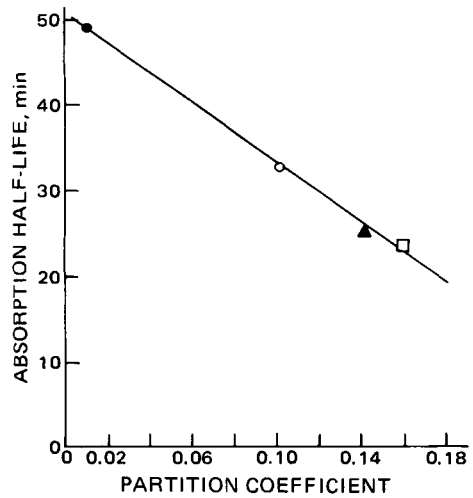

Figure 2—*Absorption half-life as a function of the heptane–phosphate buffer (pH 6.0) partition coefficient for pheniramine maleate (●), chlorpheniramine tannate (○), chlorpheniramine maleate (▲), and brompheniramine maleate (□).*

Of significance was the finding that a plot of the $t_{1/2}$ for disappearance of drug from the lumen as a function of the heptane–phosphate buffer (pH 6.0) partition coefficient was linear with a correlation coefficient of 0.998, as determined from a least-square fit to the observed data (Fig. 2). Our results are in accord with those of Schanker (3) and Kakemi *et al.* (4) who found an excellent correlation of absorption characteristics for a series of barbiturates with their corresponding lipid–water partition coefficients.

Of interest is the comparison of the absorption characteristics of these drugs to the corresponding urinary excretion data observed by Kabasakalian *et al.* (5). They found that the extent of free (unmetabolized) pheniramine excreted in the urine was greater than the extent of free chlorpheniramine excreted which, in turn, was greater than the extent of free brompheniramine excreted. Our current findings support the suggestion by Kabasakalian *et al.* (5) that the more lipid-soluble agents are to a greater extent passively reabsorbed from the kidney tubules back into the blood, allowing for further metabolism and thus resulting in decreased renal excretion of free drug. Therefore, as is true for many drugs, both absorption and excretion profiles of these alkylamine antihistamines follow patterns consistent with their overall lipid solubilities.

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Aggregation of Dantrolene to Human Serum Albumin

Keyphrases □ Dantrolene—binding to human serum albumin, fluorescence quenching study, mechanism evaluated □ Binding—dantrolene to human serum albumin, fluorescence quenching study, mechanism evaluated □ Albumin, human serum—binding to dantrolene, fluorescence quenching study, mechanism evaluated □ Relaxants, skeletal muscle—dantrolene, binding to human serum albumin, fluorescence quenching study, mechanism evaluated

To the Editor:

A recent article (1) concerning the interaction of dantrolene, 1-[[5-(4-nitrophenyl)furfurylidine]amino]hydantoin, with human serum albumin reported difficulty saturating the protein with the drug in an aqueous system. Difference spectrophotometric titrations of these two reactants resulted in a continual hyperchromism of the complexed drug's spectral band. A saturation end-point was not reached, even though the dantrolene concentration in all test solutions was increased to its solubility limit ($\sim 1.0 \times 10^{-4}$ *M*) in the pH 7.4 buffered aqueous system. Furthermore, the albumin was not saturated even in solutions that had the protein concentration reduced 100-fold from 1.45×10^{-4} to 1.45×10^{-6} *M*.

Lack of a definite end-point in the titration was attributed to two possible causes: the poor water solubility of dantrolene, limiting the saturation of available binding sites on the albumin; or association between bound and unbound drug molecules, hindering detection of a saturation point. A closer examination of this problem indicates that the latter self-association is most likely.

The purity of dantrolene[1] as the free acid was established by TLC in three different solvent systems: acetone–chloroform (7:3), R_f 0.50; methanol–chloroform (7:3), R_f 0.55; and benzene–methanol (9:1), R_f 0.80. All solvents were the highest grade commercially available.

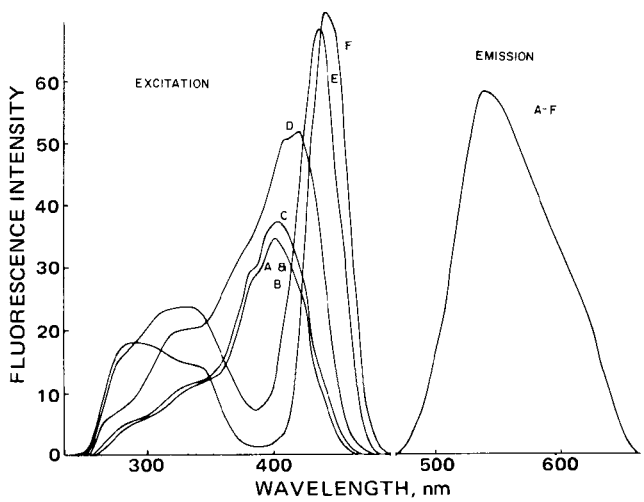

Figure 1—*Corrected fluorescence excitation and emission spectra of dantrolene in chloroform at 3.0×10^{-4} (A), 3.0×10^{-5} (B), 3.0×10^{-6} (C), 3.0×10^{-7} (D), 3.0×10^{-8} (E), and 3.0×10^{-9} (F) M. Instrumental electronic amplification was increased for each spectrum as the concentration was decreased so that the relative band shape and position of each spectrum could be seen.*

[1] Eaton Laboratories, Norwich, N.Y.

