deviation was observed suggesting that higher aggregates exist in System B. The data for the system, therefore, were treated by a nonlinear least-squares fit to Eq. 2. The best fit was obtained for the monomer–tetramer–hexamer model in the organic phase. The estimated association constants were $K'_4 = 9.96 \times 10^2\ M^{-3}$, $K'_6 = 4.79 \times 10^{28}\ M^{-5}$. The distribution isotherm based on these constants is in good agreement with the experimental data (Fig. 2). Figure 4 shows the distribution of deoxycholic acid among its various forms in Solvent System B.

In studies with the methylester of deoxycholic acid (2), it was found that the methylester exists as monomers and dimers in chloroform ($K_2 \approx 14.0\ M^{-1}$) and monomers and tetramers in carbon tetrachloride ($K_4 \approx 2.8 \times 10^5\ M^{-3}$). Although no report was found in the literature for comparison with the present observations, a qualitative comparison can be made with the data derived previously (2). In pure octanol, hydrogen bonding interactions between solvent molecules and the hydroxyl and carboxyl groups of the bile acid are considerable. Consequently, this interaction precludes any appreciable self association of steroidal monomers. Solvent System A is a solvent of low polarity. Octanol is, therefore, expected to self associate to a significant extent in this solvent system (7). Thus, there is likely to be only weak interaction between 1-octanol and bile acid molecules. This leads to self association of solute molecules either between hydroxyl groups, carboxylic acid groups, or both. It was found in the preceding study (1) that under the condition of the experiment it is the free acid form that is partitioned in the organic phase. An acid form, because of its relatively more polar nature compared to the ester form, is expected to associate more strongly.

Even higher aggregation is expected, therefore, in Solvent System B, since this solvent is even less polar than Solvent System I. The organic phase has been rendered less polar, by increasing the isooctane concentration and by substituting 1-octanol (dielectric constant 10.34) with chloroform (dielectric constant 4.81).

These results have important significance in terms of mixed micelles of bile salts with lecithin (8). In a low dielectric inert medium such as the interior of a lecithin bilayer or liposome, pairwise association of bile salt molecules hydrogen-bonded to each other through their hydroxyl and/or carboxylic acid groups is plausible. Such a mixed disk model for bile salt lecithin micelles in which hydrogen bonded bile salt anion pairs are found within the interior of the micelle has been proposed previously (9). It cannot be decided on the basis of the present or previous (9) work whether the associated species are bile salt anions or free acid molecules. Molecular models suggest that the hydroxyl groups on the trihydroxy bile acids and dihydroxy bile acids can align to form hydrogen bonded pairs. It was suggested (10) that this hydrogen-bonded pairing occurs in aqueous solvents. However, it has been shown (1, 11) that in aqueous solution, bile salts are associated by hydrophobic forces. The previous hydrogen-bonded pairing model (10) appears to be the most likely structure in a low dielectric, nonhydrogen-bonded medium. The partition of bile salts from an aqueous to a lipid membrane phase would thus involve an inversion from hydrophobic back-to-back association in the aqueous phase, to hydrogen-bonded association in the lipid phase.

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Serum Prolactin Level Increase in Normal Subjects Following Administration of Perphenazine Oral Dosage Forms: Possible Application to Bioavailability Testing

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Abstract □ Two pilot studies were performed to determine if oral phenothiazine products could generate a significant increase in serum levels of the hormone prolactin. The two studies employed three and four healthy normal male subjects, respectively. In the first study the subjects received a screening dose, a placebo, one 8-mg perphenazine tablet, and two 8-mg perphenazine tablets. In the second study, the subjects were dosed with two 10-mg amitriptyline tablets, one 10-mg amitriptyline tablet with one combination tablet containing 10 mg of amitriptyline and 4 mg of perphenazine, and two combination tablets, each containing 10 mg of amitriptyline and 4 mg of perphenazine. In both cases the drug treatments produced a significant rise in the serum prolactin levels *versus* a placebo or control. This increase was defined as a prolactin response. The possible utility of this response in bioavailability testing is discussed.

