}_2\text{-N}]}{[\text{NH}]^2} [\text{CH}_2(\text{OH})_2]$; calculated from Equation 12.

occurred within 1 min and were constant for 60 min, which indicates that equilibrium is attained rapidly in this system.

It may be shown² that the pH of solutions in which hydroxymethylamine and methylenediamine formation occur is given by Equation 11, in which N_T is the concentration of free amine

$$\text{pH} = \text{pK}'_a + \log \frac{[N_T]}{[R_2\text{NH}_2]} - \log(1 + K_1[F_F] + 2K_D[N_F][F_F]) \quad (11)$$

prior to the addition of formaldehyde, N_F is the concentration of free amine at equilibrium, and $K_D = [\text{R}_2\text{NCH}_2\text{NR}_2]/[\text{R}_2\text{NH}]^2 [\text{CH}_2(\text{OH})_2]$. The value of K_D may be obtained from the experimental results by successive approximations. Alternatively, under conditions in which the amount of free formaldehyde is negligible compared to the amount combined with amine, it may be shown that

$$\frac{[N_B]}{2[F_T] - [N_B]} = 1 + 2 \frac{K_D}{K_1} [N_F] \quad (12)$$

in which N_B is the concentration of amine which is bound as hydroxymethylamine and methylenediamine ($[N_B] = [N_T] - [N_F]$). The concentration of free amine is known from the pH and the concentration of R_2NH_2 , so that K_D may be calculated from the slope, $2K_D/K_1$, of a plot of the left side of Equation 12 against N_F . The results, shown in Fig. 8, give a value of $K_D = 15,200 \pm 600$ for the morpholine reaction. The experimental data and the values of K_D calculated at each concentration of reactants are given in Table V.

In the second method, the pH is determined with increasing total morpholine buffer concentrations in the presence of a constant total formaldehyde concentration (Table VI). Under these conditions, the observed pH is 0.7 to 0.8 unit lower than

the calculated pH, based on the total formaldehyde and free base amine concentrations and the equilibrium constant for hydroxymethylamine formation (Equation 1), which indicates that the formaldehyde binds more of the basic form of the buffer than can be accounted for by hydroxymethylamine formation. The equilibrium constant, K_D , for methylenediamine formation calculated from these data and Equation 12 is $15,400 \pm 200$ (Table VI), which agrees satisfactorily with the results of the first method. These results may be compared to values of K_D of $27,000 \pm 4,000$ at 20° (calculated for 25° , $18,300 \pm 3,000$), reported by Le Hénaff (17), and 12,300, obtained by Skell and coworkers.¹

These treatments require that a significant fraction of the methylenediamine does not become protonated under the conditions of the experiments. The absence of a significant trend of the calculated equilibrium constants, K_D , in Tables V and VI with decreasing pH indicates that this condition is met for these experiments. However, at lower pH values, a tendency toward decreasing values of K_D with decreasing pH was noted, which suggests that significant protonation of the methylenedimorpholine does occur below pH 8.0. This is not unexpected in view

of the fact that the pK values of the cyclic methylenediamine adducts of *N,N'*-biphenylethylenediamines (imidazolidines) are 0.3 to 1.6 units lower than those of the parent amines (23). The accuracy of determinations at higher pH values is limited by the buffering properties of hydrated formaldehyde itself. The accurate determination of K_D for the morpholine compounds is therefore limited to the pH range 8 to 9.

DISCUSSION

The experimental results will be discussed first from an empirical point of view, and, in a later section, the effects of perturbing factors on titrations in the presence of concentrated formaldehyde will be considered. The principal conclusions may be drawn from experiments which were carried out at moderate concentrations of formaldehyde and are supported by appropriate control experiments; the various perturbing effects in concentrated formaldehyde solutions tend to cancel each other out or give a small effect in the opposite direction from that caused by the formation of cationic hydroxymethylamines.

The leveling off of plots of ΔpH against $\log [F_F]$ or of antilog ΔpH against $[F_F]$ with increasing formaldehyde concentration is evidence for protonation of the hydroxymethyl adducts which are formed from secondary amines and formaldehyde (Equation 1, Figs. 2 and 3). The sigmoid curve which is observed in the logarithmic plot (Fig. 2) may be described as follows. At very low formaldehyde concentration, no hydroxymethyl adduct is formed and the pH of the amine buffer remains unchanged. As the formaldehyde concentration is increased, the free base form of the amine combines with formaldehyde to form the hydroxymethyl adduct, so that the ratio $[R_2\text{NH}]/[R_2\text{NH}_2^+]$ decreases and the pH decreases. At higher formaldehyde concentrations, formaldehyde also combines with the protonated amine, $R_2\text{NH}_2^+$, to form $R_2\text{NHCH}_2\text{OH}$. At very high formaldehyde concentrations, almost all of the amine, both free and protonated, is combined with formaldehyde, so that the pH is controlled only by the ratio $[R_2\text{NCH}_2\text{OH}]/[R_2\text{NHCH}_2\text{OH}]$, and a further increase in formaldehyde concentration does not affect the pH, because it does not change the concentrations of the buffer species which regulate the pH. The difference between the two horizontal segments of the curve ($\Delta\text{pH}_{\text{max}}$ of Table II) is, therefore, the difference between the pK of the amine and that of the hydroxymethyl adduct of the amine, which varies from 2.2 to 3.0 pH units (Table II).

