The Oxibase Scale and Displacement Reactions. XVIII. The Reaction of Mercaptoethylamine with Methyl Iodide and with Ethyl Tosylate[1]

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Abstract: The reaction of mercaptoethylamine (MEA) with methyl iodide to give the mono-S-methyl derivative has been studied in aqueous solution as a function of pH and temperature. The rate expression is $-d[CH_3I]/dt = k_2[CH_3I][H_3N^+CH_2CH_2S^-] + k_4[CH_3I][H_2NCH_2CH_2S^-]$ with only the thiolate forms of MEA being kinetically important. The data have been used to calculate the oxibase scale parameters of each thiolate anion. The factors influencing the reactivity of MEA as an antiradiation drug are discussed. The reaction of MEA with ethyl tosylate in aqueous buffer solutions has been studied over the pH range of 5–12 from 25 to 53°. The product is S-ethyl MEA. The oxibase scale parameters of the various forms of MEA are obtained. The relationship of these data to the antiradiation drug is discussed. It is concluded that all of the existing theories would be correlated with the oxibase scale. A prediction has been made concerning the design of better drugs. While some of the discussion is quite speculative, the theories do explain the experimental data.

Numerous compounds can offer some degree of protection against the damaging effects of ionizing radiation in mammalian tissues. One class of compounds are the aminothiols as cysteamine [or 2-aminoethanethiol or mercaptoethylamine, MEA ($H_2NCH_2CH_2SH$)], cysteine, and 3-aminopropanethiol. Numerous theories have been advanced to explain the *in vivo* protection offered by an aminothiol. Doherty[4] suggested that these materials are good free-radical scavengers. Eldjarn and Pihl[5,6] have formulated that the MEA derivatives form mixed disulfides with proteins or serve to reduce disulfides formed during the irradiation process.[7] Others have suggested that the chelation of vital metal ions as Cu(I) with MEA prevents fatal irreversible oxidation.[8,9] Others feel that pliant reduction of peroxides[10] can account for the drug's action. The ease of reduction of peroxides has been reported and discussed.[10,11] All of these theories can be expressed in terms of

$$D^- + X\cdot \longrightarrow X^- + D\cdot \quad (1)$$

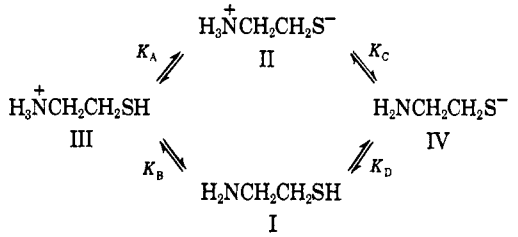
$$D^- + Cu(I)(H_2O) \longrightarrow D'\text{-}Cu(I)^- + H_2O \quad (2)$$

$$D^- + RSSR \longrightarrow RSH + D''^- \quad (3)$$

$$D^- + ROOR \longrightarrow ROH + D'''^- \quad (4)$$

where D^- is the drug and D′, D″, and D‴ are the other products. Equations 1–4 are rather general displacement reactions. It is the thesis of this investigation that the same factors in the drug which would increase the rates of reaction 1 will also increase the rate of (2), (3), and (4). We, in fact, theorize that the oxibase scale is applicable and that the higher the electrode potential and the more basic the drug, the better the material will perform all these reactions 1–4. As the oxibase scale has been used by so few investigators, it is important to obtain the *E* and *H* values of MEA derivatives in other systems. In the present study the kinetics of the reaction of methyl iodide and of ethyl tosylate with MEA are reported.

The Multiple Equilibria of MEA. The ionization equilibria of MEA in aqueous solution can be represented (without including the solvent protons) as



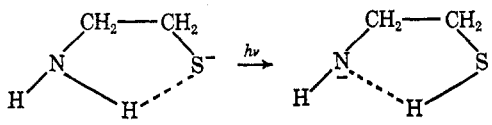
with three independent ionization constants and the fourth dependent. The two experimental ionization constants obtained from the pH titration curve are related to K_A, K_B, K_C, and K_D. Using the K_1 and K_2 values,[12,13] one can obtain the fraction[14] of each species I–IV as a function of pH.

The neutral form, $H_2NCH_2CH_2SH$, reaches its maximum concentration at pH 9.5. But since the total concentration of I at any time is so low, the value of $[k_1(H_2NCH_2CH_2SH)(\text{substrate})]$ will be very low indeed. The zwitterionic form of MEA, II, reaches also its

(1) Paper XVII: R. E. Davis, R. Nehring, W. J. Blume, and C. R. Chuang, *J. Amer. Chem. Soc.*, **91**, 91 (1969).
(2) Taken in part from the M.S. Thesis, June 1963.
(3) Postdoctoral Research Fellow.
(4) D. G. Doherty, W. T. Burnett, Jr., and R. Shapiro, *Radiat. Res.*, **7**, 13 (1957).
(5) A. Pihl and L. Eldjarn, *Pharm. Rev.*, **10**, 437 (1958).
(6) L. Eldjarn and A. Pihl, "Mechanisms in Radiobiology," Vol. 2, M. Enara and A. Forssberg, Ed., Academic Press, New York, N. Y., 1960.
(7) B. Shapiro and L. Eldjarn, *Radiat. Res.*, **3**, 255 (1955); D. B. Hope, *Biochem. Soc. Symp.*, **17**, 93 (1959).
(8) E. C. Knoblock and W. C. Purdy, *Radiat. Res.*, **15**, 94 (1961).
(9) J. Schubert, 139th National Meeting of the American Chemical Society, St. Louis, Mo., March, 1961; see *Chem. Eng. News.*, **39**, 25 (April 3, 1961).
(10) P. Alexander and D. Rosen, *Nature*, **188**, 574 (1960).
(11) R. E. Davis, unpublished data presented before the Symposium on Radiation-Protective Agents, 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962. In a preliminary report on the properties of MEA (see *Tetrahedron Lett.*, 885 (1966), we concluded that the reduction of hydrogen peroxide with MEA is a slow process. Thus little radioprotection can be offered by this reaction *in vivo*.
(12) M. Calvin, "Glutathione," Academic Press, New York, N. Y., 1954.
(13) R. E. Benesch and R. Benesch, *J. Amer. Chem. Soc.*, 77, 5877 (1955).
(14) J. T. Edsall and J. Wyman, "Biophysical Chemistry," Academic Press, New York, N. Y., 1958, Chapter 9.

