INTERACTION OF TRACE METALS WITH PHENOTHIAZINE DRUG DERIVATIVES, I. STRUCTURE-REACTIVITY CORRELATIONS*

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Trace metal elements and phenothiazine drugs separately have been subjects of great interest in contemporary biology and medicine. The former category includes normal constituents of living systems, while the latter represents unnatural substances of pharmacological importance. In both cases, a broad spectrum of biological activity has been defined, but the underlying molecular biology remains largely undetermined.1–7

An interrelationship between certain trace metals and phenothiazine derivatives was first suggested when it was observed that a thiazine drug (chlorpromazine) could suppress markedly the binding of divalent manganese by tissue homogenates and soluble protein, although it was not then clear whether this represented a direct reaction or competition for binding sites.8 In the present communication, a chromogenic reaction between thiazines and manganese (and some other metals) is examined in an effort to establish some molecular interactions of possible importance in situ within organisms.

Materials.—Reagents: Metal salts and other reagents used were all of analytical grade. Co+++ was prepared as Na2Co(CO3)2·6H2O.9

Phenothiazine derivatives: Smith Kline and French Laboratories provided chlorpromazine hydrochloride, chlorpromazine sulfoxide, prochlorperazine dimaleate, and trifluoperazine dihydrochloride; Warner-Chilcott Laboratories, mepazine hydrochloride and etopropazine hydrochloride; and Wyeth Laboratories, promethazine hydrochloride. The structural formulas of these congeners are depicted in Figure 1. Fresh aqueous stock solutions were made up at 0.010 M (or occasionally higher) and stored in the dark.

Apparatus: A Beckman Model H-2 pH meter was provided with a glass electrode and a calomel, saturated-KCl reference electrode. Ratio-recording, direct-writing spectrophotometers (Beckman DK-2 and Cary Model 14) were used for recording visible and ultraviolet absorption spectra.

During titrations a Rehberg microburet served to add concentrated solutions of alkali or acid to solutions of more dilute reactants under constant stirring.

Results.—Formation of a colored product: Chlorpromazine was insoluble in water at pH ≥ 6.5. Upon admixture of manganous salts, weakly acid solutions of chlorpromazine (2 < pH < 6.5) remained colorless. If mixed solutions of divalent manganese and the drug were titrated first to the physiological pH range of 6.8–7.5 with NaOH or KOH and then back-titrated to pH < 6.5 with HCl (or other mineral acid) a characteristic rose-red color promptly appeared.8 This occurred over a wide range of concentrations of the reactants.

The dominant absorption band in the visible region of the colored product was at 523 mμ (Fig. 2). Increasing concentrations of reactants under otherwise similar conditions gave rise to greater intensity of color. The effects of the concentration and stoichiometry of the reactants, as well as some other variables, are treated in
### Phenothiazine Derivatives

<table>
<thead>
<tr>
<th>Derivative</th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
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<tbody>
<tr>
<td>Phenothiazine</td>
<td>-H</td>
<td>-H</td>
<td>-</td>
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<tr>
<td>Chlorpromazine</td>
<td>-( \text{CH}_3\text{CH}_2\text{N} )( \text{CH}_3 )</td>
<td>-( \text{Cl} )</td>
<td>-</td>
</tr>
<tr>
<td>Chlorpromazine Sulfoxide</td>
<td>-( \text{CH}_3\text{CH}_2\text{N} )( \text{CH}_3 )</td>
<td>-( \text{Cl} )</td>
<td>=( \text{O} )</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>-( \text{CH}_2\text{CH}_2\text{N} \rangle \text{NCH}_3 )</td>
<td>-( \text{Cl} )</td>
<td>-</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>-( \text{CH}_2\text{CH}_2\text{N} \rangle \text{NCH}_3 )</td>
<td>-( \text{CF}_3 )</td>
<td>-</td>
</tr>
<tr>
<td>Mepazine</td>
<td>-( \text{CH} \langle \text{CH}_3 )</td>
<td>-( \text{H} )</td>
<td>-</td>
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<tr>
<td>Promethazine</td>
<td>-( \text{CH}_2\text{CHN} \langle \text{CH}_3 )( \text{CH}_3 )</td>
<td>-( \text{H} )</td>
<td>-</td>
</tr>
<tr>
<td>Ethopropazine</td>
<td>-( \text{CH}_2\text{CHN} \langle \text{CH}_3 )( \text{CH}_3 )( \text{CH}_2\text{H} )</td>
<td>-( \text{H} )</td>
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**Fig. 1.**—Structural formulas of phenothiazine derivatives used in these experiments and of the parent compound, phenothiazine.