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Table I—Corrected Fluorescence Excitation and Emission Maxima of Dantrolene in Solutions of Varying Acidity, Polarity, and Hydrogen Bonding Capability

	Excitation, nm		Emission, nm	
	Monomer	Aggregate	Monomer	Aggregate
Dilute sulfuric acid, pH 3	360	380	520	520
Dilute sodium hydroxide, pH 12	387	387	530	530
Chloroform	390	430	540	540
Nitromethane	400	430	580	580

Anal.—Calc. for $C_{14}H_{10}N_4O_5$: C, 53.45; H, 3.21; N, 17.83. Found: C, 53.53; H, 3.23; N, 17.78.

The corrected fluorescence excitation and emission spectra of dantrolene were recorded on a spectrofluorometer[2] in aqueous solution at pH 3 and 12 and in chloroform and nitromethane.

In aqueous solutions, the dantrolene concentration was varied from 8.0×10^{-9} to 8.0×10^{-5} *M;* in chloroform and nitromethane, the concentrations ranged from 3.0×10^{-9} to 3.0×10^{-4} *M*. The long wavelength excitation and emission spectral maxima of the highest and lowest concentrations of dantrolene employed in these solvents are reported in Table I.

As the dantrolene concentration in chloroform solutions was decreased, the long wavelength excitation maximum shifted from 390 to 430 nm (Fig. 1). Accompanying the shift was a gradual loss in vibrational structure, terminating in the diffuse spectral band with a maximum at 430 nm. Over the same concentration range, the emission spectrum demonstrated no change in band shape or position whether the wavelength of exciting light was 390 or 430 nm.

The shift to longer wavelengths of the corrected excitation spectrum with the subsequent loss of vibrational structure is consistent with the association of molecules in the ground state to form polymeric complexes (usually dimers) containing two or more monomer molecules (2, 3). Such complexes are frequently nonfluorescent (2). This situation is evidently the case with dantrolene since the emission spectrum is not altered in any way by an increase in drug concentration or a change in wavelength of exciting light. Therefore, the emission spectrum with the maximum at 540 nm must be that of the dantrolene monomer.

[2] Perkin-Elmer MPF-4, Perkin-Elmer Corp., Norwalk, Conn.

Results similar to these were obtained for solutions of dantrolene in nitromethane and in aqueous solutions at pH 3. However, in aqueous solutions at pH 12, neither the fluorescence excitation nor emission spectra exhibited any change in band shape or position as the dantrolene concentration in solution was increased. Since dantrolene has a pKa at 7.5[3], this result seems to indicate that the free acid forms polymeric aggregates while the anion does not.

The interaction of dantrolene with human serum albumin is believed to occur through complexation of the anion at a binding site containing both an electropositive and a hydrophobic region (1). If it is assumed that electrostatic attraction between the anion and the electropositive region partially neutralizes the anion, then conditions are suitable for association between unbound molecules of free acid and neutralized, bound anion. An association of this nature would explain why solutions of human serum albumin could not be saturated regardless of their dantrolene concentration.

Such associations of molecules in solution may be due to hydrogen bonding and/or van der Waals interactions. The dependence of aggregate formation upon solution acidity suggests that hydrogen bonding may occur between molecules of the free acid in aqueous solution. Apparently, the dissociable hydrogen with pKa 7.5 may be important not only to the self-association of dantrolene free acid molecules in aqueous solution but to the association of the free acid to previously bound molecules.

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[3] Literature on dantrolene sodium (Dantrium), Eaton Laboratories, Norwich, N.Y.

BOOKS

REVIEWS

Survey of Organic Syntheses, Vol. 2. By CALVIN A. BUEHLER and DONALD E. PEARSON. Wiley-Interscience, 605 Third Ave., New York, NY 10016, 1977. 1105 pp. 16 × 24 cm. Price $25.00.

Every synthetic chemist should appreciate this book, particularly those who are not working in the field but need to plan the synthesis of an organic compound. There is much that recommends this book, notably its convenience in leading one into the vast and seemingly inhibiting realm of the organic chemical literature and the organized way in which functional group preparations are presented.

Volume 2 spans the literature from 1969 to 1975. Over 3000 references from journal articles, reviews, and books are cited. Addenda are included at the end of each chapter, listing references to synthetic methods not available when the chapter was first written.

The organization of this volume is similar to the first volume in that there are 20 chapters, each dealing with different functional groups. Much