values allows a rational selection of the more energetic polymorphic forms of these drugs for pharmacological absorption studies, and also gauges their probable stability in various dosage forms.

It is suggested that large differences in the free energy content of the polymorphs, as was demonstrated in the case of chloramphenicol palmitate may affect significantly the absorption and resulting blood levels. On the other hand, a small difference as was seen with mefenamic acid does not appear to affect the absorbability of the drug.

It is hoped that future studies with polymorphs of other drugs will allow a closer correlation between the free energy differences and drug availability of the polymorphs.

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Quantitative Determination of Dantrolene Sodium and Its Metabolites by Differential Pulse Polarography

PHILLIP L. COX*, J. P. HEOTIS†, DONALD POLIN, and GORDON M. ROSE

Abstract □ A differential pulse polarographic method has been developed for the simultaneous determination of dantrolene sodium and its nonreduced and reduced metabolites. The compound and its metabolites are extracted from urine or plasma with ethyl acetate and the ethyl acetate removed by evaporation. The compounds are dissolved in a small quantity of *N,N*-dimethylformamide and diluted with 0.2 *M* pH 4 acetate buffer. Dantrolene sodium plus the reduced and nonreduced metabolites are quantitatively determined by the polarographic reduction of the azomethine linkage at a cell potential of −0.86 v. The nitro compounds are quantitatively determined as dantrolene equivalents by the reduction of the nitro group at a cell potential of −0.26 v. The difference between the two determinations represents the reduced metabolites. Levels as low as 0.1 mcg./ml. can be determined by the reduction of the nitro group or the azomethine linkage.

Keyphrases □ Dantrolene Na and metabolites—analysis □ Plasma, urine—dantrolene, metabolite determination □ Polarography, differential pulse—analysis

Dantrolene sodium, 1-{[5-(*p*-nitrophenyl)furfurylidene]amino}hydantoin sodium salt hydrate, was reported by Snyder *et al.* (1) as a muscle relaxant of potential clinical usefulness. The compound is currently undergoing clinical investigation.

Research in these laboratories has shown that dantrolene sodium is metabolized by nonreductive and reductive pathways (Fig. 1). *Via* the latter route the nitro group is reduced to the amine (F-405), and in some animals, including man, the amine is acetylated (F-490). By the nonreductive pathway the compound is metabolized to a metabolite designated *A*. Metabolite *A* spontaneously degrades to Compound *B*. Both Metabolite *A* and Compound *B* retain the nitro group and the azomethine linkage.

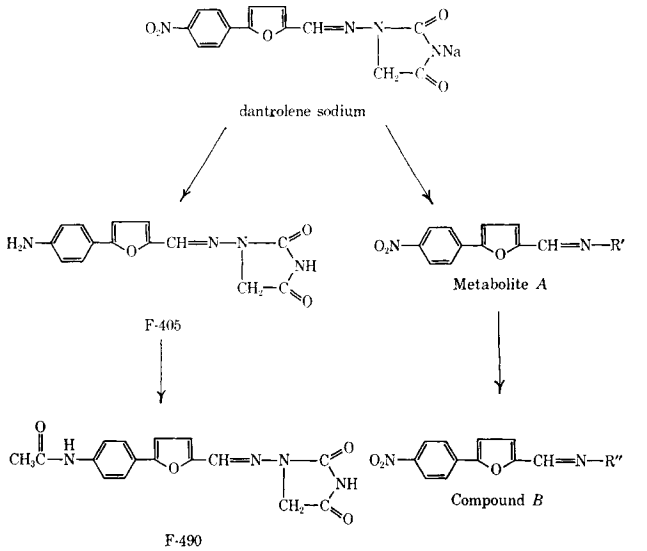

Figure 1—*Metabolic pathway of dantrolene sodium.*

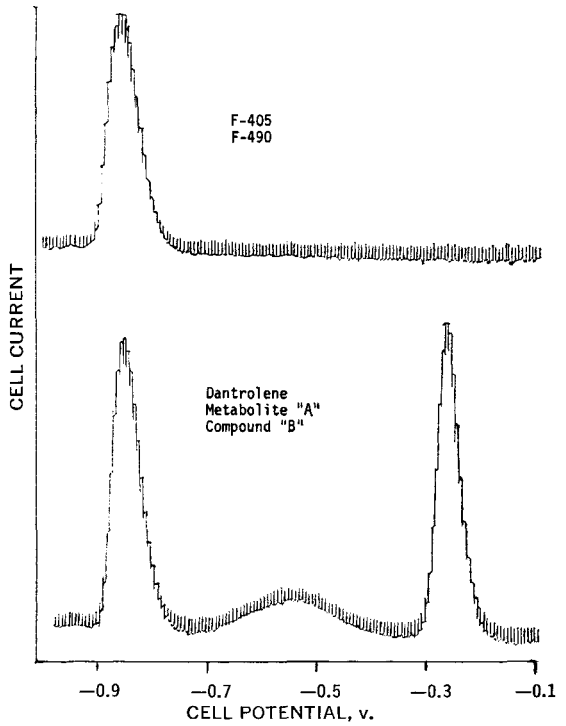

Figure 2—*Differential pulse polarogram.*

The nitro group of dantrolene, Metabolite *A*, and Compound *B* can be reduced polarographically at a cell potential of −0.26 v. The azomethine linkage, which is common to dantrolene, its metabolites, and the degradation product, can be reduced polarographically at a cell potential of −0.86 v. Figure 2 shows polarograms characteristic of each compound as determined by differential pulse polarography in pH 4 acetate buffer. The peak height is proportional to concentration.