That the leveling off of these curves with increasing formalde-

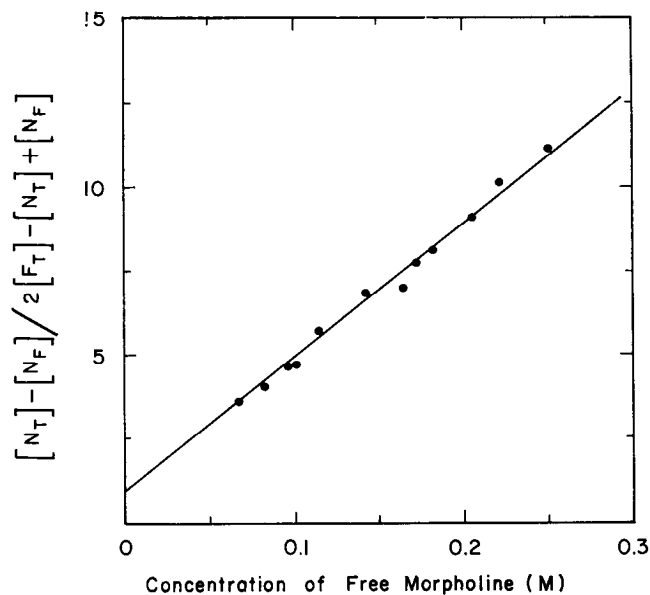


FIG. 8. Graphical method for the determination of the equilibrium constant for methylenediamine formation from morpholine and formaldehyde, based on Equation 12.

TABLE VI

Calculated equilibrium constant for methylenedimorpholine formation at formaldehyde concentration 0.5 M; morpholine buffer 80% free base at 25° ; ionic strength, 1.0 M

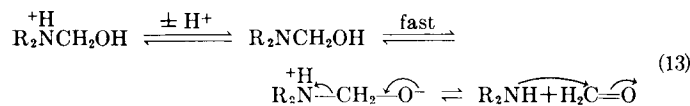
Solution	pH observed	pH expected for N_H formation only	$\text{pH}_{\text{obs}} - \text{pK}'_a$	Total buffer	Total base $[N_T]$	Bound base ^a $[N_B]$	Free base $[N_F]$	Free formaldehyde; calculated $[F_F]^b$	K_D^b
				M	M	M	M	M	M ⁻²
Control 1	9.45	9.48	0.00	0.05	0.040		0.040		
Control 2	9.49	9.48	0.00	1.20	0.96		0.960		
A	8.03	8.83	-0.85	0.80	0.64	0.618	0.022	0.01910	15,400
B	8.31	9.06	-0.57	1.00	0.80	0.746	0.054	0.00562	15,600
C	8.56	9.16	-0.32	1.20	0.96	0.845	0.115	0.00169	15,300

^a Calculated from $\text{pH} = \text{pK}'_a + \log [\text{base}]/[\text{acid}]$; $\text{pK}'_a = 8.88$; and $[N_B] = [N_T] - [N_F]$.

^b Calculated from $K_D = [N_B] - K_H[N_F][F_F]/2[N_F]^2[F_F]$ and $[N_T] = [N_F] + [N_H] + 2[N_D]$.

hyde concentration is caused by protonation of the hydroxymethyl adduct and not by some medium effect of the concentrated formaldehyde solutions is shown by the fact that no such leveling off is observed with primary amines, nor with secondary amines which, for steric reasons, have lower affinity constants for formaldehyde and therefore cannot be studied in formaldehyde solutions that are sufficiently concentrated to form the protonated hydroxymethyl adducts. In the case of primary amines, a second formaldehyde molecule adds to the free amine to form the dihydroxymethyl adduct at relatively low formaldehyde concentrations, before protonation of the monohydroxymethyl adduct occurs. If the addition of one hydroxymethyl group lowers the basicity of an amine by 2 to 3 pK units, the addition of two hydroxymethyl groups might be expected to lower the basicity by such an amount that protonation of the dihydroxymethyl adduct would not be easily detectable in the experimentally accessible range of formaldehyde concentrations. The medium effects which are observed with tertiary amines at very high formaldehyde concentrations cause downward deviations of the plots, rather than the leveling off which is attributed to protonation of the hydroxymethylamines.