The solubility of phenothiazine derivatives in acid media is due chiefly to protonation of the substituted amine side chain ("\( R_1 \)" in Fig. 1). Alkalization to the point where the charge on the thiazine molecule becomes neutralized should give rise to a

**Fig. 2.**—Absorption spectra in the visible region of the colored reaction products produced from the phenothiazine derivatives listed in Figure 1. For a given congener, the spectrum was qualitatively identical regardless of whether the Mn-titration system or other generating reactions discussed in the text were used. Note that the dominant absorption bands possess but one of three wavelengths, depending only on the nature of the thiazine ring substituent, "\( R_2 \)" in Figure 1: 513 \( \mu \) (—\( \text{H} \)), 523 \( \mu \) (—\( \text{Cl} \)), or 497 \( \mu \) (—\( \text{CF}_3 \)).
relatively nonpolar uncharged phenothiazine base of low solubility. Indeed, when solutions of phenothiazine derivatives were titrated with alkali, white precipitates of the drug molecules were seen invariably. The titration curves (Fig. 3) clearly revealed the anticipated requirement of one equivalent of alkali per mole of monobasic side chain, while two separate titration steps were observed with trifluoperazine due to the presence of a dibasic alkyl piperazinyl group (Fig. 1).

When the titrations were performed in the presence of equimolar divalent manganese, there was no apparent departure from the curves of Figure 3, and the white precipitates appeared as usual. However, if slight excess of alkali was added following neutralization of the charged groups, insoluble manganese precipitates promptly appeared which redissolved only very slowly upon reacidification. Nevertheless, the intensity of red color produced by back-titration depended upon whether alkaline titration of the drug had been completely brought about or not.

With aqueous solutions of 0.005 M chlorpromazine and 0.005 M MnCl₂ under air, the apparent molar extinction coefficients (εₚₑₚₑ) at 523 nm following such titrations did not exceed approximately 400. Surprisingly, when all solutions were bubbled with 100% oxygen, apparent εₚₑₚₑ's up to 700–800 were achieved. Conversely, when nitrogen or helium were bubbled through these solutions, the chromogenic reaction was almost totally suppressed.

The dependence of the chromogenic reaction on oxygen and on the titration procedures was explained by postulating that chlorpromazine reacts with trivalent manganese and not with the divalent species. However, at pH ≈ 9 (Fig. 3) the autoxidation equilibrium between Mn²⁺ and Mn³⁺ produced by dissolved oxygen is overwhelmingly in favor of the lower valence form, and the population

![TITRATION OF PHENOTHIAZINE DRUGS](image-url)
of ions in the manganic state is exceedingly small. Reaction with receptor molecules will not tend to occur unless factors are favorable for the approach of the trivalent ions to the immediate vicinity of the drug molecules. Alkalization facilitates this close approximation by neutralizing the charge repulsion between the thiazine and manganese ions.\textsuperscript{13}

This hypothesis was supported by experiments designed to obviate the titration and oxygenation: manganous salts were converted to the trivalent (or higher) oxidation state prior to mixing with the phenothiazine by alkalization of oxygenated manganous salt solution, thus forming the brown hydrated oxide MnO(OH).\textsuperscript{14} This was added anaerobically in excess or in equal concentration to a slightly acid solution of 0.01 $M$ chlorpromazine, and the typical color appeared spontaneously. The reaction was slow, presumably because of the slight solubility of MnO(OH). MnO\textsubscript{2} also slowly gave rise to production of red color in deoxygenated chlorpromazine solutions.

\textit{The effect of metal cations of physiological importance:} The requirement for oxygen in the reaction of chlorpromazine with divalent manganese and the spontaneous reaction with MnO(OH) and MnO\textsubscript{2} suggest that an oxidation step directly involving chlorpromazine is necessary in the formation of color with manganese. Terpositive manganese is recognized as a strong oxidizer, and the quadrivalent state, which is unstable except as MnO\textsubscript{2}, also can act as an oxidizing agent.\textsuperscript{14} Hence, other oxidizing metal ions also might be expected to react similarly with chlorpromazine and its congeners.

