and, as the CH coupling was removed, the ABX pattern simplified to a AB quartet centered at 198 Hz.

Similarly, thiation of 4-benzyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine 1,1-dioxide[2] (**36**) (2.87 g, 0.01 mole) by P_2S_5 (2.22 g, 0.01 mole) in refluxing dioxane (50 ml) gave 4-benzyl-2H-1,4-benzothiazine-3(4H)-thione 1,1-dioxide (**37**).

2H-1,4-Benzothiazine-3(4H)-thione[4] (**42**) was obtained as yellowish green crystals in 39% yield from **1** (R = R^1 = H) and P_2S_5 using dioxane as a solvent instead of toluene as reported by Kiprianov, *et al.*,[4] mp 126–128° (EtOH).

4-Benzyl-3,4-dihydro-2H-1,4-benzothiazine-3-thione (**45**) was prepared from 4-benzyl-3-oxo-3,4-dihydro-2H-1,4-benzothiazine[2,5] (**44**) (5.1 g, 0.02 mole) by the method used for the preparation of **32**, except that the product was isolated by extraction with Et_2O.

(9) Compound **44** was obtained in 55% yield by refluxing **1** (R = R^1 = H) for 6 hr with 10% excess benzyl chloride and NaOH in 60% EtOH. Reporter[5] method required the use of NaH in DMF.

Acknowledgments.—The author deeply appreciates the technical assistance given by Mr. A. Fung and Mrs. K. Tietje. The author is also grateful to Drs. J. H. Short and D. L. Garmaise for many helpful discussions and critical reading of the manuscript of the paper, and to Dr. Thomas Darby, Mr. Leo Wiemeler, and Mr. Charles Shannon of the Pharmacology Department of Abbott Laboratories, North Chicago, Ill., for pharmacological investigations and permission to use their data.

The Activity of Phenothiazine Anthelmintics as Related to Semiquinone Formation

THOMAS N. TOZER, L. DALLAS TUCK, AND J. CYMERMAN CRAIG

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco Medical Center, San Francisco, California 94122

Received October 2, 1968

Substitutions of various groups on the phenothiazine nucleus have been studied with respect to their effects on standard electrode potential, semiquinone free-radical stability, and anthelmintic activity. The electrode potentials of the two oxidation steps are correlated on Hammett plots. An expression is derived for the relative semiquinone concentration occurring in a biological system having a definite oxidation potential and pH. The anthelmintic activity is shown to be related to the semiquinone concentration.

Craig, and coworkers,[1] studied a series of substituted phenothiazines with regard to potentiometric titration, electrode potentials, and their correlation with anthelmintic activity as measured in the biological assay using mixed infestations of *Syphacia obvelata* and *Aspiculuris tetraptera* in mice. From these studies it appeared that two factors were necessary for activity, namely, the ability to form a high proportion of a stable semiquinone radical (as measured by the index potential in aqueous AcOH), and the presence of a free 3 or 7 position.

In addition to the two factors above, Craig, *et al.*,[1] also noted that only those compounds with electrode potentials in the range of 550–850 mV in aqueous AcOH had significant activity. If the toxic or paralyzing effect of the phenothiazines is due to an inhibition by the semiquinone of an oxidation–reduction system in the parasite, it would seem reasonable that the active phenothiazines would have reduction potentials corresponding to those of the oxidation–reduction enzyme system or systems which they inhibit. At the similar potentials the semiquinone concentration would be maximal and thus facilitate or compete with the electron transfers in the enzyme system involved. For example, it has been suggested that the semiquinone of chlorpromazine is responsible for the inhibition of certain oxidoreductases *in vitro*[2-4] and that some of the biological activities of phenothiazines correlate with the formation of their semiquinones *in vivo*.[5,6]

In this report the substituted phenothiazines studied by Craig, *et al.*,[1] are examined to discover a possible relationship between the calculated relative concentration of their semiquinones *in vivo* and their anthelmintic activities. It is first established that the electrode potentials for the two one-electron oxidation steps leading to the phenazothionium ion are linear functions of the Hammett substituent constants. The semiquinone concentration then becomes a function of the Hammett constant and the prevailing electromotive force and pH at the site of action. The results are shown to be not inconsistent with the supposition that the biological action is a result of the interference of the semiquinone with an essential oxidation-reduction system in the parasite.

Reduction Potentials.[7]—Let E represent the local

(1) (a) J. Cymerman-Craig, M. E. Tate, G. P. Warwick, and W. P. Rogers, *J. Med. Pharm. Chem.*, **2**, 659 (1960); (b) J. Cymerman-Craig, M. E. Tate, F. W. Donovan, and W. P. Rogers, *ibid.*, **2**, 669 (1960); (c) J. Cymerman-Craig and M. E. Tate *Progr. Drug Res.*, **3**, 76 (1961); (d) W. P. Rogers, J. Cymerman-Craig, and G. P. Warwick, *Brit. J. Pharmacol.*, **10**, 340 (1955).