Keyphrases □ Prolactin—serum prolactin level increase following administration of perphenazine oral dosage forms, application to bioavailability testing □ Bioavailability—application to testing, serum prolactin level increase following administration of perphenazine □ Perphenazine—oral dosage forms, serum prolactin level increase following administration, application to bioavailability testing

An adequate methodology for determining the bioavailability and bioequivalence of phenothiazine dosage forms has been sought for some time. The phenothiazines are a vital psychopharmaceutic tool in combating mental and emotional illnesses. In addition, these drugs are produced and marketed in a very large number of dosage

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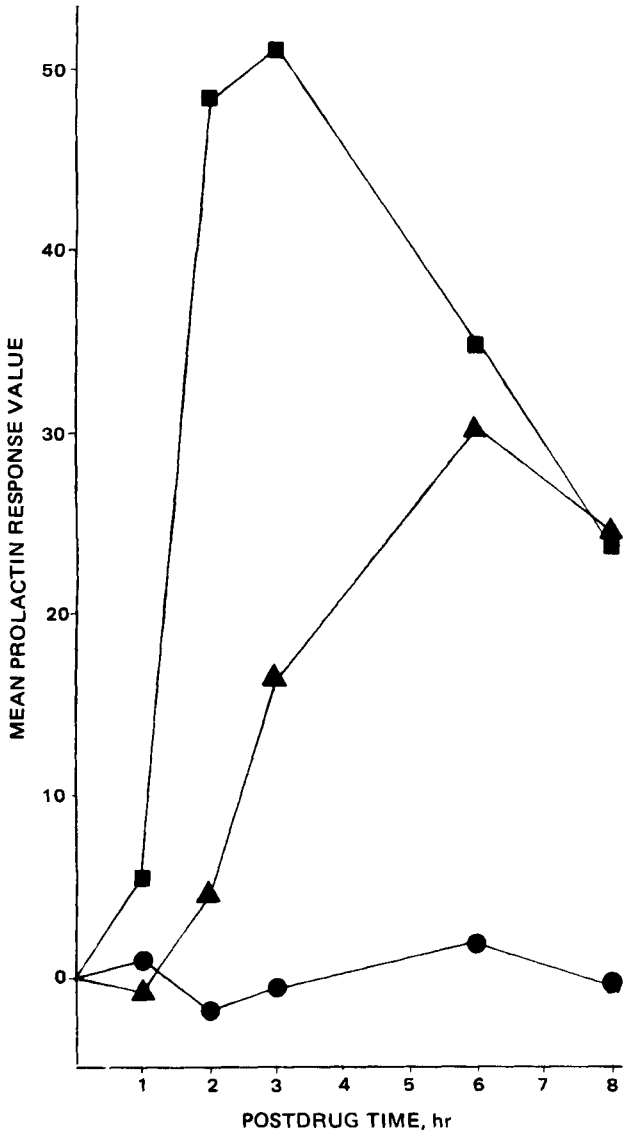

Figure 1—*The mean prolactin response* versus *time over an 8-hr postdrug period for three healthy, normal male subjects dosed with a placebo capsule (●); an 8-mg perphenazine tablet (▲); two 8-mg perphenazine tablets (■).*

forms. Also, several of the most frequently prescribed phenothiazines recently have lost patent protection or shall lose this protection in the near future. Hence, an adequate testing methodology for the phenothiazines is of critical importance. The present report presents data that should lead to the establishment of a meaningful, reliable, reproducible methodology of phenothiazine bioavailability and bioequivalence evaluations.

Traditionally, methods of assessing the major tranquilizers have centered around detection of the parent drug and metabolites in plasma by chemical assay. This approach has been based on the assumption that detectable plasma drug levels are adequately reflective of drug concentrations at critical sites in the central nervous system. There has been work published that suggests that this assumption may be incorrect (1, 2).

The mechanism of action of the phenothiazines has received considerable attention. There is much evidence that the phenothiazines act through a blockade of dopaminergic and noradrenergic receptors (3–9). Blockade of dopaminergic receptors with phenothiazines has been positively