maximum at pH at 9.5 when 84.6% of the MEA is in this form. Form III predominates below pH 8.5. Form IV rapidly increases in concentration above pH 10 and above pH 12.0 accounts for the majority of the total amount of MEA in solution.

Ultraviolet Spectrum of MEA as a Function of pH. The ultraviolet spectrum of MEA as a function of pH has been measured in this study and found to resemble the spectrum of cysteine[13,15-17] as a function of pH. The thiolate anions are much more colored in the ultraviolet than the thiol forms. The spectrum of the thiolate IV is different enough from the ethanethiolate spectrum to suggest that the electrons on the nitrogen can participate in the electronic transition(s). Quan-



(5)

tum mechanical calculations are currently being carried out on these molecules.

The ultraviolet spectra of MEA solutions are quite stable at low pH with little change in the optical density after 1 hr. At higher pH values the solutions under air are quite unstable and the optical density will change quite rapidly unless the solutions are protected from oxygen. In a single kinetic experiment at pH 8.13 in air-saturated aqueous methanol at 35°, the rate of change of the spectrum of MEA at 2.3×10^{-3} M obeyed half-order kinetics with a k of 3.4×10^{-5} $M^{1/2}$ sec^{-1} (the first half-time was 680 sec).

$$\text{rate} = k[\text{MEA}]^{1/2} = 3.4 \times 10^{-5}\ M^{1/2}\,\text{sec}^{-1}[\text{MEA}]^{1/2}$$

Therefore, it is quite important to remove oxygen from the solutions.

Methyl Iodide as a Substrate. Methyl iodide has served as the substrate in relatively few SN2 reactions at 25° in purely aqueous media. The relative data needed to compute the substrate parameters, α and β, are presented in Table I. An estimate of α is 2.955

Table I. Displacement Reactions with Methyl Iodide at 25° in Water

$$CH_3\text{–}I + N^z \longrightarrow CH_3N^{z+1} + I^-$$

N^z	k_2, M^{-1} sec^{-1}	Ref	E_a, kcal/mol	Log (k/k_{H_2O})	E^e	H^e
$S_2O_3^{2-}$	3.15×10^{-2}	*a*	18.9	7.448	2.52	3.60
HO^-	6.52×10^{-5}	*b*	22.2	4.747	1.65	17.48
Br^-	4.95×10^{-5}	*c*	19.3	4.644	1.51[h]	7.2[f]
H_2O	1.41×10^{-9}	*d*	28.1	0	0[g]	0[g]

[a] E. A. Moelwyn-Hughes, *J. Chem. Soc.*, 1576 (1933). [b] E. A. Moelwyn-Hughes, *Proc. Roy. Soc., Ser. A*, **196,** 540 (1949). Extrapolated from data at 30, 39, 49, 60, and 70°. [c] E. A. Moelwyn-Hughes, *J. Chem. Soc.*, 779 (1938). Interpolated from data at 20, 35, 30, 39, 49, 60, and 70°. [d] E. A. Moelwyn-Hughes, *Proc. Roy. Soc., Ser. A*, **164,** 295 (1938). [e] Taken from J. O. Edwards, *J. Amer. Chem. Soc.*, **76,** 1540 (1954), except for *f*. [f] J. C. McCoubrey, *Trans. Faraday Soc.*, **51,** 743 (1955). [g] Defined. [h] $E = \epsilon_{X^-} + 2.60$ V for $2X^- \rightleftharpoons X_2 + 2e^-$ in water at 25°. $H = pK_a + 1.74$ for $X^- + H^+ \rightleftharpoons HX$.

(15) G. A. Anslow and M. L. Foster, *J. Biol. Chem.*, **97,** 37 (1932).
(16) L. J. Saidel, A. R. Goldfarb, and S. Waldman, *ibid.*, **197,** 285 (1952).
(17) H. A. Rothschild and E. S. G. Barron, *ibid.*, **209,** 511 (1954).

and of β is -0.00333. It is of interest to note that methyl iodide *is very sensitive* to the oxidative dimerization potential of the nucleophile and only very slightly sensitive to the basicity of the nucleophile. In this case the oxibase scale

$$\log (k/k_0) = \alpha E + \beta H \tag{6a}$$

reduces to (if $\beta = 0$):

$$\log (k/k_0) = \alpha E \tag{6b}$$

which more nearly resembles the two-parameter Swain[18]

$$\log (k/k_0) = sn \tag{7}$$

equation. It is of interest to note the β is negative indicating the *more basic* the nucleophile the *slower* the rate with constant E values.

However, we still feel that the four-parameter scale should be used. If one plots log (k/k_0) *vs.* E, the bromide ion point is high and the hydroxide point is below the best straight line. Including the βH term improves the correlation.