The most direct evidence for the existence of the hydroxymethyl derivative comes from direct titration at low temperature (Fig. 4). If morpholine or piperidine is completely converted to the hydroxymethyl adduct, the solution may be rapidly acidified without hydrolysis of the adduct because of the kinetic stability of the protonated adduct in acid solution. As in other reactions of aldehydes with basic amines (24), it is the species with no net charge that undergoes hydrolysis (Equation 13). Measured



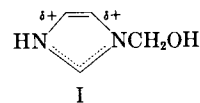
amounts of base may then be added to this acidified and diluted solution to convert the adduct partially to the free base form. Rapid hydrolysis follows, but, by working at 1° and extrapolating the pH readings to zero time, it is possible to measure the pH of the solution that contains only the free and protonated hydroxymethylamine adducts and hence to determine the pK' of this species.

If an equilibrium exists between a protonated secondary amine and formaldehyde to form the protonated hydroxymethyl adduct, $\text{R}_2\text{NHCH}_2\text{OH}^+$, it would be expected that a similar equilibrium should exist between a protonated tertiary amine and its hydroxymethyl adduct, $\text{R}_3\text{NCH}_2\text{OH}^+$ (Equation 6), and that this equilibrium should be detectable in sterically favorable cases. Evidence for such a reaction was, in fact, found for the tertiary amines pyridine, *N*-methylimidazole, and triethylenediamine, in which steric hindrance is minimal because the substituents on the amine group are pinned back by the ring structures. The effect of this equilibrium is to cause an increase, rather than a decrease, in pH, because the net effect of the reaction is to remove the protonated species of the amine buffer from the equilibrium which controls the pH (Equation 6, Figs. 5 and 6). The situation is analogous to that with buffers prepared from thiols and acidic imines, with which formaldehyde also combines to cause an increase in pH by decreasing the concentration of the acidic species of the buffer (25). The

equilibrium constants for the reactions of formaldehyde with protonated tertiary amines are near 1.0 and are similar to those for the corresponding reactions of protonated secondary amines.

It should be kept in mind that the existence of an equilibrium between two compounds does not imply anything about the kinetic pathway for the interconversion of the two compounds. In particular, although it is convenient to discuss the equilibrium for the addition of formaldehyde to protonated secondary and tertiary amines to form cationic hydroxymethyl adducts, this does not imply that the protonated amine reacts directly with formaldehyde. In fact, in sufficiently acidic solution, equilibrium is not reached rapidly with these and other amines, and it is certain that the reactions occur, as with other basic amines (24), through a reaction of the free base form of the amine and the species of the hydroxymethyl adduct which has no net charge (Equation 13); the addition of protons to these species presumably occurs rapidly through diffusion-controlled reactions.

Imidazole—Levy found a small decrease in the pH of 4(5)-methylimidazole and imidazolelactic acid buffers on the addition of formaldehyde and noted that this could be explained either by a solvent effect in the absence of a reaction of these compounds with formaldehyde or by a reaction of both the basic and acidic forms with imidazole (26). In the formol titration of histidine and of proteins, it seems to have been generally assumed that the imidazole residues do not react with formaldehyde (1, 26, 27). Our results (Fig. 6) strongly suggest that imidazole and *N*-methylimidazole do react readily with formaldehyde, although the resulting changes in pH are not large.⁶ The complex changes in pH observed on the addition of formaldehyde to imidazole buffers (Fig. 6) reflect the several different equilibria for the addition of formaldehyde and protons to this compound (Equation 8). The relatively small decrease in pH on the addition of formaldehyde and the ΔpK of only 0.7 unit between free imidazole and its hydroxymethyl adduct undoubtedly reflect the fact that with this amine protonation of the monohydroxymethyl adduct occurs at a different nitrogen atom from that which is hydroxymethylated (Equation 8). The effect of the hydroxymethyl group is therefore attenuated and results in only a small decrease in the basicity of the second nitrogen atom. An equivalent explanation of the same effect is that the positive charge of the protonated hydroxymethyl adduct of imidazole is distributed over 2 nitrogen atoms by resonance (I), instead of being localized on a single nitrogen atom as in ordinary amines; this will tend

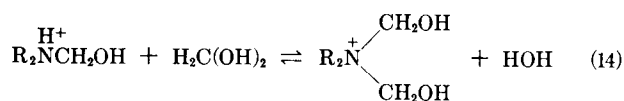


to decrease the destabilizing effect of this substituent. This conclusion suggests that the assumption that protonation and hydroxymethylation occur at the same site in the formol titration of adenine (9) does not have general validity and may be incorrect even for adenine. The addition of the hydroxymethyl group and the proton to different nitrogen atoms of adenine would account for the small ΔpK which is observed with this

⁶ L. J. Saidel and R. L. Carino have recently reported in a preliminary communication (*Federation Proc.*, **25**, 796 (1966)) that the reaction of formaldehyde with the imidazole group of amino acids and proteins may be followed spectrophotometrically at 230 m μ .

compound. Similarly, the considerable stability of the product of this reaction, compared to that of other protonated hydroxymethylamines, may be attributed to the delocalization of its positive charge over 2 nitrogen atoms.