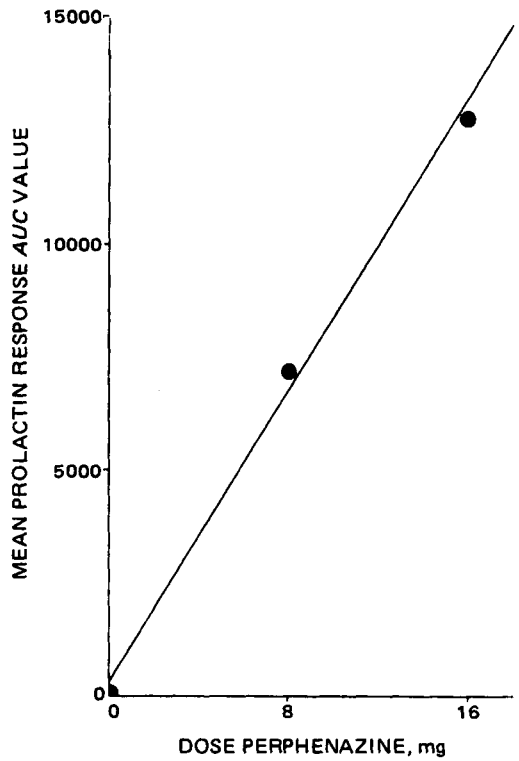

Figure 2—*The mean 60–480 min* AUC's *for the prolactin response for three healthy normal male subjects dosed with a placebo, one 8-mg perphenazine tablet, and two 8-mg perphenazine tablets* ($y = a_1x + a_0$; $y = 792.94x + 374.17$; $r^2 = 0.996$).

correlated with the antipsychotic effect of the phenothiazines (10–14).

Dopaminergic neurons also tonically inhibit the release of prolactin from the pituitary gland (15, 16). Intramuscular and intravenous administration of various phenothiazine products has generated a reproducible, dose-dependent response: an increase in serum prolactin level (17, 18). This increase has been attributed to the blockade of dopaminergic neurons (19). The change in plasma prolactin concentration to these neuroleptic agents correlates with their clinically observed antischizophrenic potency (13).

The present study was initiated to determine whether oral phenothiazine dosage forms also generate a prolactin response, and to preliminarily assess this response as a bioavailability/bioequivalence test procedure for phenothiazine dosage forms.

The results indicated that the oral phenothiazine doses dependably generated a prolactin response that was significantly greater than a placebo generated response. The method has much potential for bioavailability testing.

EXPERIMENTAL

Two studies were conducted to investigate changes in serum prolactin levels after administration of several oral phenothiazine dosage forms.

The first study investigated the changes in serum prolactin after the administration of tablet perphenazine to healthy normal subjects.

Subjects—Males (18–35 years of age) were employed as experimental subjects. All were classified as healthy and normal on the basis of the following: interview, physical examination, electrocardiograph, exercise stress test, chest X-ray, intraocular pressure measurement, microscopic urinalysis, hematology, and blood chemistry. Hematology included the following: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and white blood cell count with differential. Blood chemistry included the following: triglycerides, serum

Table I—Prolactin Blood Level (ng/ml), Prolactin Response Values [a], and *AUC* Values [b]

Postdrug Time	Subject 1 Blood Level	Subject 1 Prolactin Response	Subject 2 Blood Level	Subject 2 Prolactin Response	Subject 3 Blood Level	Subject 3 Prolactin Response
			Placebo			
0	*ND*[c]		21.8		9.9	
60	11.5	2.6	11.0	0.3	10.7	0.6
120	7.4	−1.5	8.3	−2.4	10.5	−1.7
180	5.8	−3.1	11.6	0.9	12.8	−0.4
300	9.5	0.6	14.6	3.9	11.9	1.5
480	10.4	1.5	8.1	−2.6	13.2	0.1
60–480 *AUC*		12		306		135
			8-mg Perphenazine Tablet			
0	16.5		20.1		7.0	
60	9.4	0.5	10.3	−0.4	5.0	−2.2
120	14.8	5.9	15.4	4.7	13.3	4.0
180	32.5	23.6	23.3	12.6	14.4	12.9
300	42.1	33.2	40.5	29.8	31.0	27.4
480	43.6	34.7	29.5	18.8	18.5	20.1
60–480 *AUC*		10611		7554		7188
			Two 8-mg Perphenazine Tablets			
0	23.0		15.8		7.3	
60	15.1	6.2	16.6	5.9	9.9	3.9
120	72.3	63.4	53.0	42.3	19.8	37.9
180	74.5	65.6	57.8	47.1	32.1	44.3
300	47.5	38.6	46.5	35.8	28.4	30.3
480	44.0	35.1	27.4	16.7	19.6	19.9
60–480 *AUC*		19029		14004		12806

[a] Prolactin response = [prolactin in sample for subject] − [subject's mean placebo prolactin over 60–480-min postdrug]. [b] *AUC*'s (60–480 min) for three healthy male subject's dose with a placebo, one 8-mg perphenazine tablet, and two 8-mg perphenazine tablets. [c] No data.