The data of Table I are represented in Figure 1 in the more convenient form[1]

$$E^{-1} \log (k/k_0) = \alpha + \beta(H/E) \tag{8}$$

in which $E^{-1} \log (k/k_0)$ is plotted *vs.* (H/E). The slope is β and the intercept is α when $H/E = 0$.

Kinetics. The kinetic behavior of MEA and methyl iodide in aqueous buffer solutions from pH 5 to 12.5 can be formally expressed as

$$\text{rate} = [CH_3I][k_1[I] + k_2[II] + k_3[III] + k_4[IV]] \tag{9}$$

or

$$\text{rate} = [CH_3I][k_2[II] + k_3[III] + k_4[IV]] \tag{10}$$

since $k_1[I]$ is so small for the system

$$H_2NCH_2CH_2SH + CH_3I \xrightarrow{k_1} H_2NCH_2CH_2SCH_3 + H^+ + I^- \tag{11}$$

$$r_1 = k_1[CH_3I][H_2NCH_2CH_2SH] \tag{12}$$

$$r_1 = k_1[CH_3I][I] \approx 0 \tag{13}$$

$$H_3\overset{+}{N}CH_2CH_2S^- + CH_3I \xrightarrow{k_3} H_3\overset{+}{N}CH_2CH_2SCH_3 + I^- \tag{14}$$

$$H_3\overset{+}{N}CH_2CH_2SH + CH_3I \xrightarrow{k_2} H_3\overset{+}{N}CH_2CH_2SCH_3 + H^+ + I^- \tag{15}$$

$$H_2NCH_2CH_2S^- + CH_3I \xrightarrow{k_4} H_2NCH_2CH_2SCH_3 + I^- \tag{16}$$

Experimentally, the reaction is first order in methyl iodide and first order in MEA. One might expect that the most reactive nucleophiles would be the thiolate anions, II and IV. At a constant pH the rate expression

$$\underset{\text{II}}{H_3\overset{+}{N}CH_2CH_2S^-} \qquad \underset{\text{IV}}{H_2NCH_2CH_2S^-}$$

is

$$\text{rate} = k_0[CH_3I][\text{MEA}] \tag{17}$$

where [MEA] is the total stoichiometric concentration and k_0 is the observed second-order rate constant. A plot of log k_0 *vs.* pH is presented in Figure 2. One can quickly detect that the pH profile is quite simple and

(18) C. G. Swain and C. B. Scott, *J. Amer. Chem. Soc.*, **75,** 141 (1953).

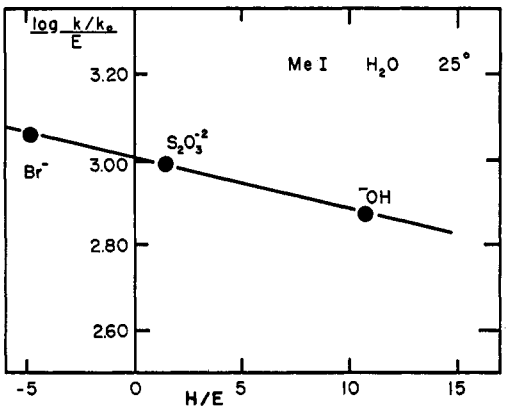


Figure 1. Plot of E^{-1} log (k/k_0) for various nucleophiles with methyl iodide in water at 25° *vs.* *H/E*.

that the two anions are of nearly equal effectiveness. Refinement of the data gives

$$k_2 = 0.468 \pm 0.074\ M^{-1}\ \text{sec}^{-1}$$

and

$$k_4 = 2.14 \pm 0.06\ M^{-1}\ \text{sec}^{-1} \text{ at } 25°$$

Thus the rate expression 9 reduces to the practical, observed rate

$$\text{rate} = k_2[CH_3I][H_3N^+CH_2CH_2S^-] + k_4[CH_3I][H_2NCH_2CH_2S^-] \quad (18)$$

Estimates of k_1 and k_3 can be made; they are each less than $10^{-5}\ M^{-1}\ \text{sec}^{-1}$ (see eq 9).

Using the α and β values of methyl iodide and the pK_a values of MEA,[12,13] the E values of the two thiolate forms have been computed and listed in Table II.

Table II. *E* Values of the MEA Thiolate Anions Using CH_3I

Thiolate	*E*, V
$H_2NCH_2CH_2S^-$	3.06 ± 0.04
$H_3N^+CH_2CH_2S^-$	2.84 ± 0.04

The kinetic studies have been made at 0.80, 13.5, and 25° and as a function of pH. More effort was made to obtain accurate activation parameters for the zwitterionic form, II, as in the human body this is the thiolate form in larger relative concentration. The temperature data also allow one to compute the rate constant at 37°, near body temperatures. In Figure 3 a plot of log k_2 *vs.* $1/T$ is presented.

The parameters are given in Table III from the data of Figures 2 and 3. It is obvious from the data that a *better* antiradiation drug would be one whose relative thiolate anion concentration reached its maximum near physiological pH (7.4 or so) and would still have a very high *E* value. Clearly the presence of the amino group lowers the pK_a of the thiol.