The other equilibria that involve imidazole are described in Equation 8 and "Results." Of all the secondary amines examined, only imidazole shows evidence for the combination of the protonated hydroxymethyl adduct with a second mole of formaldehyde. The failure to detect such a reaction with other secondary amines (Equation 14) is presumably caused principally



by the steric hindrance which is introduced by the addition of the first hydroxymethyl group. The absence of a detectable reaction with hydroxymethylmorpholine, for example, is not surprising in view of the absence of an observable reaction of formaldehyde with *N*-methylmorpholine.

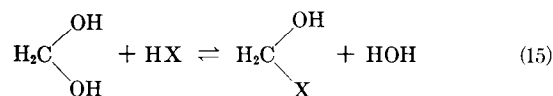
Methylenediamines—The equilibrium constant $K_M = K_D/K_1 = [\text{R}_2\text{NCH}_2\text{NR}_2]/[\text{R}_2\text{NCH}_2\text{OH}][\text{R}_2\text{NH}]$ for the formation of methylenedimorpholine from hydroxymethylmorpholine and a second molecule of morpholine is 19.4 M^{-1} . This may be compared to the value of $K_1 = 800 \text{ M}^{-1}$ for the formation of hydroxymethylmorpholine from morpholine and formaldehyde and indicates that the tendency of a hydroxymethylamine molecule to combine with an amine is some 40-fold less than that of methylene glycol. This difference may be attributed to some degree of crowding in the methylenedimorpholine molecule and to the destabilizing effect of the two electronegative *gem*-hydroxyl groups in methylene glycol, which is relieved by methylenediamine formation. The magnitude of this equilibrium constant indicates that methylenediamine formation will occur and the results of the formol titration will be dependent on the amine concentration only in relatively concentrated solutions of amine.

Fernandez and Butler (28) found that the heat evolution on mixing 1 eq of formaldehyde with 2 eq of morpholine in water is much larger than that observed on the addition of a second equivalent of formaldehyde and concluded that the preponderant product in these solutions is the methylenediamine. This conclusion rests on assumptions regarding the heats of hydroxymethylamine and methylenediamine formation. Although the exact experimental conditions are not given, the composition of the solutions examined by Fernandez and Butler may be estimated roughly from their reported experimental procedure and the values of K_1 and K_D reported here. Such an estimate indicates that after the addition of the first equivalent of formaldehyde the solution examined by these workers probably contained approximately 20% free amine, 15% hydroxymethylamine, and 65% methylenediamine, and that after the addition of the second equivalent of formaldehyde it contained approximately 2% free amine, 72% hydroxymethylamine, and only 26% methylenediamine. Although these values are not exact, they do suggest that caution should be exercised in the use of measured heats of reaction to determine the composition of solutions of amines and formaldehyde in the absence of knowledge of the heats of the several reactions which may occur in such solutions.

Effect of Structure on Association Constants

Steric Effects—The steric requirements of the amine are of primary importance in determining the equilibrium constant for the addition of formaldehyde to the amine (1). This is evident from the decrease in association constant on the introduction of substituents α or β to the amine nitrogen atom (Tables I to III and Reference 1) and is strikingly illustrated by logarithmic plots of association constants against the pK of amines (not shown). Such plots resemble scatter diagrams and show no relationship of the affinity constants to amine basicity, but it is evident that there is a regular increase in association constant with decreasing steric demands of the amine. Steric effects are of particular importance in two respects for the results reported here. The reduced steric demands of dimethylamine and of cyclic amines, such as piperidine and morpholine, result in a large increase in the affinity of these compounds for formaldehyde compared to diethylamine and other open chain secondary amines (Table II). It also makes possible the measurement of the comparatively favorable equilibrium constants for the formation of the protonated hydroxymethyl adducts of dimethylamine and cyclic secondary amines (Table II) and for the formation of the cationic hydroxymethyl derivatives of cyclic tertiary amines (Table III); the equilibrium constants for the addition of formaldehyde to noncyclic tertiary amines are too small for detection by the methods used here. Secondly, the equilibrium constants for the addition of a second mole of formaldehyde to primary amines are one to two orders of magnitude smaller than those for addition of the first mole (Table I, Reference 1) and the equilibrium constants for the addition of a second mole of formaldehyde to secondary amines are too small to be detected (except for the special case of imidazole). This may be ascribed to the increased steric requirements which are introduced by the addition of the first hydroxymethyl group in each case.