(2) M. Wolleman and P. Elodi, *Biochem. Pharmacol.*, **6**, 228 (1961).

(3) M. Wolleman and T. Keleti, *Arzneimittel-Forsch.*, **12**, 360 (1962).

(4) L. Levy and T. N. Burbridge, *Biochem. Pharmacol.*, **16**, 1249 (1967).

(5) H. Laborit, U. S. Psychopharmacology Service Center Bulletin, Vol. 2, 1962–1963, p 34.

(6) I. S. Forrest and F. M. Forrest, *Exp. Med. Surg.*, **21**, 231 (1963).

(7) The following treatment of the electrode potentials makes use of the conventions and definitions adopted at the XVIIth Conference of the International Union of Pure and Applied Chemistry, Stockholm, 1953; see J. A. Christiansen, *J. Amer. Chem. Soc.*, **82**, 5517 (1960). In addition, all potentials are referred to the standard H electrode, and the electrode potentials for the two univalent oxidation steps are defined as follows: E_1 is the normal electrode potential under the condition that phenothiazine and its semiquinone are of equal activity; E_2 is the normal electrode potential at a specified pH under the condition that the semiquinone and the phenazothionium ions are of equal activity. As considered in greater detail in eq 5 and 6, the potentials E_1 and E_2 are related to the standard electrode potentials $E_1°$ and $E_2°$ and the bivalent midpoint electrode potential E_M (called the "mean normal potential" by L. Michaelis and M. P. Schubert, *Chem. Rev.*, **22**, 437 (1938)) as follows.

$$E_1 = E_1° \quad E_2 = E_2° + (RT/F) \ln [H^+] \quad E_M = \tfrac{1}{2}(E_1 + E_2)$$

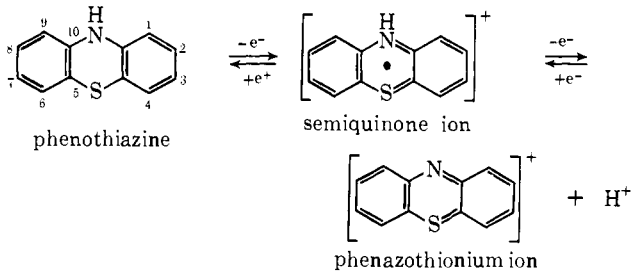
In the factor RT/F, R is the gas constant, T the absolute temperature, and F is the value of the faraday, all expressed in consistent units.

TABLE I

Letter[f]	Compd	Electrode potential,[a] mV	Anthelmintic act., %[b] ± std error	Hammett substituent parameter
A	Methylene blue	355[c]	2 ± 0.3	−1.20[e]
B	Thionine	378[c]	5 ± 1.5	−1.32[e]
C	3-Aminophenothiazine	451[c]	2 ± 0.5	−0.66
D	3,7-Dimethoxyphenothiazine	475	0 ± 0	−0.54[e]
E	3,4,6,7-Dibenzophenothiazine	548	1 ± 0.5	
F	3-Ethoxyphenothiazine	580	29 ± 9	−0.25
G	3-Methoxyphenothiazine	590	72 ± 6.5	−0.27
H	3,7-Dimethylphenothiazine	590	1 ± 0.5	−0.34[e]
I	3,4-Benzophenothiazine	628	7 ± 3.5	
J	3-Methylphenothiazine	651	40 ± 11	−0.17
K	2-Chloro-7-methoxyphenothiazine	662	100 ± 0.2	+0.10[e]
L	4-Chloro-7-methoxyphenothiazine	668	100 ± 0.2	+0.10[e]
M	3-Phenylphenothiazine	679	1 ± 1	+0.01
N	Phenothiazine	696	70 ± 7	0.00
O	3-Fluorophenothiazine	722	23 ± 11.5	+0.06
P	3-Chlorophenothiazine	763	70 ± 5.5	+0.23
Q	3-Bromophenothiazine	766	24 ± 6.5	+0.23
R	3-Iodophenothiazine	758	6 ± 2.5	+0.28
S	3-Nitrophenothiazine	900[d]	1 ± 0.5	+0.78

[a] Reference 1, measured at 20° in 80% v/v AcOH (pH ~2). [b] Reference 1, and J. Cymerman-Craig, unpublished data. For the method of determination of biological activity, see ref 1d. [c] L. Michaelis in "The Enzymes," Vol. II, Part I, J. B. Sumner and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1951. [d] Approximate value only; potential became unstable between 25 and 50% of the univalent titration step, and value was obtained by extrapolation. [e] For derivatives having more than one substituent the constants are added. [f] See Figures 1, 4, and 6.