Table III. Activation Parameters with Methyl Iodide

	E_a, kcal/mol	$\Delta S^{\ddagger}$, gibbs
$H_3N^+CH_2CH_2S^-$	19.3	4.4

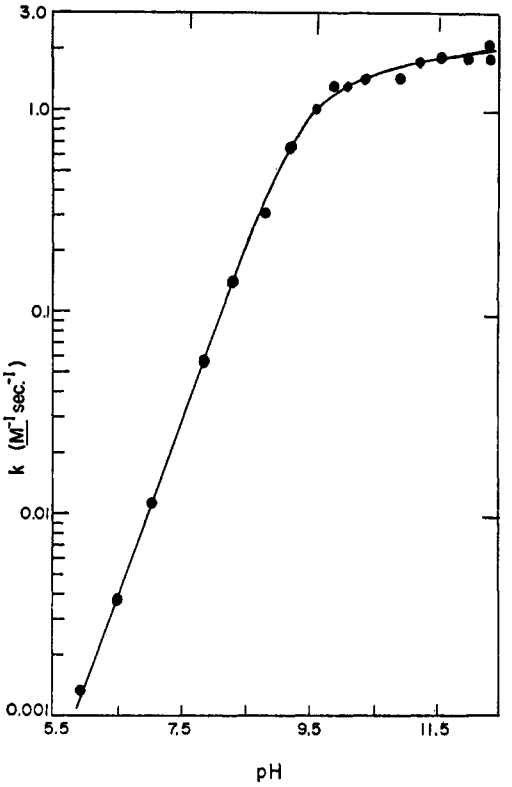


Figure 2. Plot of log k_0 *vs.* pH for MEA with methyl iodide in water at 25°. [MEA] has been varied from 5.8 to 28.7 × 10^{-4} *M*. The [CH_3I] has been varied from 5 to 18 × 10^{-4} *M*. Much of these data are reported with all the experimental details and data in the thesis of S. P. Molnar.

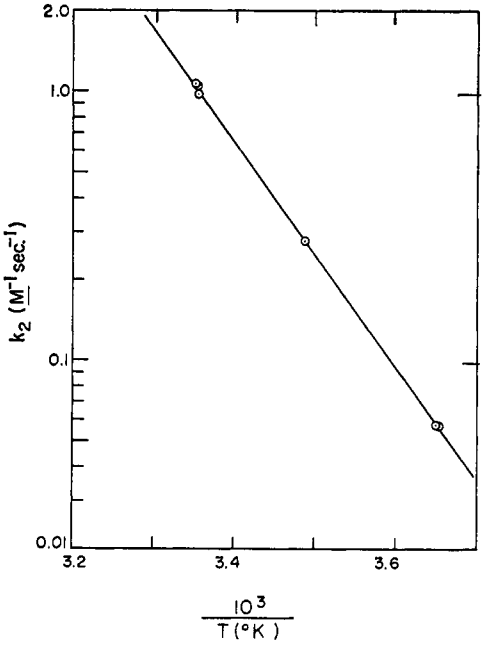


Figure 3. Temperature dependence of the reaction of H_3N^+-$CH_2CH_2S^-$ with methyl iodide in aqueous solution. Log k_2 *vs.* $1/T$ in a buffer of pH 9.90 ± 0.02.

Ethyl Tosylate. The *E* value of the tosylate group (about 0.5 V) is low. Therefore the α of the alkyl substrate is low. The *E* value of iodide ion (2.06 V) is high so the α of methyl iodide is high. Methyl iodide with its very high α value has very high rates with high *E* nucleophiles. Very low rates are observed with low *E*

Table IV. Kinetic Data on the Reaction of MEA with Ethyl Tosylate

MEA, $10^2 M$	EtOTs, $10^3 M$	pH[a]	Temp, °C[b]	$10^4 k$, sec^{-1} [c]
1.81	2.17	9.75	35	2.28
2.43	1.68	9.75	35	2.63
3.59	2.80	9.75	35	3.54
1.70	2.40	9.75	35	2.23
3.62	1.11	9.75	35	4.17
5.12	2.88	11.20	35	5.73
4.31	2.34	11.20	35	4.90
4.40	21.0	7.90	36	1.47
5.29	2.74	7.90	36	1.59
3.97	2.41	11.20	38	5.45
3.99	2.49	4.97	41	0.565
5.38	2.87	4.97	41	0.574
4.97	2.22	4.97	41	0.592
4.50	3.09	6.75	41	0.879
4.99	2.49	6.75	41	0.894
4.39	3.25	6.75	41	0.931
3.87	2.42	7.96	41	2.17
4.59	0.194	7.96	41	2.52
3.78	2.39	7.96	41	1.89
3.66	2.05	8.56	41	3.84
5.02	5.27	8.56	41	4.70
3.69	1.36	8.56	41	3.96
3.40	2.57	8.56	41	4.70
3.92	2.37	9.80	41	3.96
2.25	2.44	9.82	41	4.56
3.96	2.30	9.82	41	6.10
2.62	1.66	9.82	41	5.31
3.92	2.37	9.80	41	7.18
3.02	2.09	9.82	41	5.32
3.61	0.235	10.64	41	8.59
2.69	1.58	10.64	41	5.37
3.76	2.28	10.64	41	8.00
3.45	2.27	11.20	41	6.57
3.70	2.65	11.20	41	8.29
43.6	2.71	5.77	41	0.883
5.71	2.52	5.77	41	0.561
7.60	2.29	5.77	41	0.689
3.75	2.52	12.00	41	0.98
4.53	2.26	12.00	41	8.99
4.85	2.64	12.00	41	8.67
3.97	2.64	11.20	46	10.96
4.86	2.66	7.90	46	4.22
3.36	1.83	9.75	47	9.32
3.86	2.77	9.75	47	12.04
4.29	2.32	7.90	50	6.30
3.62	2.67	11.20	50	13.64
2.42	2.72	9.82	53	12.76
2.60	6.73	9.82	53	13.07
4.04	0.215	9.82	53	19.18
0	1.30	5.92	35	0.110
0	1.30	8.9	35	0.110
0	1.06	4.97	41	0.432

[a] ±0.01 at each temperature. [b] ±0.01° (NBS). [c] Apparent first-order rate constant for the disappearance of the ethyl tosylate.