Polar Effects—The equilibrium constants and the corresponding free energies of the reaction for the addition of different classes of compounds to formaldehyde have been summarized in Table VII. These equilibrium constants are based on water molarity, rather than the convention that the activity of pure water is 1.0, in order to make possible a comparison of affinities relative to water. Since formaldehyde is hydrated in water, the equilibria refer to the reaction of Equation 15 and reflect the



differences in energy obtained on cleaving a C—O and X—H bond and forming a C—X and O—H bond. Two of these changes are constant for all of the reactions, so that the equilibrium constants and free energies are primarily a measure of the relative affinity of the group X for a proton and for the carbon atom of the hydroxymethyl group (3, 24).

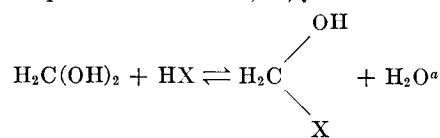
There is very little effect of the polar character of the group HX, as reflected in its acidity or basicity, on the equilibrium constant for addition to formaldehyde and other carbonyl compounds. The equilibrium constants for the addition of amines to the carbonyl group of pyruvate anion vary only 6-fold in the series semicarbazide, methoxyamine, hydroxylamine, and hydrazine, whereas the basicity of the amines varies by 10^4 (35). Still more striking is the difference of only 3-fold in the equilib-

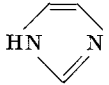
rium constants for the addition to formaldehyde of a series of acidic carbon compounds, $\text{HC}(\text{NO}_2)_2\text{R}$, over a range of 10^{11} in the acidity of the addend (34). The absence of a correlation of amine basicity with affinity for formaldehyde has been noted above and is especially clearly reflected in the approximately 2-fold difference in the affinities of piperidine and morpholine, which are of similar structure, but vary 200-fold in basicity, and in the similar affinities of aliphatic primary amines (pK 10 to 11) compared to urea, thiourea, and amides (pK 0 to 1). Hine and Weimar (3) have pointed out that this insensitivity to polar effects is not unexpected, because hydrogen and the hydroxymethyl group exhibit similar polar properties (3). The σ_p values are 0 and -0.01 , and the σ^* values are 0.49 and 0.56 for hydrogen and hydroxymethyl, respectively (36, 37). However, these σ values may be influenced by electron donation from the hydroxymethyl group by hyperconjugation and the decreased basicity of hydroxyethylamine (pK'_a 9.74; cf. the pK'_a of methoxyethylamine = 9.45) compared to that of ethylamine (pK'_a 10.81 (38)) suggests that in the absence of hyperconjugation the hydroxymethyl group has somewhat more electron-withdrawing effect than is suggested by the σ values.

The problem, then, is to account for the fact that the basicity of hydroxymethylamines is two to three orders of magnitude less than that of free amines. This is the same as accounting for the fact that the affinity of protonated amines for formaldehyde is two to three orders of magnitude less than that of free amines, because these equilibria are different expressions of the same free energy differences (Equation 1). Steric, polar, and solvation effects may be considered as possible explanations for these differences. Although there has been controversy regarding this point, it is probable that a lone pair of electrons on nitrogen has slightly smaller steric requirements than a hydrogen atom (39). This provides a partial explanation for the decreased basicity of hydroxymethylamines, but this effect is almost certainly not large and would not be expected to account for more than a small fraction of the observed decrease in basicity. There is a very large difference in the polar nature of a free and protonated amine; for example, the difference in the pK_a of free and protonated amines is about 10^{20} (40). Although the polar effect probably makes a significant contribution, the insensitivity of equilibria for hydroxymethyl compound formation to polar effects and the similar polar properties of hydrogen and the hydroxymethyl group noted above suggest that even this large difference cannot account for a difference of several orders of magnitude between the equilibria for the protonation of free and of hydroxymethylamines.

Solvation effects, therefore, should be considered as a possible cause of the differences in these equilibria. The solvation energy of a protonated, cationic amine is larger than that of the free amine and increases with an increase in the number of protons on the amine, so that tertiary amines are weaker bases than would otherwise be expected (41-44). The large decrease in the basicity of methoxyamine (pK_a 4.6) compared to hydroxylamine (pK_a 6.0) and of *N,N*-dimethylhydrazine (pK_a 7.2 (45)) and tetramethylhydrazine (pK_a 6.3) compared to hydrazine (pK_a 8.1 (46)) is difficult to explain on any other basis than by a decrease in the solvation energy of the cation caused by substitution of a methyl group for hydrogen, which may be on an atom adjacent to that which undergoes protonation. Hall's correlation of amine basicity with σ^* (42) suggests that the difference in solvation of