Experiments with Fe\textsuperscript{+++} and Co\textsuperscript{+++} confirmed the expectation of spontaneous interaction between thiazine derivatives and these ions.\textsuperscript{16, 15} In each instance, the absorption spectrum of the colored product from equimolar concentrations (0.005 $M$) of phenothiazines and ferric or cobaltic\textsuperscript{9} salts was identical qualitatively with that produced by manganese (Fig. 2). On the other hand, other metal cations of biological interest were inert: Ni\textsuperscript{++}, Cu\textsuperscript{++}, Zn\textsuperscript{++}, Al\textsuperscript{+++}, Cr\textsuperscript{+++}, Ca\textsuperscript{++}, and Mg\textsuperscript{++} at 0.0025 $M$ did not react with 0.0025 $M$ chlorpromazine or mepazine either spontaneously or following a titration-back-titration step.\textsuperscript{16} Furthermore, these ions did not interfere in the chromogenic reactions of chlorpromazine with manganese or iron. This held true whether the spontaneous interaction or the titration-dependent type were investigated and even when these metals were present in three or four times the concentration of the manganese (or iron) and chlorpromazine (0.0017 $M$ of each).\textsuperscript{17} Co\textsuperscript{++} was similarly inactive, and even Mn\textsuperscript{++} did not affect the spontaneous reaction with Fe\textsuperscript{+++}. However, Fe\textsuperscript{++} inhibited color formation with Fe\textsuperscript{+++} or manganese ions or quenched color if added after the thiazine-metal reaction.\textsuperscript{15, 16} However, Fe\textsuperscript{++} alone did not produce colors with phenothiazines and did not alter their respective absorption spectra.

\textit{Some characteristics of the colored product from chlorpromazine:} The chlorpromazine reaction product was metastable and photosensitive regardless of how it was obtained. Appreciable spontaneous bleaching occurred over the first few hours even in the dark, and this continued more slowly over several days. The photosensitivity was most marked in ultraviolet light.

Unlike native chlorpromazine, the colored product was nonfluorescent and gave rise to an ultraviolet absorption spectrum characteristically different from those of either the drug itself or of its sulfoxide (Fig. 4).
When a red product was generated from a titrated manganese chlorpromazine solution, there was slight diminution in the intensity of the major ultraviolet absorption band corresponding to chlorpromazine with the concomitant appearance of a small absorption peak or "shoulder" in the 274 mμ region, where the colored product absorbed strongly (Fig. 4). Conversely, upon bleaching of the chromophore by ultraviolet light, there was a parallel decrease in the absorption bands at 274 and 523 mμ (ref. 10) and a reciprocal enhancement of the main chlorpromazine peak. Similar data were obtained with mepazine and trifluoperazine. Hence, a small fraction of thiazine was converted to the chromophoric form.

Structure-reactivity correlations with a series of substituted phenothiazine drug derivatives: It was noted earlier that colored reaction products were formed from thiazine drugs other than chlorpromazine; however, with any given derivative spectrally identical products were obtained from all manganese-generating systems and with ferric iron (or Co++, where tested) (Fig. 2). When the major visible-region absorption peaks of the resultant colors were correlated systematically with the molecular structure of the drugs (Fig. 1), a meaningful pattern was disclosed. The position of the dominant absorption band was found to shift slightly according to changes in substituents directly on the resonating planar ring system ("R₂" in Fig. 1), much as Michaelis had noted two decades ago with a series of substituted Wurster's dyes.19, 20 On the other hand, the nature of the side chains connected to the thiazine nucleus by single bonds through an N-alkylamine bridge ("R₁" in Fig. 1) did not alter the wavelength of the main absorption peaks. Hence, chlorpromazine and prochlorperazine were seen to possess identical spectra (Fig. 2) despite their different substitutions at "R₁" (Fig. 1). However, the characteristic peak for these derivatives at 523 mμ was distinct from that of trifluoperazine, which differs in its ring substituent at "R₂" (Fig. 1), while mepazine, promethazine, and ethopropazine—which are unsubstituted at "R₂" (Fig. 1)—demonstrated their greatest absorbancy at another wavelength (Fig. 2).