Table V. Activation Parameters of Various Forms of MEA with Ethyl Tosylate at 25°

Form	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, gibbs
$H_2NCH_2CH_2S^-$	14.7	−20.5
$H_3{}^+NCH_2CH_2S^-$	16.6	−14.4
$H_3{}^+NCH_2CH_2SH$	22.3	+1

nucleophiles. Thus with methyl iodide only the rates with the thiolate anions could be measured. The use of ethyl tosylate allows us to obtain E values on H_3N^+-CH_2CH_2SH and compare the kinetic E values obtained with methyl iodide.

The data are presented in Table IV. In Table V, the activation energy parameters have been computed at 25°. In Table VI, the E and H values of the two thiolate forms have been listed. The values compare quite well with the E and H values for the thiolates obtained using methyl iodide as a substrate. Thus the two E values of $H_2NCH_2CH_2S^-$ are 3.11 and 3.06 V with a difference of less than 2%.

Table VI. Oxibase Parameters for the Various Forms of MEA from Ethyl Tosylate

Form	E, V	H	H/E
$H_2NCH_2CH_2S^-$	3.11 ± 0.03	12.5[a]	4.02
$H_3{}^+NCH_2CH_2S^-$	2.90 ± 0.04	10.35[b]	3.56

[a] Computed from the pH at which one-half of the MEA present is in the form of the monothiolate. [b] Computed from the pK_a.

Discussion

The E values of the thiolates of MEA are high (Table VI), but not that different from numerous other thiolate anions.[19] Yet numerous compounds are *not* antiradiation drugs. Clearly the amino group is required to first lower the pK_a of the thiol so that more thiolate anions are present at physiological pH values and second to interact with thiolate anion in intermolecular hydrogen bonding, in chelating, and other special effects suggested by numerous investigators.[5-11]

Site Potentials. It is obvious why the first alkylation with methyl iodide occurs solely on the thiolate sulfur rather than on the basic nitrogen. While few E values are yet available for typical amines,[19b] ammonia has an E of 1.84 (and an H of 11.22) and aniline has an E of 1.78 (and an H of 6.28). Using the values of 1.8 as typical of the E value of the basic nitrogen of MEA, one can see that relative rates of S-alkylation *vs.* N-alkylation would be of the order of nearly 5400 to 1 with methyl iodide at 25°. The α

$H_2N—CH_2—CH_2—S^-$
(1.8) 3.06 E value
1 5400 relative rate with methyl iodide

is 2.96. The log of the relative rate of S- *vs.* N-alkylation is

$$\log (rs/rn) = \Delta E\alpha = (1.26)(2.96) = 3.73 \quad (19)$$

An important project will be to obtain numerous E and H values for different functional groups. In this manner one can judge selective synthesis in a much more quantitative manner.

Attention can be drawn to the effect of the terminal NH_2 or NH_3^+ group on the E value of the thiolate anion. The presence of the ammonium ion in II serves only to reduce the oxidative dimerization of II compared to IV.

$$2H_3N^+CH_2CH_2S^- \overset{\epsilon^0_{II}}{\rightleftharpoons} H_3N^+CH_2CH_2SSCH_2CH_2N^+H_3 + 2e^- \quad (20)$$

$$E_{II} = \epsilon^0_{II} + 2.60 \text{ V} = 2.87 \text{ V}$$

$$2H_2NCH_2CH_2S^- \overset{\epsilon^0_{IV}}{\rightleftharpoons} H_2NCH_2CH_2SSCH_2CH_2NH_2 + 2e^- \quad (21)$$

$$E_{IV} = \epsilon^0_{IV} + 2.60 \text{ V} = 3.08 \text{ V}$$

(19) (a) R. E. Davis, in "Organic Sulfur Compounds," N. Kharasch, Ed., Pergamon Press, New York, N. Y., in press. (b) R. E. Davis in "Survey of Progress in Chemistry," Vol. II, A. Scott, Ed., Academic Press, New York, N. Y., pp 189–238. See Tables III and IV in that paper.

It is somewhat harder to remove two electrons from two molecules of II compared to IV due to the electrostatic attraction in II, thus $E_{IV} > E_{II}$.

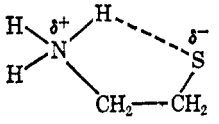
Calculation of Effective Charge Density. If one assumes that the $\Delta S^{\pm}$ (Table V) observed is mostly due to the $\Delta S^{\pm}_{el}$, which is generally true for most reactions between polar molecules and ions in water,[20] then using Kirkwood's model,[21] one can calculate the change on the thiol sulfur atom assuming (1) a constant distance between the S and C and between C and the O in each transition state, (2) a constant S–C–O angle in each transition state, and (3) that the microscopic dielectric constant is the same. The results of the calculations are that the relative charge density on the sulfur in $H_2NCH_2CH_2S^-$ is 4.2 times that of the sulfur in $H_3NCH_2CH_2SH$ and $^+H_3NCH_2CH_2S^-$ forms a transition state with 3.5 times the amount of negative charge density of the sulfur atom than the transition state formed from $^+H_3NCH_2CH_2SH$ (see Table VII). Thus the presence of the extra protons has an important effect of the relative charge densities.