ambient electromotive force of the physiological system; let [R], [S$^+$], and [T$^+$] represent the thermodynamic activities of the reduced, semiquinone, and totally oxidized forms of phenothiazine, respectively. The relationships between E and the standard potentials $E_1°$ and $E_2°$ for the two univalent steps 1 and 2 are shown in eq 3 and 4.



$$S^+ + e^- = R \quad (1)$$

$$T^+ + H^+ + e^- = S^+ \quad (2)$$

$$E = E_1° + (RT/F) \ln ([S^+]/[R]) \quad (3)$$

$$E = E_2° + (RT/F) \ln ([T^+]/[S^+]) + (RT/F) \ln [H^+] \quad (4)$$

Adding (3) and (4) to eliminate [S$^+$], one obtains

$$E = {}^1/_2(E_1° + E_2°) + (RT/2F) \ln ([T^+]/[R]) + (RT/2F) \ln [H^+] \quad (5)$$

When T$^+$ and R have the same activity, E equals the bivalent midpoint electrode potential E_m, so that

$$E_m = {}^1/_2(E_1° + E_2°) + (RT/2F) \ln [H^+] \quad (6)$$

The difference ΔE between E and E_m at 30° then becomes

$$\Delta E = E - E_m = (RT/2F) \ln ([T^+]/[R]) = 0.030 \log ([T^+]/[R]) \quad (7)$$

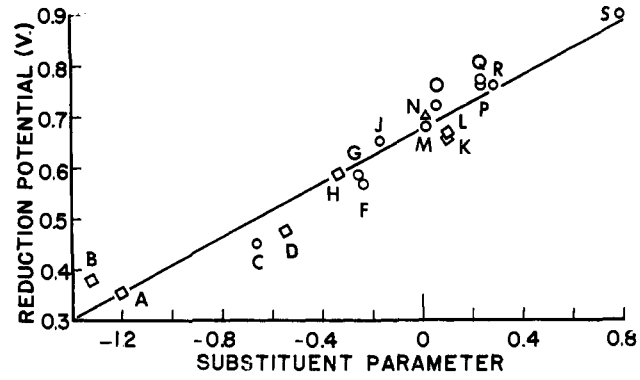


Figure 1.—Reduction potential of the first univalent oxidation step of the substituted phenothiazines as a function of the Hammett substituent parameter: △, unsubstituted; ○, monosubstituted; ◇, disubstituted. For identifying letters, see Table I.

The stability of the semiquinone in the rapidly reversible chemical reaction R + T$^+$ + H$^+$ = 2S$^+$ (reverse of the disproportionation reaction) is expressed by the semiquinone formation equilibrium constant K. This

$$K = \frac{[S^+]^2}{[R][T^+][H^+]} \quad (8)$$

equilibrium constant is related to the standard reduction potentials $E_1°$ and $E_2°$ as follows.

$$E_2° - E_1° = (RT/F) \ln K \quad (9)$$

The subsequent algebra is simplified by letting $K' = K[H^+]$, from which also

$$E_2 - E_1 = (RT/F) \ln K' \quad (9')$$

The electrode potentials of the first oxidation step (E_1) for the phenothiazine derivatives are given in Table I. It is of considerable interest and of predictive value that the reduction potentials yield a linear Hammett plot (Figure 1) when the *para* substituent con-

TABLE II
ASSIGNMENT OF SUBSTITUENT CONSTANTS

	Assignment			Cor coeff	Slope ± std error
	para	*meta*	*n*		
	Phenothiazine Derivatives from Table I				
A[a]	3,7	2,4	17	0.976	0.270 ± 0.033
B[b]	2	3,7	16[c]	0.938	0.474 ± 0.055
C	All positions av of *meta* and *para*		16[c]	0.959	0.352 ± 0.044
D	All positions *para*		17	0.981	0.275 ± 0.028
	Promazine[d] Derivatives from Billon[9]				
A[a]	3	2	12	0.888	0.278 ± 0.045
B[b]	2	3	12	0.943	0.238 ± 0.033
C	All positions av of *meta* and *para*		12	0.973	0.286 ± 0.023
D	All positions *para*		12	0.984	0.236 ± 0.017

[a] With reference to nitrogen. [b] With reference to sulfur. [c] Data for 4-chloro-7-methoxyphenothiazine not included since the 4 position is *ortho* in these assignments. [d] For consistency only the derivatives of 10-dimethylaminopropylphenothiazine (promazine) are correlated here.