The nonreduced metabolites are measured as dantrolene equivalents based on the assumptions of equivalent molecular polarographic response, and relatively minor difference in molecular weight. The metabolite has not yet been isolated and characterized, due to its instability and low urinary excretion.

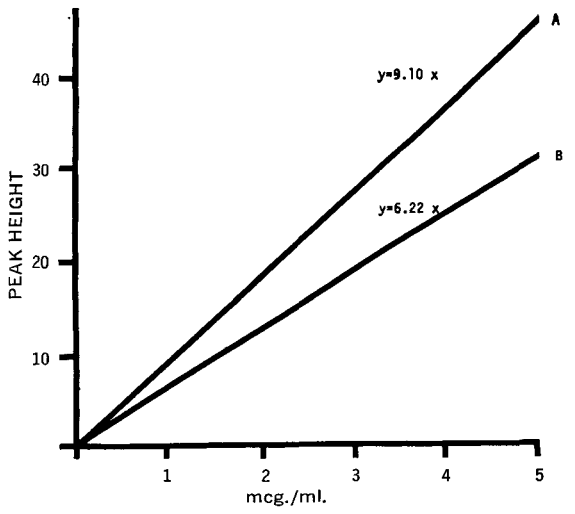

Figure 3—*Standard curves for:* A, *dantrolene (at −0.26 v.);* B, *F-490 (at −0.86 v.); in urine.*

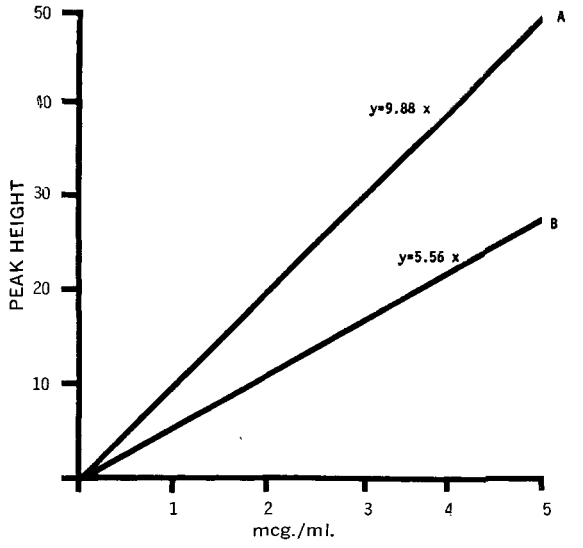

Figure 4—*Standard curves for:* A, *dantrolene (at −0.26 v.);* B, *F-490 (at −0.86 v.); in plasma.*

F-405 (the amine) has not been detected in human urine or plasma but the acetylated metabolite (F-490) is present. Also present in human samples are dantrolene, Metabolite *A*, and traces of Compound *B*, but the major materials are Metabolite *A* and F-490 (the acetamido compound). Advantage was taken of the well-separated reduction potentials of the nitro group and the azomethine linkage for the development of a differential pulse polarographic procedure to determine simultaneously the nitro compounds and F-490.

EXPERIMENTAL

Instrument and Reagents—A pulse polarographic analyzer (Melabs CPA-3) was used in the differential pulse mode, in which pulses of uniform amplitude are superimposed on the voltage sweep and the combination applied to the cell during a series of mercury drops. This instrument uses three-electrode, controlled-potential circuitry with potentials referred to a saturated-calomel reference electrode. Measurements were performed at room temperature. An automatically controlled mercury drop rate of 2 sec. per drop was used. Solutions were deoxygenated by bubbling prepurified grade nitrogen through the solution for 5 min. and then maintaining an atmosphere of nitrogen over the surface of the solution.

Reagents included: spectroquality ethyl acetate; reagent grade *N,N*-dimethylformamide, anhydrous sodium acetate, glacial acetic acid, and sodium tungstate.

Procedure—Dantrolene, its reduced and nonreduced metabolites, and its degradation product were extracted from aqueous solution with ethyl acetate. To determine simultaneously the nonreduced compounds and F-490 in urine, 5-ml. samples were adjusted to pH 7 using either 1 *N* HCl or NaOH. Approximately 2 g. of granular sodium chloride was added to prevent formation of emulsions and the samples were extracted with 5 ml. of ethyl acetate Four and one-half milliliters of the ethyl acetate phase were removed and evaporated to dryness.

Five milliliters of plasma was mixed with 20 ml. of $^1/_{12}$ *N* H_2SO_4 followed by the addition of 3 ml. of 10% sodium tungstate to precipitate the proteins, which were removed by centrifugation. The samples were extracted with 20 ml. of ethyl acetate and 15 ml. of the ethyl acetate phase was removed and evaporated to dryness.