Table VII. Relative Negative Charge Densities Computed from an Electrostatic Model in the Transition State[a]

Form	Relative charge density
$H_2NCH_2CH_2S^-$	4.2
$^+H_3NCH_2CH_2S^-$	3.5
$^+H_3NCH_2CH_2SH$	1.0

[a] Computed using the $\Delta S^{\pm}$ from Table V on ethyl tosylate. The assumptions used to calculate the charge density are listed in the text.

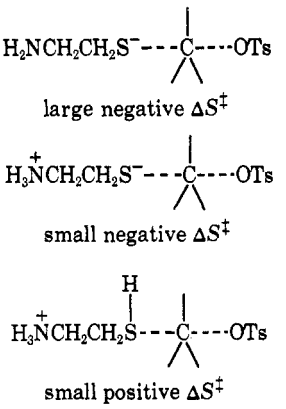
The drop from 4.2 to 3.5 in relative charge density by adding a proton on to an atom four atoms away is perhaps surprising. However, the most probable configuration for the zwitterion would be in internally hydrogen-bonded form. The hydrogen-bonded form



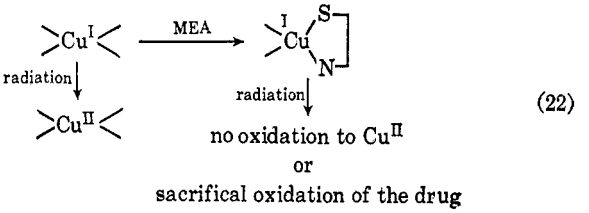
has been used to explain the difference in the ultraviolet spectrum of MEA compared to ethylthiol as a function of pH. It appears that the thiolate ultraviolet transition does place charge on the nitrogen (see reaction 5).

It is of interest to note the regular increase in the empirical activation energy and the steady increase in the entropy of activation as protons are added to the aminothiolate. Both effects are in complete accord with the decreased nucleophilic character of the thio sulfur and the change in the degree of solvation in each activated complex. A plot of $\Delta H^{\pm}$ *vs.* $\Delta S^{\pm}$ from Table V is a fair straight line.

Assuming that in each model the tosylate leganion is receiving nearly equal solvation from the solvent, the degree of positive charge character in the aminothiol forms increases as the $\Delta S^{\pm}$ increases and that appropximately one less water molecule is required in each activated complex.



The Mechanisms of Antiradiation Drug Protection. The mechanisms by which ionizing radiation damage tissue and lead to death are not understood. It is known that if rats are exposed to X-rays or β rays, urinary excretion of taurine, $H_2NCH_2CH_2SO_3H$, is greatly increased.[22,23] Risse[24] in 1929 suggested that X-rays produced H· and ·OH radicals from water. Bacq and Alexander[25] and Muller[26] have reviewed these topics with regard to the antiradiation problem. Hope[27] also summarized the main theories which include (a) the drug's ability to induce anoxia by removal of oxygen in the cell, (b) the compound's action as an antioxidant to remove free radicals, and (c) combination of the drug with some radiosensitive molecule which then prevents direct and/or indirect damage. In particular, chelation of copper(I) or other metals with the drug has been suggested which prevents the fatal irreversible oxidation.[8,9]



(22)

Complex Ion Formation. The oxibase scale has been applied by Edwards[28] in his first paper to the problem of complex ion equilibria. He concluded that the oxibase scale does best when the complex formed has a high degree of *covalent* character. Using Bjerrum's values[29] on the copper(I) complexes, a figure (Figure 4) has been prepared by plotting E^{-1} log K_f *vs.* H/E. The α is $+4.95 \pm 0.10$ and β is $+0.162 \pm 0.009$. The fact that α is so large and positive means that anions of very high E values are very tightly bound to copper (I). The positive charge density of the copper(I) ion is reflected in the large and positive β.

While the large α and β for copper(I) would be reflected in the binding of a chelate, the problem is that the effective α and β probably change as chelation occurs. The first addition would produce a complex

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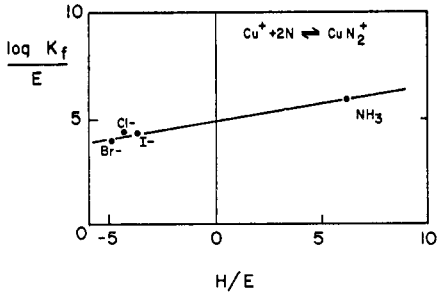
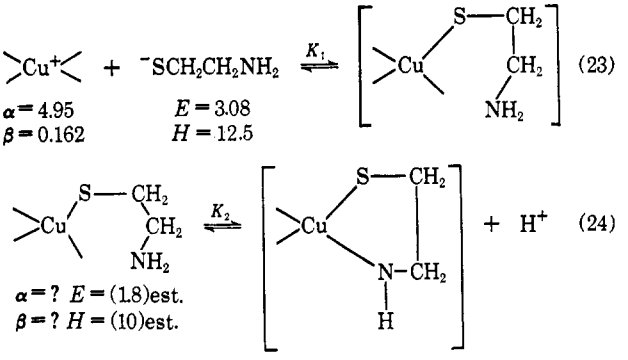


Figure 4. Oxibase scale plot of the complex ion formation constants, K_f, for the $Cu^+ + 2N \rightleftharpoons CuN_2^+$ equilibria. Plot of E^{-1} log K_f *vs.* H/E in water at 25°.

whose formation would reflect the α and β of hydrated copper(I). Thus



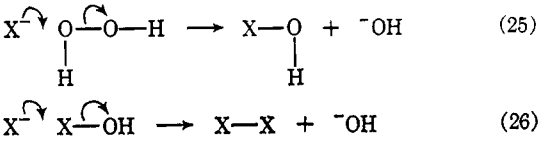
However, the second group to enter has a different type of copper(I) center upon which it reacts. The α and β of the new partially chelated copper(I) would not be known.