stants are assigned to the 3 and 7 positions and the *meta* to the 2 and 4 positions. Some of the derivatives have more than one substituent and for such compounds the substituent parameter σ is the sum of the substituent constants. The data are in agreement with the previously observed additivity of the substituent constants for aromatic compounds with more than one substituent.[7] These results are expressed algebraically (least-squares calculation) by the equation

$$E_1 = 0.675 + 0.27\sigma \qquad (10)$$

Alternate assignments of substituent constants have been tested and are summarized in Table II. It can be seen that either assignment A or D gives a good Hammett correlation. Additional statistical analyses were carried out on the polarographic potential data of Billon[9] for 10-dimethylaminopropyl derivatives of phenothiazine in acetonitrile solution. For Billon's data, assignment D gives the best correlation. The assignment of the *para* substituent constants to the 2, 3, 7, and 8 positions is consistent with the combined data of Billon and Craig, and appears to be independent of N substitution.

It is useful to show that the electrode potential of the second oxidation step E_2 also obeys a Hammett relationship. In spite of the potentiometric instabilities of their titration curves, Craig, *et al.*,[1c] were able to obtain E_2 values directly for a few compounds. These are used in a subsequent paragraph, together with values estimated in another way, to obtain a quantitative Hammett relationship. We first show from kinetic evidence that such a linear relationship probably exists.

Using esr techniques, Tozer and Tuck[10] observed that the concentrations of the semiquinones of phenothiazines monosubstituted in the 3 position decrease with time after their initial formation by the addition of 1 equiv of the oxidant Ce^{4+} to an aqueous AcOH solution of the phenothiazines. The over-all rate of disappearance depends on the substituent and is of second order. Using *para* substituent constants, a Hammett plot of the log of the second-order decay rate constants shows

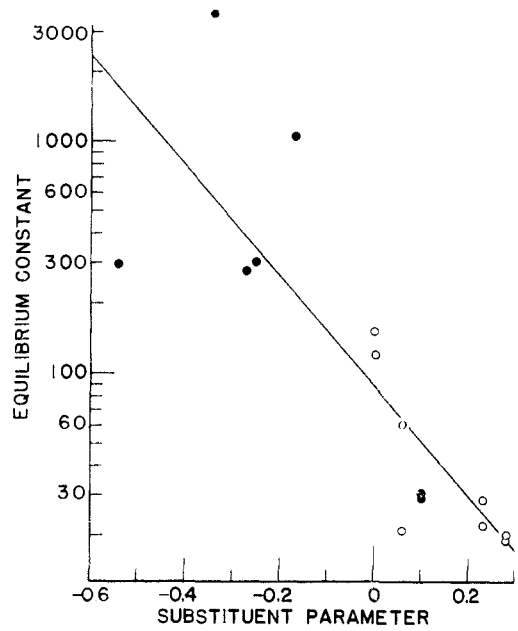


Figure 2.—Semiquinone formation equilibrium constant as a function of the substituent parameter. The solid circles are the calculated equilibrium constants for the compounds for which E_1 and E_2 have been measured (ref 1c, Table 14). The open circles are estimates of the equilibrium constants obtained from the data in Figure 2 of ref 1a.

linearity; the semiquinones of the derivatives of higher E_1 have the higher rates. It has been shown[11] that phenothiazine semiquinone itself is stable in this solution. The decay of the semiquinone occurs because of solvolysis of the two-electron oxidation product of phenothiazine, phenazothionium ion. The rate of disappearance of the semiquinone thus depends upon two factors: the disproportionation equilibrium of the semiquinone with its totally oxidized and reduced forms, and the susceptibility of the phenazothionium ion to solvolysis. Because the decay rates of the semiquinones are correlated in a Hammett plot, both the semiquinone formation constant (reciprocal of the disproportionation constant) and the reactivity of the phenazothionium ion must be related to the substituent effects.

By reference to eq 9 it is seen that since E_1° and the logarithm of K are linearly related to the substituent constant, it follows that E_2° also must be so related.

Because of the lack of adequate E_2 data from the second step of the titration it was necessary to determine values of E_2 from the shape of the first univalent step of the potentiometric titration curve. Using a graphical method based on the equation of Clark,[12] values of K' are estimated from the values of the electrode potentials at the $^1/_4$, $^1/_2$, and $^3/_4$ equivalent points on the titration curves. Values of log K' so determined, as well as a few directly calculated by eq 9′ from available E_1 and E_2 data, are plotted in Figure 2 against the Hammett substituent parameter. Using the method of least squares the best straight line found to represent the data at pH 2 is

$$\log K' = 1.94 - 2.38\sigma \qquad (11)$$

(8) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).
(9) J. P. Billon, *Ann. Chim.* (Paris), **7**, 183 (1962).
(10) T. N. Tozer and L. D. Tuck, *J. Pharm. Sci.*, **54**, 1069 (1965).
(11) T. N. Tozer, Ph.D. Dissertation, University of California, San Francisco, Calif., 1963.
(12) W. M. Clark, "Oxidation-Reduction Potentials of Organic Systems," Williams and Wilkins Co., Baltimore, Md., 1960, p 189, eq 20.