Discussion.—The correlations of spectral type with chemical substitution on
the phenothiazine ring rather than with N-amino substituent groups implicate the thiazine nucleus itself in the interaction with metal ions. The locus on the planar ring system whose properties are most propitious for a reactive site would appear to be the heterocyclic sulfur atom (thioether), with its lone pair of electrons available for donation to the metal. This interpretation was reinforced by many observations that chlorpromazine sulfoxide, with its thioether locus blocked by oxygen ("R₂" in Fig. 1), could not be made to develop a colored product with any of the oxidizing systems that were chromogenic with native chlorpromazine. In a following theoretical part of these reports, many of the pharmacological characteristics common to all substituted phenothiazines will be correlated with the reactive properties of the thiazine rings and their bridging sulfur atoms.

Chromophores of unidentified chemical species have been generated previously from phenothiazines in the presence of trivalent iron and strong acid. These have been used for analytical detection of phenothiazine congeners. The published spectra of these products coincide with those of Figure 2, but, in the present investigations, similar spectra were obtained with and without sulfuric acid. Hence, the experiments reported here indicate that the strong acid per se is not essential for the characteristic chromogenesis. A stabilizing role of strong acid on resonating free radical forms of thiazines was shown many years ago by Michaelis et al.

Therefore, concentrated acid could serve in preserving the oxidizing metal ions.

Summary.—Colored products were produced from chlorpromazine and several other phenothiazine derivatives by the action of the trivalent cations Fe³⁺⁺, Co⁺⁺⁺, and Mn⁺⁺⁺, which are of biological interest. With a given phenothiazine derivative, the colored product formed was the same, regardless of which cation was used for the reaction. In the case of manganese, the colored product could be formed by reaction of Mn⁺⁺ and atmospheric oxygen at physiological pH ranges.

The ions Ni⁺⁺, Cu⁺⁺, Zn⁺⁺, Al⁺⁺⁺, Ca⁺⁺, and Mg⁺⁺ did not react to form colored products.

The divalent sulfur of the thiazine nucleus appears to be essential for the formation of the colored products, since none was formed from chlorpromazine sulfoxide.

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The effect of alkalinization was not to facilitate the autoxidation of manganese on the interface provided by precipitated phenothiazine. For example, with dibasic trifluoperazine plus manganous salts, aerobic alkalinization produced color as the first positive charge was neutralized, corresponding to the initial step of the titration curve of Figure 3. Precipitation, per se, occurred only after the second proton was titrated (Fig. 3).


Yamamoto et al. (Jap. J. Pharm., 10, 38 (1960)) also noted a pink color with maximum absorption at 525 mA (sic) upon mixing chlorpromazine with FeCl₃, but FeCl₂ and CuCl₂ did not react.

The apparent inactivity of these ions is in concordance with the observation of Yamamoto et al. that chlorpromazine inhibits cysteine oxidation by Fe³⁺ but not by Cu²⁺ (Jap. J. Pharm., 6, 138 (1957)), since the present experiments elucidate an Fe³⁺-chlorpromazine interaction but no reaction with Cu²⁺. See also Yamamoto's later article (Jap. J. Pharm., 10, 38 (1960)) reporting chlorpromazine inhibition of enzymes containing Fe³⁺ but not of enzymes containing Cu²⁺ or Fe²⁺.

The ultraviolet absorption spectra of mepazine and trifluoperazine appear virtually identical with that of chlorpromazine (Fig. 4), except that all absorption peaks are shifted ~2 mA towards shorter wavelengths. The ultraviolet absorption spectra of the chromophoric reaction products from both mepazine and trifluoperazine also are essentially congruent with that of the red product from chlorpromazine (Fig. 4) except that they are shifted ~2 mA to have their peaks at ~272 mA.


With chromophores generated somewhat differently, in strong H₂SO₄ in the presence of Fe³⁺, Rieder found the major absorption from promazine at 510-512 mA. This is in accord with the structure-reactivity correlations drawn from the present data, because promazine is structurally identical with chlorpromazine except for its lacking chlorination at "R₂" (Fig. 1).


INTERACTION OF TRACE METALS WITH PHENOTHIAZINE DRUG DERIVATIVES, II. FORMATION OF FREE RADICALS*

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In the preceding paper, it was reported that several biologically important transition-group metals can act on chlorpromazine and its congeners to develop characteristic chromophores even under mild conditions. The nature of this chemical process and the characterization of the colored reaction products are the subject of this communication.