After the evaporation of the ethyl acetate, the residue from urine or plasma samples was dissolved in 0.2 ml. of *N,N*-dimethylformamide and mixed with 5 ml. of 0.2 *M* pH 4 acetate buffer. The samples were deoxygenated by bubbling nitrogen through the buffered solutions for approximately 5 min. The voltage scan was then recorded from 0 to −1.0 v. using the differential pulse mode of the polarographic analyzer.

Table I—Simultaneous Recovery of Dantrolene and F-490 from Human Urine

Added, mcg./ml.		Recovered, mcg./ml.			
Dantrolene	F-490	Dantrolene	*SD*	F-490	*SD*
0.10	0.10	0.09	±0.006	0.11	±0.009
0.10	1.00	0.10	±0.002	1.03	±0.031
0.10	5.00	0.10	±0.015	4.96	±0.086
0.10	10.00	0.11	±0.007	10.83	±0.079
1.00	1.00	1.02	±0.082	1.06	±0.030
1.00	5.00	1.04	±0.036	5.11	±0.059
1.00	10.00	1.03	±0.017	10.58	±0.118
5.00	1.00	5.17	±0.405	0.97	±0.093
5.00	5.00	5.11	±0.069	5.51	±0.225
5.00	10.00	5.03	±0.354	11.27	±0.116

The concentration of dantrolene and Metabolite *A* plus Compound *B* was determined and expressed as dantrolene by comparing the height of the nitro peak at −0.26 v. to the appropriate standard curve for dantrolene in plasma or urine. The azomethine linkage and the nitro group produced peaks of equal height at their respective potentials for all the nonreduced compounds. Since the nitro peak and azomethine peak were of equal height for the nonreduced compounds, the azomethine peak at −0.86 v. less the nitro peak at −0.26 v. represented F-490. The F-490 concentration was determined by comparing the corrected azomethine peak height to the appropriate F-490 standard curve in plasma or urine.

Standard curves for dantrolene and F-490 were determined by adding to control plasma and urine samples varying concentrations of dantrolene and F-490 from 0.1 to 5.0 mcg./ml. and treating the samples as described in the procedure.

Standard curves are shown in Fig. 3 for dantrolene and F-490 in urine determined by assaying urine samples with varying concentrations of dantrolene or F-490 from 0.1 to 5.0 mcg./ml. The lower slope for the F-490 standard curve is due to its higher molecular weight and a slightly lower partition coefficient into ethyl acetate.

Figure 4 shows standard curves for dantrolene and F-490 determined in plasma. The standard curves are linear from 0 to 5 mcg./ml. in both urine and plasma. At higher concentrations the polarographic response starts to deviate from linearity, making it necessary to dilute the final buffer solution to stay within the linear response of the polarographic assay. All dilutions must be made with a 1:25 mixture (by volume) of *N,N*-dimethylformamide and 0.2 *M* pH 4 acetate buffer.

RESULTS

The recovery of dantrolene and F-490 from urine samples containing varying ratios of the two compounds is presented in Table I. These data are based on three determinations for each combin tion. The data show that low levels of dantrolene can be determined in the presence of high concentrations of the reduced metabolite. This is due to the detection of the nitro group of dantrolene or the nonreduced compounds by the reduction potential at −0.26 v. It is not possible to accurately determine the reduced metabolite at very low concentrations in the presence of high concentrations of the nonreduced compounds since this involves showing a very small change in a large peak for the reduction of the azomethine linkage at −0.86 v.

Table II—Simultaneous Recovery of Dantrolene and F-490 from Dog Plasma

Added, mcg./ml.		Recovered, mcg./ml.	
Dantrolene	F-490	Dantrolene	F-490
0.10	0.10	0.11	0.10
0.10	1.00	0.11	1.16
0.10	5.00	0.11	5.53
1.00	1.00	0.96	1.02
1.00	5.00	1.06	5.78
5.00	1.00	5.08	1.18
5.00	5.00	4.93	5.68

The simultaneous recovery of dantrolene and F-490 at various levels from plasma samples is shown in Table II. Each value is based on a single determination. These recoveries compare favorably with the recoveries from urine samples (Table I).

SUMMARY

A differential pulse polarographic method has been developed for the simultaneous determination of dantrolene and its nonreduced and reduced metabolites. The compound and its metabolites were extracted from urine or plasma with ethyl acetate and the ethyl acetate removed by evaporation. The compounds were dissolved in a small quantity of *N,N*-dimethylformamide and diluted with 0.2 *M* pH 4 acetate buffer. Dantrolene plus the reduced and nonreduced metabolites were quantitatively determined by the polarographic reduction of the azomethine linkage at a cell potential of −0.86 v. The nitro compounds were quantitatively determined by the reduction of the nitro group at a cell potential of −0.26 v. The difference between the two determinations represents the reduced metabolite. Levels as low as 0.1 mcg./ml. can be determined by the reduction of the nitro group or the azomethine linkage.

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