In the tissue most metal ions as copper and iron functional in redox cycles are also chelated. One can conclude, however, that they also would have large and positive α values and probably positive β values.

Displacements at the Sulfur–Sulfur Bond. Unfortunately there is not enough quantitative rate data at present to establish the α and β of a displacement reaction on a typical sulfur–sulfur bond. The literature on these reactions has been reviewed by Pryor,[30] Kharasch,[31] and the present author.[19]

The reactivity order of thiophiles $RS^- > {}^-CN \approx C_6H_5S^- > SO_3^{2-} > RSO_2^- \gg S_2O_3^{2-} > RSO_2S^- > (RO)_2POS^- > {}^-SCN$ on a typical sulfur–sulfur bond in a disulfide would require a large positive α and a positive β. The fact that the rate of reduction of oxidized glutathione is used as a preliminary test for antiradiation drug activity also supports the conclusion that a disulfide has both $\alpha > 0$ and $\beta > 0$.

Reduction of Peroxides. The reactions of nucleophiles[28,32] with hydrogen peroxide occur by simple SN2 displacement reactions (eq 25 and 26). In Figure



(30) W. A. Pryor, "Mechanisms of Sulfur Reactions," McGraw-Hill Book Co., Inc., New York, N. Y., 1962.
(31) A. J. Parker and N. Kharasch, *Chem. Rev.*, **59**, 583 (1959).
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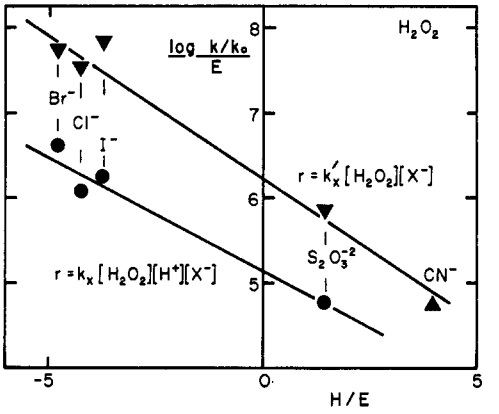
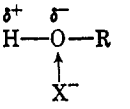


Figure 5. Oxibase scale plot of the reactions of various nucleophiles with hydrogen peroxide in water. The upper line is for the data in basic solution with a rate expression: rate = $k_x'(H_2O_2)(X^-)$. The lower line represents the data obtained in an acidic medium where the rate expression is $k_x(H_2O_2)(H^+)(X^-)$.

5 an oxibase scale plot is made of the data.[28,32] It is of interest to note that α is very large for both hydrogen peroxide and the protonated peroxide.

H—O—O—H	$H-O-\overset{+}{O}H_2$
$\alpha = 6.22$	$\alpha = 5.17$
$\beta = -0.43$	$\beta = -0.29$

The β is negative for both. The addition of a proton does increase β. The large α is a reflection of the very weak oxygen–oxygen bond and the large negative heat of formation of hydroxide ion. The sign of β is perhaps understood best in terms of the small oxygen atom, rich in electrons due to its high electronegativity. Thus the nucleophile is approaching a rather small negative center.



β predicted to be negative

If hydrogen peroxide was reduced by MEA in the same manner, the calculated rate constants would be those listed in Table VIII. The very large α and the large

Table VIII. Rate Constants for Reaction of Various Nucleophiles with Hydrogen Peroxide at 25° in Water

Nucleophile	E	H	k_2, M^{-1} sec^{-1}
H_2O	0	0	$(4.6 \times 10^{-17})^a$
Cl^-	1.24	−5.26	1.1×10^{-7}
Br^-	1.51	−7.26	2.3×10^{-5}
I^-	2.06	−7.76	6.9×10^{-1}
$S_2O_3^{2-}$	2.52	3.60	2.5×10^{-2}
HO^-	1.65	17.48	$(2 \times 10^{-13})^a$
$H_2NCH_2CH_2S^-$	3.11	12.5	$(3.3 \times 10^{-2})^a$
$H_3N^+CH_2CH_2S^-$	2.90	10.34	$(5.8 \times 10^{-3})^a$

[a] Calculated using the oxibase scale.

E favor a facile reaction between the thiolates of MEA and hydrogen peroxide even though the high basicity of the thiolates slows the rate by several thousand-fold! The specific rate constants of reaction of the thiolates with hydrogen peroxide are about 10^{-2} M^{-1} sec^{-1} assuming the oxibase scale is correct. If the drug and the peroxide are about 10^{-3} M in the tissue the first

$t_{1/2}$ of disappearance of peroxide would be about 10^{5+} seconds. This seems to be a rather long time; however, this does not disprove the antiradiation protection mechanism involving hydrogen peroxide reduction. But it does cast doubt on this theory of protection.

Free-Radical Reactions. The free-radical reaction (27) is also a displacement reaction with the novel sug-

$$RS^- + \cdot OH \longrightarrow RS\cdot + {}^-OH \quad (27)$$

$$Y^- + AX \longrightarrow YA + X^- \quad (28)$$

gestion that as the electrons of the nucleophile are donated to the substrate, the group transferred (A) back is the hole of the half-filled molecular orbital. The advantage to be gained by the use of the oxibase scale is that the free energy of the hole can be defined as zero and that the very good nucleophiles (high E values) would be good scavaging materials. Unfortunately, quantitative data in the literature are scarce and a good test of this novel idea can not be made. Experimental work is in progress.