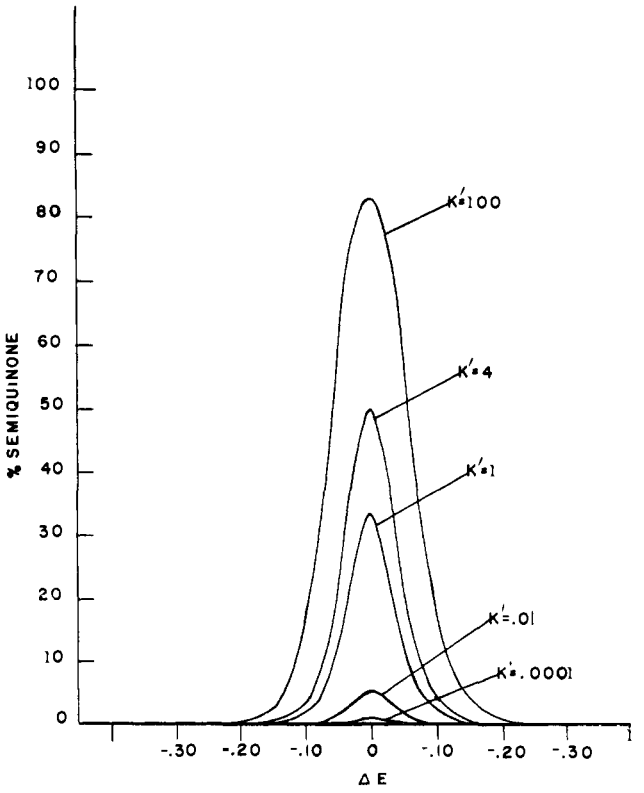


Figure 3.—Per cent semiquinone as a function of the difference ΔE between the poised potential of the solution E and the bivalent midpoint electrode potential E_m for various values of the semiquinone formation equilibrium constant.

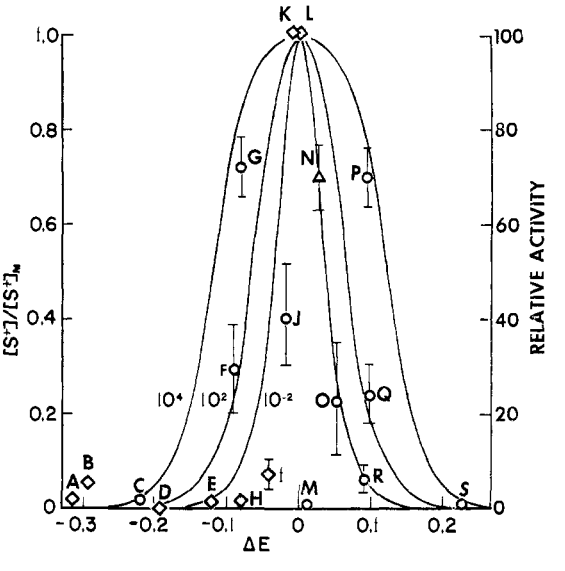


Figure 4.—Ratio of semiquinone concentration to its maximum value (left scale) as a function of ΔE for various values of the semiquinone formation equilibrium constant K'. The anthelmintic activities (right scale) of the phenothiazine derivatives are superimposed as a function of E_1 so that the ΔE at maximum activity is zero: △, unsubstituted; ○, monosubstituted; ◇, disubstituted. For identifying letters, see Table I. Vertical bars represent standard errors where these are $>\pm 2$.

The correlation coefficient is -0.834 ($P < 0.001$), and the slope and its standard error are -2.38 ± 0.44. Since this analysis is based on only a small fraction of the compounds studied, and taking into account the sources of error involved in the methods of determination of K', a high precision cannot be expected for the above numerical values. For example, the equilibrium involving protonation of 3-amino, 3,7-diamino, or 3,7-bis(dimethylamino) derivatives[13] is undoubtedly responsible for their not possessing the values of log K' which would be predicted from Figure 2. Assignment of a substituent constant for these compounds is complicated because, in contrast to the amino group, the ammonium ion has a large positive substituent constant.

