Free Radical Studies by Electron-Spin Resonance of Some Derivatives of Phenothiazine

By DAVID W. SCHIESER† and L. DALLAS TUCK

The existence of stable free-radical semiquinoid intermediates in the oxidation of several N-substituted phenothiazine derivatives of therapeutic importance has been demonstrated by means of electron-spin resonance. The spectra differ from those of the parent compound in the lack of hyperfine structure.

By means of electron-spin resonance, stable semiquinone free radicals have been shown to be formed upon oxidation of certain N-substituted derivatives of phenothiazine. This is an extension of work reported elsewhere (1) on phenoxazine, phenothiazine, and some ring-substituted derivatives of the latter. The compounds of the present study, which are of medical importance because of their tranquilizing, antihistaminic, and antinauseant effects, are (I) prochlorperazine, (II) chlorpromazine, (III) triflupromazine, and (IV) perphanazine. As is seen by their structural formulas in Fig. 1, compounds I and IV contain the common structure of an *n*-propyl chain on the nitrogen, terminated by a piperazine ring, and differing from each other by the substitution in the 4-position. Compounds II and III contain a dimethylaminopropyl chain on the nitrogen, and differ from each other by the nature of the substituent β to the nitrogen.

EXPERIMENTAL

The spectrometer used in this research is described elsewhere (1). Measurements were made as the derivative of the absorption at a frequency of 9400 megacycles per second and at a magnetic field strength of approximately 3300 gauss.

All of the free radical solutions were prepared by dissolving approximately 100 mg. of the solid parent compound in 10 ml. of concentrated sulfuric acid. In all cases but one, it was found that the sulfuric acid was able to oxidize rapidly the drugs to the free radical state. In the case of triflupromazine, the free radical was produced only after addition of 0.1 ml. of 30% hydrogen peroxide to the acid solution.

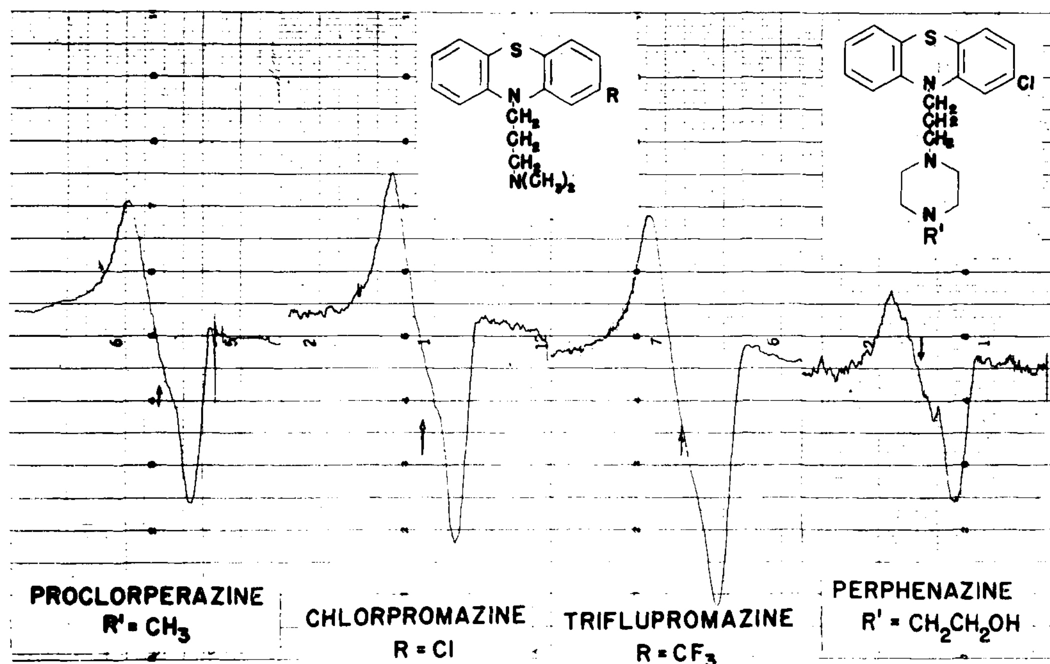

Fig. 1.—Electron-spin resonance spectra of free radicals formed by oxidation of prochlorperazine, chlorpromazine, triflupromazine, and perphenazine. Each horizontal division is 7.4 gauss. Low magnetic field is to the right on this figure.