TABLE VII
Equilibrium constants, K , for reaction



HX	K	$-\Delta F$	Reference
		cal/mole	
H_2O	1	0	
HOCH_2OH	4.6	800	29
HOOH	1,600	4,400	30
HSR	34,000	6,200	12
H_2NR	300-3,000	3,400-4,700	This work; 1
$\text{HN}(\text{CH}_2\text{OH})\text{R}$	15-200	1,600-3,100	This work; 1
HNR_2	100-2,000	2,700-4,500	This work; 1
HNR_2 (cyclic)	4,500-90,000	5,000-6,700	This work; 1
	800	4,000	This work
H_2NR_2 (cyclic)	25-400	1,900-3,500	This work
HNR_3 (cyclic)	50	2,300	This work
H_2NCNH_2	2,800	4,700	31
H_2NCNH_2	1,000	4,100	32
H_2NCR	1,200	4,200	33
$\text{HC}(\text{NO}_2)_2\text{R}$	$0.6-2.0 \times 10^6$	8,000-8,500	34

^a At 20-25°. $K = [\text{H}_2\text{C}(\text{X})\text{OH}][\text{H}_2\text{O}]/[\text{H}_2\text{C}(\text{OH})_2][\text{HX}]$ with water concentration expressed in molar units; *i.e.* $K = 55.5 K'$, for which $K' = [\text{H}_2\text{C}(\text{X})\text{OH}]/[\text{H}_2\text{C}(\text{OH})_2][\text{HX}]$ and the activity of pure water is taken as 1.0.

secondary and tertiary protonated amines results in a base-weakening effect of some 2.5 pK units, which agrees well with the observed differences between the pK values of free and hydroxymethylated secondary amines. The difference of 0.06 unit in the σ^* values of hydrogen and the hydroxymethyl group corresponds to a base-weakening effect of only about 0.2 pK unit. We conclude, therefore, that the decrease in solvation energy brought about by the replacement of a hydrogen atom by a hydroxymethyl group is the principal cause of the decreased basicity of hydroxymethylamines and, conversely, of the reluctance of protonated amines to add a hydroxymethyl group, and that polar and steric effects of the hydroxymethyl group contribute significant but smaller influences in the same direction.

Other Correlations—The data summarized in Table VII confirm and extend previously reported correlations of structure with the relative affinity of compounds for hydrogen and for addition to the carbonyl group (3, 24). The order of nucleophilic reactivity toward the carbonyl group of $\text{RS}^- > \text{RO}^- > \text{HO}^-$, which reflects relative transition state stabilities, parallels the order of the equilibrium constants for the addition of the conjugate acids of these compounds to formaldehyde. The

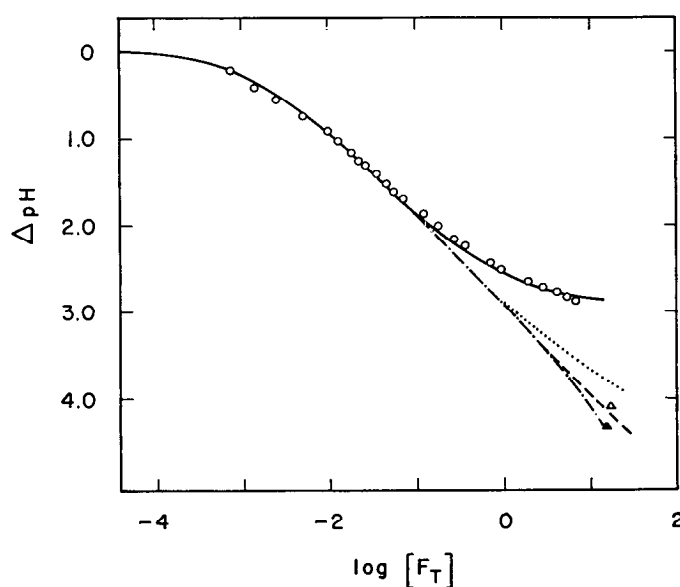


FIG. 9. The influence of various factors on the relationship between ΔpH and the logarithm of the total formaldehyde concentration, $[F_T]$, in concentrated solutions. The lines are calculated for morpholine buffers, as explained in the text. ---, effect of the change in water concentration, based on

$$\Delta\text{pH} = \log (1 + K_1[F_T]/[\text{HOH}])$$

Δ , correction for activity coefficient and electrode effects for morpholine in 13 M formaldehyde, based on measurements with *N*-methylmorpholine. \blacktriangle , average activity coefficient-electrode correction for amine buffers in 13 M formaldehyde. \cdots , effect of formaldehyde polymerization, based on $\Delta\text{pH} = \log (1 + K_1[E])$ (see Equation 16). ---, theoretical curve based on a summation of the above effects, but assuming no protonation of the hydroxymethyl adduct; $\Delta\text{pH} = \log (1 + K_1\lambda[F_T]/[\text{HOH}] - \log \gamma_N/\gamma_{\text{NH}^+})$. —, theoretical curve for morpholine based on a summation of the above effects and including protonation of the hydroxymethyl adduct, based on the equation

$$\Delta\text{pH} = \log (1 + K_1\lambda[F_T]/[\text{HOH}]) / (1 + K_2\lambda[F_T]/[\text{HOH}]) - \log \gamma_N/\gamma_{\text{NH}^+}$$