Combination of eq 9′, 10, and 11 yields

$$E_2 = 0.791 + 0.127\sigma \qquad (12)$$

Concentration of Semiquinone.—The concentration of the semiquinone of each of the phenothiazines at the active site in the parasite cannot be measured directly. However, concentrations *in vivo* can be calculated from the following information: (1) the total phenothiazine concentration (phenothiazine + phenothiazine semiquinone + phenazothionium ion), (2) the difference between the bivalent midpoint electrode potential at the physiological pH and the local potential of the affected physiological system, and (3) the inherent stability of the semiquinone as determined by the semiquinone formation equilibrium constant at physiological pH.

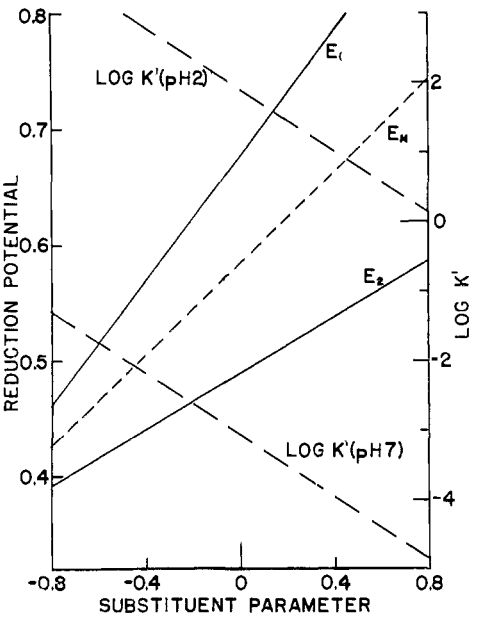


Figure 5.—Experimental values of E_1 and log K' at pH 2 and calculated values of E_2, E_m, and log K' at pH 7 as functions of the substituent parameter.

Ignoring the distinction between concentration and thermodynamic activity and using A to represent the total concentration of the substituted phenothiazine, $A = [R] + [S^+] + [T^+]$, and Q the ratio $[T^+]/[R]$, the semiquinone concentration is then obtained by substitution into eq 8.

$$[S^+] = \frac{A(K'Q)^{1/2}}{(K'Q)^{1/2} + Q + 1} \qquad (13)$$

(13) S. Granick, L. Michaelis, and M. P. Schubert, *J. Amer. Chem. Soc.*, **62**, 1802 (1940); L. Michaelis and S. Granick, *ibid.*, **64**, 1861 (1942).

From eq 7 it follows that Q equals $10^{\Delta E/0.03}$ at 30°. On substituting in (13), the equation becomes

$$[S^+] = \frac{A(K')^{1/2}}{(K')^{1/2} + 10^{\Delta E/0.06} + 10^{-\Delta E/0.06}} \quad (14)$$

Figure 3 shows the percentage of semiquinone ($100[S^+]/A$) as a function of the difference ΔE between the ambient physiological potential and the bivalent midpoint reduction potential for various values of K'. The curves in Figure 4 show the calculated ratio of the semiquinone concentration to its value at $\Delta E = 0$ as a function of ΔE for various values of K'. Increasing the value of K' has the effect of broadening the region of ΔE where the semiquinone concentration is appreciable. It can be seen from Figures 3 and 4 that (except for extremely large values of K') the semiquinone concentration is relatively negligible for compounds with an E_m greater than 150 mV above or below E. That is to say, beyond these limits the compounds exist substantially in either their reduced or oxidized forms.

Correlation with Anthelmintic Activity.—The anthelmintic activities of the substituted phenothiazines studied by Craig, *et al.*,[1] are given in Table I.[14] These activities are also shown as points in Figure 4 as a function of their first univalent midpoint electrode potentials E_1; the figure is drawn so that the compounds of maximum activity correspond to a ΔE of zero. It is then evident that anthelmintic activity appears to be concentrated in the region $-0.1 < \Delta E < 0.1$, *i.e.*, in the arc of maximum semiquinone concentration. If the semiquinone were the active anthelmintic species, the bivalent midpoint electrode potential of the active phenothiazines would be expected to be close to the physiological potential of the system with which they interfere, since the concentration of the semiquinone at the site of action would become insignificant for larger magnitudes of ΔE.

However, there is a theoretical basis for a correlation with E_m rather than E_1. Using our tentative Hammett relations, eq 10 and 12, with eq 6, an expression is derived for the bivalent midpoint electrode potential E_m at pH 7, using this as an approximation for the pH at the active site in the parasite. A similar correction is applied to eq 11. For pH 7, these quantities now have the following dependences on the substituent parameter.

$$E_m = 0.583 + 0.198\sigma \quad (15)$$

$$E_2 = 0.491 + 0.127\sigma \quad (16)$$

$$\log K' = -3.07 - 2.38\sigma \quad (17)$$

Potential E_1 is independent of pH. These functions are graphically expressed in Figure 5.