Conclusion

One method of improving the drug properties of MEA derivatives would be to place more electron-rich groups in the molecule which can increase the effective charge density on the thiolate anion and thus increase the ease of oxidation. As a corollary of our work, the true mechanisms of protection must be obtained in an *in vivo* system.

Experimental Section

Materials. MEA hydrochloride was obtained from Evans (Waterloo, N. Y.) and was recrystallized from ethanol–diethyl ether and dried *in vacuo*. Pure MEA was prepared from 0.5 mol of sodium methoxide in 200 ml of methanol and 0.5 mol of MEA·HCl in 100 ml of methanol at 0° under nitrogen. The precipitated sodium chloride was removed and the solution distilled *in vacuo*. The residue was twice recrystallized from ethanol under nitrogen. The pure MEA had a melting point of 98–100° (lit.[33] 99–100°).

Eastman Kodak Yellow Label ethyl tosylate was distilled under vacuum, bp 127° (2.5 mm). Methyl iodide (Columbia Organic Chemicals) was redistilled immediately before use.

Reagents used for the buffer solutions were "Baker Analyzed" or "Fisher Certified" reagents. The following materials were used in the pH range of 6–8 (potassium hydrogen phosphate and potassium dihydrogen phosphate) and 8–11 (bicarbonate–carbonate, hydroxide), and calcium hydroxide was used at 12.3. The ionic strength of the buffers were adjusted to μ equals 1.2–5.8 $\times 10^{-2}$ M. Redistilled, deionized water was used. Measurement of pH were made using E-2 glass electrodes with a Beckman Model G meter.

Stock solutions were prepared (in deoxygenated buffer solutions) using a Cahn microbalance. Methyl iodide was injected into a melting point capillary tube using a Hamilton microsyringe and then reweighing the tube on a microbalance. The tube was then crushed under the surface of the air-free buffer solution.

Kinetic Measurements. All kinetic experiments were made on a calibrated Beckman DU spectrophotometer using 1.000 ± 0.003 cm quartz cells in a brasss thermostated block (±0.01° NBS). The rate constants were estimated from properly constructed graphs and then calculated using an IBM 7090 computer to obtain a least-square fit. The MEA concentration was varied from 1×10^{-4} to 18×10^{-4} M while the methyl iodide was varied from 2×10^{-4} to 15×10^{-4} M. EDTA at 4 to 6×10^{-4} M had no effect upon the rate. Variation of the lot and the amount of the buffers produced no strange results; therefore, we conclude metal ions have little or no catalytic role in the reaction. All flasks, solvents, and solutions were kept under pure nitrogen. Kinetic measurements were made using a thermostated spectrophotometer. The wavelength was set at 275 mμ for the tosylate and at 240 or 260 mμ for the methyl iodide studies.

Products. MEA hydrochloride (0.202 mol) was dissolved in 150 ml of water under nitrogen and sodium hydroxide (0.40 mol) added. Methyl iodide (0.28 mol) was added slowly and the mixture stirred rapidly under nitrogen for 10 hr at 25°. The homogeneous solution was acidified with hydrochloric acid and then most of the water was removed *in vacuo*. Solid potassium hydroxide was then added very slowly and then the basic solution was extracted for 6 hr with ether in a continuous solvent extractor. The ether layer was dried and then evaporated *in vacuo*. The residue was then distilled and the fraction boiling at 148–149° (n^{25}D 1.4905) was collected (lit.[34] 146–148° (7.0 mm)), yield 80%. The product had amino nitrogen frequencies. The spectrum resembled that of S-ethyl MEA. *Anal.* Calcd for C_3H_9NS: C, 39.52; H, 9.95; N, 15.37; S, 35.17. Found: C, 40.08; H, 10.10; N, 15.25; S, 34.30.

The S-methyl-MEA was converted to the hydrochloride by passing gaseous hydrogen chloride into an ether solution. The oily residue that separated crystallized on standing. The very hygroscopic salt was recrystallized from dry ethanol–diethyl ether. After several recrystallizations the melting point was constant at 144–146° (lit.[34] *ca.* 120°). *Anal.* Calcd for $C_3H_{10}NSCl$: C, 28.23; H, 7.90; N, 10.98; S, 25.12; Cl, 27.78. Found: C, 28.48; H, 8.20; N, 10.77; S, 25.40; Cl, 27.56.

Alkylation with an Excess of Ethyl Tosylate. A solution of 0.10 mol of MEA in 30 ml of dioxane and 20 ml of water and 0.30 mol of ethyl tosylate in 270 ml of dioxane and 200 ml of water. The mixture was stirred for 1 day at room temperature. Ethyl tosylate was present in the residue in large amounts. Iodometric titrations in acetic acid showed that the complete absence of free thiol in the final reaction mixture.

Vacuum distillation (vpc showed the presence of 0.1 mol) gave S-ethylthioethylamine, bp 75° (27 mm), n^{25}D 1.4834; hydrochloride, mp 145–147° (lit.[35] 147°). *Anal.* Calcd for $C_4H_{12}NSCl$: C, 33.90; H, 8.54; N, 9.88; S, 22.63; Cl, 25.02. Found: C, 34.20; H, 8.62; N, 9.95; S, 22.62; Cl, 24.80.

Acknowledgment. The authors wish to thank the Research Corporation for a Cottrell grant, the Sloan Foundation, the Walter Reed Army Institute of Research for a grant, WRAMC-MD-2107, the National Science Foundation for a grant, NSF-GI5750, and the National Institutes of Health for grants GM-07655 and RH-00279 over the past 7 years which have supported these investigations.

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