\circ , experimental points, obtained with methanol-free formaldehyde.

unusual nucleophilic reactivity of hydrogen peroxide, an "α effect" compound, is similarly reflected in the large equilibrium affinity of hydrogen peroxide for the carbonyl group.

Perturbing Effects on Formol Titration in Concentrated Formaldehyde Solutions—It is remarkable that the results of formol titrations should appear to follow any simple quantitative relationship in concentrated formaldehyde solutions without correction for the changes in water concentration, the polymerization of formaldehyde, the presence of methanol in formalin, and medium effects on activity coefficients and electrodes, which might be expected to have significant effects in such solutions. An attempt will be made to evaluate the effects of these factors in this section; the results are summarized in Fig. 9.

Polymer Formation—In concentrated solutions, the amount of formaldehyde which is available for reaction with amines is less than the stoichiometric amount of formaldehyde in solution because a significant fraction of the total formaldehyde exists as polyoxymethylene polymers (6). An attempt was made to evaluate the effect of formaldehyde polymerization on the binding

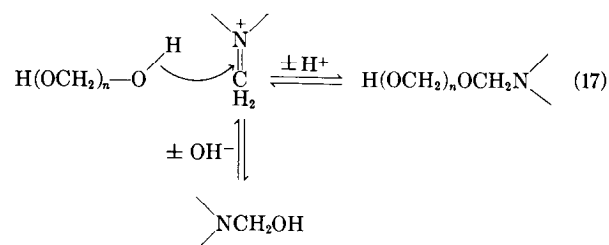
of formaldehyde to amines by assuming that each molecule of polymer has the same tendency to react with a molecule of amine as does a molecule of hydrated formaldehyde (methylene glycol). The terminal hydroxymethyl units of the polymer, $\text{HOCH}_2\text{O}(\text{CH}_2\text{O})_n\text{H}$ are similar in structure to hydrated formaldehyde and each molecule of polymer has two hydroxyl groups which can be replaced by amines, as does methylene glycol. Those formaldehyde molecules which are in the interior of the polyoxymethylene chain are not available for reaction with amines. The total number of molecules which are available to react with amines, E , may then be defined according to Equation 16 in which λ is

$$E = \sum_{n=1}^{\infty} [\text{HO}(\text{CH}_2\text{O})_n\text{H}] = \lambda[F_T] \quad (16)$$

$$\lambda = \sum_{n=1}^{\infty} \left(\frac{\text{Fraction of } F_T \text{ as } \text{HO}(\text{CH}_2\text{O})_n\text{H}}{n} \right)$$

the fraction of the total formaldehyde, F_T , which exists in reactive forms, E . The value of E at different formaldehyde concentrations was calculated from the data of Table 17 of Reference 6, which gives the fractions of total formaldehyde present as monomer through decamer in formaldehyde solutions of up to 16 M. This table is based on two sets of mutually consistent results from measurements of partial pressures and bisulfite analyses and of the average molecular weights of the solute species. Measurements with nuclear magnetic resonance have given similar results (29). The effect of formaldehyde polymerization on the equilibrium between an amine and formaldehyde, based on these calculations, is shown as the dotted line in Fig. 9. The effect of polymerization is to cause an upward deviation in the $\Delta\text{pH} - \log [F]$ relationship from what would be expected if no such polymerization occurred.

The rate of depolymerization of formaldehyde is slow at pH 2.6 to 4.3, but increases rapidly at higher and lower pH values (47). The fact that pH readings were constant after the initial rapid change in pH in the most acidic solutions examined in these experiments shows that equilibrium was attained rapidly and that a time-dependent depolymerization of formaldehyde was not affecting the results. No kinetic barrier would be expected for the reaction of amines with formaldehyde polymers, because the cationic imine formed by dehydration of a molecule of



hydroxymethylamine would be expected to react rapidly with a molecule of polymer (Equation 17).