Substitution into eq 14 yields a general relationship between $[S^+]$ and σ of the form

$$\frac{[S^+]}{A} = \frac{10^{B\sigma}}{10^{B\sigma} + (C \times 10^{D\sigma}) + (F \times 10^{-D\sigma})} \quad (18)$$

(14) The reason for the larger standard errors in some compounds of low and moderate activity is that most such compounds were tested only on a group of five animals while those showing high activity were tested on larger groups of animals. These standard errors are shown in Figures 4 and 6, and it is seen that they do not affect the general argument.

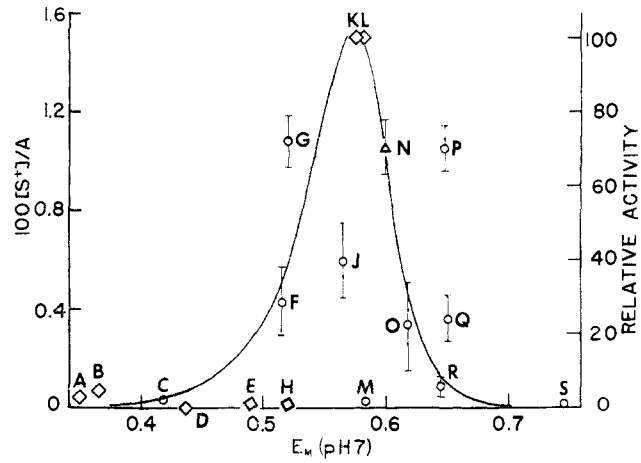


Figure 6.—Calculated per cent semiquinone (solid line) at pH 7 as a function of the bivalent midpoint electrode potential E_m for a system poised at 0.583 V. The anthelmintic activities (points) of the derivatives are shown at their calculated E_m values at pH 7: △, unsubstituted; ○, monosubstituted; ◇, disubstituted. For identifying letters, see Table I. Vertical bars represent standard errors where these are > ±2.

The symbol A is defined above, $2B$ is the slope of the plot of log K' *vs.* σ, $0.060D$ is the slope of a plot of E_m *vs.* σ, and the constants C and F are defined by $0.03 \log C = E - E_2$ and $0.03 \log F = E_1 - E$ for the compound for which $\sigma = 0$. The relative concentration of semiquinone $[S^+]/A$ at pH 7 and $E = 0.583$ V is found by substitution of appropriate numbers into eq 18 to give

$$\frac{[S^+]}{A} = \frac{10^{-1.19\sigma}}{10^{-1.19\sigma} + (34.3 \times 10^{-3.30\sigma}) + (34.3 \times 10^{+3.30\sigma})} \quad (19)$$

The ordinate for the curve of Figure 6 is based on calculations from this equation, and the abscissa is calculated from eq 15. Figure 6 also shows the anthelmintic activities at their calculated E_m values at pH 7. It is seen that, allowing for the various uncertainties inherent in the measurements, active phenothiazine derivatives appear to have E_m values in the region of greatest semiquinone concentration, as shown by the slightly skew bell-shaped curve, suggesting that semiquinone concentration may indeed be a major determinant of anthelmintic activity.

Such a conclusion is consistent with the earlier observations[1] that only those compounds having an E_1 between 550 and 850 mV (at pH 2) were active. The additional requirement for a free 3 or 7 position, discussed elsewhere,[1] is reflected in the lack of activity of the 3,7-disubstituted compounds (A, B, D, E, and H in Table I).

The above relations still contain a degree of uncertainty arising because of the necessity for extrapolating the essential emf data, obtained in an aquo-acetic acid solvent, to water.

The choice of 0.583 V for E is based upon the bell-shaped curve of anthelmintic activities as a function

of E_m at pH 7. Although this potential is greater than the potential of normal biological substrates, correction for the solvent system may, in fact, make the electrode potentials E_1, E_2, and E_m smaller by as much as 200–300 mV, resulting in a potential within the range of the isolated cytochromes, and considerably below the electrode potential of oxygen–water (0.810 V) at pH 7. One might speculate that the electron-transport particle is the site of action of these anthelmintics. Moreover, such a correction would result only in a bulk shift of the data as well as the curves as they appear in Figures 4 and 6, and would not affect any conclusions derived from these figures.

It should be emphasized that in the foregoing discussion the site and mechanism of action of the phenothiazine anthelmintics are hypothetical, and little is therefore known about the possible effects of other factors such as distributive and metabolic parameters. However, the observed correlation appears interesting and significant enough to encourage further investigation of systems in which semiquinone free radicals are suspected to be the biologically active species.