RESULTS AND DISCUSSION

Figure 1 shows the spectra of the four free radicals and the structural formulas of the parent compounds (reduced state). Table I presents their g values

Received November 25, 1960, from the School of Pharmacy, University of California, San Francisco 22.
Accepted for publication October 6, 1961.
† Present address: Directorate of Medical Research, Army Chemical Research and Development Laboratory, Army Chemical Center, Md.

TABLE I.—g VALUES OF FOUR FREE RADICALS

	g Value
Prochlorperazine	2.0036
Chlorpromazine	2.0034
Triflupromazine	2.0034
Perphenazine	2.0029

determined by comparison with diphenylpicryl hydrazyl, whose resonant position in the magnetic field is indicated in each spectrum by an arrow.

It is seen that the nature of the substituent has almost no perceptible effect on the appearance of the spectrum and has only a minor effect on the g value. These spectra appear to consist of a single absorption line, different from that of phenothiazine and its ring-substituted derivatives, all of which give a spectrum of four well-resolved lines. The latter spectra have been interpreted in terms of the hyperfine splitting by the nitrogen nucleus together with the proton on this position in acid solution. In each of the present spectra, the slight inflection point suggests that the spectrum is the summation of three equally weighted broad component lines arising from splitting by the nitrogen nucleus. The breadth of the lines in this degree appears to be due to the hyperfine splitting by the protons in the side rings and in the complex chain attached to the nitrogen. Other contributory factors to the line breadth are the nature of the solvent system and the magnetic dipole-dipole interaction from neighboring molecules. However, these factors must not play an important role in the present case, as the conditions were comparable with those under which four well-resolved lines were obtained with phenothiazine and other compounds.

Further discussion of these effects will appear in a subsequent publication in which a series of N-alkyl-substituted radicals are studied (2).

Further work is planned to ascertain the concentration of free radical under various conditions of solvent and acidity, and therefrom the semiquinone formation constants (dismutation constants) will be computed.

REFERENCES

(1) Tuck, L. D., and Schieser, D. W., *J. Phys. Chem.*, in press.
(2) Tuck, L. D., and Tozer, T. N., to be published.

Simplified Method for the Measurement of Actomyosin Contraction

By DUANE G. WENZEL and HARBANS LAL

ACTOMYOSIN, the principal contractile protein in muscle, exists in the form of contractile filaments. It is possible to extract these filaments, fragmented transversely but otherwise undamaged, from muscle fiber. The microscopic filaments may then be realigned into a three-dimensional network capable of sustaining tension. It was demonstrated some years ago that threads formed from this actomyosin responded to the addition of adenosine triphosphate (ATP) with spectacular shortening (1). These first threads, however, were composed of poorly oriented actomyosin filaments and did not develop any appreciable tension (2). Close packing of the filaments by treatment with glycerol, drying, and stretching produced threads capable of developing tension (3). It was later demonstrated that relatively strong fibers could be formed by lateral compression of surface-spread layers of actomyosin (4).

The actomyosin model is similar, in many respects, to its intact muscle counterpart. Histologically, of course, the original muscle fiber also possesses a sheath or sarcolemma. The response of the model to ATP is, for all practical purposes, the same as that of the muscle fiber. Because of the relative simplicity of this system and because a specific muscle component may be studied independently, the actomyosin threads or fibers have become popular models for the study of muscle pharmacology.

Received August 17, 1961, from the University of Kansas, School of Pharmacy, Lawrence.
Accepted for publication September 7, 1961.

A number of methods have been employed to measure the contractile response of actomyosin. A simple procedure introduced by Hayashi (5) and later modified by Robb, *et al.* (6), employs a vertical fiber supporting a glass weight. This fiber was prepared in a Langmuir trough and then transferred to a beaker containing the bath solution. The response of the system was determined by measuring the movement of the weight with a microscope having a calibrated eyepiece. This method has several disadvantages. First, the actomyosin fiber must be transferred from the trough to the contraction chamber and must further be extensively manipulated in order to be loaded. Second, the vertical position of the fiber can affect the response to ATP through variable buoyancy and the weight of the thread itself.

In an attempt to eliminate the above disadvantages, a new method for the preparation of the actomyosin fiber and a new measuring apparatus were introduced (7). In this method the surface film of actomyosin is not completely compressed to a thick fiber, but instead is formed into a wider band of a constant width and minimal thickness. This assures a uniform diffusion of ATP into the center of threads or fibers which are still thick enough to support a weight. It has been suggested that during the isotonic contraction of actomyosin, the "unplasticized" core of the fiber not reached by ATP may interfere with the shortening (8). This method utilizing the wide band of actomyosin also permits horizontal attachment of the band between a fixed support extending from the bottom of the