Water Concentration—Since water is a product of the reaction of amines with (hydrated) formaldehyde (Equation 1), the decreased concentration of water in concentrated formaldehyde solutions is expected to shift the equilibrium toward hydroxymethylamine formation. The concentration of water was estimated from the weight per cent composition, the degree of formaldehyde polymerization (Equation 16), and the density of formaldehyde solutions, and the effect of the decreasing water

concentration on the amine-formaldehyde equilibrium is shown as the *lower dot-dashed* line in Fig. 9. The effect of the decreasing water concentration is to cause a downward deviation from the line of slope 1.0, which is similar in magnitude and opposite in sign compared to the deviation caused by formaldehyde polymerization.

Methanol—The 10 to 11% methanol which is present in commercial formalin solutions might be expected to affect the formaldehyde-amine equilibrium by causing hemiacetal formation with formaldehyde, by decreasing the water concentration, by shifting the polymer equilibria, and by affecting activity coefficients. Although *concentrated* methanol does affect the apparent pH of amine buffers (Table IV), the concentrations of methanol present in the experiments reported here (up to 10%) have very little effect on the apparent pH of triethylamine buffers. Furthermore, identical results were obtained with formaldehyde which had been prepared from paraformaldehyde in the absence of methanol (Fig. 2). Evidently, the perturbing effects of methanol on the equilibrium either are not significant or cancel each other out at the concentrations which were present in these experiments. Similarly, Guthe has found that the amount of methanol in commercial formalin does not significantly affect the equilibria for the reaction of formaldehyde with the amine groups of hemoglobin (48).

Medium Effects—The pH of amine buffer solutions is given by Equation 5, and, at a given buffer ratio, the pH varies directly with $\log(\gamma_{R_3N}/\gamma_{R_3NH^+})$. Similarly, the pH of a solution containing only the hydroxymethylated amine derivatives varies with $\log(\gamma_{R_2NCH_2OH}/\gamma_{R_2NHCH_2OH})$ and the pH of a solution which contains both free and hydroxymethylated amines will depend on the activity coefficients of both of these species and their conjugate acids. In addition, the fractions of amine and protonated amine which are hydroxymethylated will depend on the activity coefficients of these species and of formaldehyde. All of these activity coefficients would be expected to be dependent on the formaldehyde concentration and to differ appreciably from unity in concentrated formaldehyde solutions. Furthermore, concentrated formaldehyde solutions may cause changes in the response of the system used for the measurement of pH. The largest activity coefficient effects are expected to be on the ratio of the neutral and acidic species of the buffers, because these species differ in charge, but effects on these ratios should be similar for the amine and hydroxymethylamine buffers, because these species do not differ greatly in structure. Activity coefficient effects on the equilibria for the addition of formaldehyde to amines and protonated amines should also be relatively small, because the reactants and products have the same charge and similar structure.

The magnitudes of these activity coefficient effects can be estimated from the effects of methanol and ethylene glycol, which are models for methylene glycol, on the pH of triethylamine buffer solutions (Table IV), and from the effect of concentrated formaldehyde on the pH of buffers prepared from tertiary amines with relatively severe steric requirements which show no evidence for a chemical reaction with formaldehyde (Table III). These results suggest that 13 M formaldehyde causes a decrease of from 0 to 1 pH unit in the observed pH because of such solvent effects; the average decrease is about 0.4 pH unit. This decrease is shown as the *solid triangle* in Fig. 9. It should be noted that the changes in pH caused by

activity coefficient effects are in the opposite direction to those which are caused by protonation of the hydroxymethylamine.

The *dashed line* in Fig. 9 is a calculated line for a hypothetical amine which is similar to morpholine in all respects except that

$$\Delta pH = \log \left(1 + \frac{K_1}{[HOH]} [E] \right) - \log \frac{\gamma_N}{\gamma_{NH}} \quad (18)$$

its hydroxymethyl adduct does not add a proton. This calculated line is based on Equation 18 and takes into account the effective formaldehyde concentration, the concentration of water, and nonspecific solvent effects of concentrated formaldehyde, based on the observed solvent effects of formaldehyde on *N*-methylmorpholine. It is apparent that the different effects cancel each other out almost completely to give a straight line with a slope of 1.0, the same as would be expected if these effects were ignored. With other amines, nonspecific solvent effects cause a moderate downward deviation at the highest formaldehyde concentrations. It is possible that this solvent effect masks a relatively small amount of formation of cationic hydroxymethylamines at the highest formaldehyde concentrations with amines which have unfavorable equilibria.

The *upper solid line* of Fig. 9 is calculated from Equation 19

$$\Delta pH = \log \left(\frac{1 + \frac{K_1}{[HOH]} [E]}{1 + \frac{K_2}{[HOH]} [E]} \right) - \log \frac{\gamma_N}{\gamma_{NH}} \quad (19)$$

which takes into account the above factors and also protonation of the hydroxymethyl derivative of morpholine. This theoretical line shows satisfactory agreement with the experimental data for this amine except for the deviation caused by solvent effects at the highest formaldehyde concentrations (Fig. 9, ○).

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