α,α,α-Trifluorotoluamides as Anticoccidial Agents

DEAN E. WELCH, ROBERT R. BARON, AND BRUCE A. BURTON

Research Division, Salsbury Laboratories, Charles City, Iowa 50616

Received September 3, 1968

The preparation and anticoccidial activity of a number of α,α,α-trifluorotoluamides and related compounds are reported. Several active compounds were obtained, but the most active were the amide, dimethylamide, ethylamide, and diethylamide in which the trifluoromethyl and a nitro group are in a 3,5 relationship. One other amide with 2-chloro-5-trifluoromethyl showed similar activity.

The use of nitrated and halogenated benzamides (**1–3**) as feed additives for the control of poultry coccidiosis has been known for several years.[1–4] In these compounds the nitro group is known to be essential for significant anticoccidial activity.[5] Certain aminobenzoic acids and related compounds are also known to have anticoccidial activity.[6] These compounds are believed to act as *p*-aminobenzoic acid (PABA) antagonists because simultaneous administration of PABA is reported to reduce their efficacy. Also, it has long been recognized that certain coccidia are sensitive to known PABA antagonists such as the sulfonamides and 4,4′-diaminophenyl sulfones.[7–11] In contrast there is no direct evidence that compounds such as **1–3** act as PABA antagonists.

During the past 20 years a substantial effort has been devoted to the replacement of hydrogen, nitro, halogen, or methyl by fluorine or trifluoromethyl in prototype molecules which are known to have chemotherapeutic activity.[12–14] This work has led to some compounds with interesting and often more powerful and varied biological activity.

As part of a continuing search for new and improved anticoccidial agents and prompted by previous work on organofluorine drugs, we became interested in trifluoromethylbenzamides similar to **1–3**. The object of the study was to determine if replacement of a nitro group by a trifluoromethyl group would give a compound with anticoccidial activity, and, if so, what structural requirements were necessary for this activity.

Chemistry.—The compounds initially prepared for testing are listed in Table I. Most of the amides were prepared from the acid chloride using commercially available α,α,α-trifluoro-*m*-toluic acid (**50**) as a starting point. However, several attempts to prepare the N-aminoethyl- and N-hydroxyethylamides by this route always gave the disubstituted derivatives **22** and **23**. Amides **31**, **34**, and **35** were obtained from hydrolysis of the appropriate nitriles.

The amino derivative **26** and the *o*-hydroxyamide (**27**) were prepared from the esters **46** and **48** and concentrated NH_4OH under pressure. A cursory attempt to prepare **26** from the *o*-amino ester **45** was not successful. The preparation of **24** was best accomplished by catalytic reduction of **7** rather than ammonolysis of the ester **49**. The other amides were prepared by the acid chloride–NH_3 route.

During the course of this investigation it was of interest to determine if a change in the amide portion of the molecule would give compounds with anticoccidial activity. Consequently the thioamide **53**, sulfonamide **55**, nitriles **51** and **56**, and amidine derivatives **52** and **54** were prepared as described in the Experimental Section.

(1) N. F. Morehouse and W. C. McGuire, *Poultry Sci.*, **36**, 1143 (1957); **37**, 1228 (1958); **38**, 410 (1959).
(2) T. A. Hymas and G. T. Stevenson, *ibid.*, **39**, 1261 (1960).
(3) R. R. Baron, M. W. Moeller, and N. F. Morehouse, *ibid.*, **45**, 411 (1966).
(4) S. J. Ball and E. W. Parnell, *Nature*, **199**, 612 (1963).
(5) Salsbury Laboratories, unpublished results.
(6) E. F. Rogers, R. L. Clark, H. J. Becker, A. A. Pessolano, W. J. Leanza, E. C. McManus, F. J. Andriuli, and A. C. Cukler, *Proc. Soc. Exp. Biol. Med.*, **117**, 488 (1964).
(7) C. Horton-Smith and E. Boyland, *Brit. J. Pharmacol.*, **1**, 139 (1946).
(8) L. P. Joyner and S. B. Kendall, *ibid.*, **11**, 454 (1956).
(9) E. Waletzky and C. O. Hughes, *Amer. J. Vet. Res.*, **7**, 365 (1946).
(10) E. H. Peterson, *ibid.*, **9**, 77 (1948).
(11) L. C. Grumbles, J. P. Delaplane, and T. C. Higgins, *Poultry Sci.*, **27**, 605 (1948).
(12) For a review of the trifluoromethyl group in medicinal chemistry and references to its inductive and hyperconjugative comparison to other groups, see H. L. Yale, *J. Med. Pharm. Chem.*, **1**, 121 (1959).
(13) A. Burger, "Medicinal Chemistry," Interscience Publishers, Inc., New York, N. Y., 1960, p 82.
(14) R. E. Bambury, H. K. Yaktin, and K. K. Wycoff, *J. Heterocycl. Chem.*, **5**, 95 (1968).