Table II—ANOVA for Postdrug *AUC* Values [a]

Analysis of Variance for Dependent Variable 1

Source	Error Term	*F*	Sum of Squares	Degrees of Freedom	Mean Square	Expected Mean Square	
(1) Mean	*S*	11.0292	4.0517664E + 08	1	4.0517664E + 08	9.000(1)	3.000(3)
(2) *D*	*DS*	10.0762	2.4186097E + 08	2	1.2073047E + 08	3.000(2)	1.000(4)
(3) *S*			7.3480106E + 07	2	3.6740053E + 07	3.000(3)	
(4) *DS*			4.8006148E + 07	4	1.2001537E + 07	1.000(4)	

Estimates of Variance Components
(1) 4.0731379E + 07
(2) 3.6309650E + 07
(3) 1.2246694E + 07
(4) 1.2001531E + 07
Mean 11729.25000

Cell Means

D =	(1) Placebo	(2) One 8-mg perphenazine	(3) Two 8-mg perphenazine
	135.00000	7188.00000	12806.00000

S =	(1)	(2)	(3)	(4)
	7804.00000	7288.00000	2957.00000	

Cell Deviations

X(0.) − X(. .)			
D =	(1)	(2)	(3)
	−6574.66667	478.33333	6096.33333

X(S.) − X(. .)			
S =	(1)	(2)	(3)
	3174.33333	578.33333	−3752.66667

[a] 60–480 min for three healthy normal male subjects dosed with a placebo, 8 mg, and 16 mg of perphenazine.

glutamic oxalacetic transaminase, serum glutamic-pyruvic transaminase, phosphorus, alkaline phosphatase, uric acid, albumin, blood urea nitrogen, chloride, potassium, lactic dehydrogenase, calcium, bilirubin, total protein, glucose, sodium, albumin–globulin ratio, and creatinine. Each test value had to fall within the acceptable limits for the test for the subject to be accepted in the study. Each subject also signed an informed consent form prior to first participation in the study. Subjects abstained from any drug or drug product for the entire study. Each subject agreed to abstain from alcohol for 48 hr prior to each experiment. Subjects were not permitted tobacco for 12 hr prior to each experiment. Also, subjects were not to take anything orally, except water, for 10 hr prior to each experiment.

The subjects reported to the facility once a week for 4 consecutive weeks. They arrived at the facility at ~8 am. Each subject was briefly examined. The oral temperature, pulse, respiration, and blood pressure of each subject was determined and recorded. The predrug blood sample then was drawn.

Doses and Experimental Design—All three subjects received the doses in the same order. The doses were as follows: (*a*) one milligram of perphenazine as a liquid concentrate[1], (*b*) one 8-mg perphenazine tablet[2], (*c*) one placebo capsule, (*d*) two 8-mg perphenazine tablets. The first dose was used as a screening dose to prevent any hypersensitive individuals from receiving a larger dose. The perphenazine concentrate was administered mixed in ~175 ml of orange juice. No blood samples were taken from the subjects before or after the administration of this screening dose. The subjects were observed for any reaction that might constitute evidence of an allergic or hypersensitive response to the perphenazine. All of the subjects received all of the doses.

Blood Samples—Blood samples were obtained periodically from the volunteers during each of the other three experiments. Prior to each dose administration, a predrug blood sample was obtained. Five postdrug samples were obtained during each experiment at 1, 2, 3, 6, and 8 hr postdose.

Each blood sample was obtained from a vein in the antecubital space

[1] Trilafon concentrate, 16 mg/5 ml, Schering Corp., Kenilworth, NJ 07033.
[2] Trilafon tablets, 8 mg, Schering Corp., Kenilworth, NJ 07033.

Table III—Prolactin Blood Level (ng/ml), Prolactin Response [a], and *AUC* Values [b]

Postdrug Time	Subject 1 Blood Level	Subject 1 Response Value	Subject 2 Blood Level	Subject 2 Response Value	Subject 3 Blood Level	Subject 3 Response Value	Subject 4 Blood Level	Subject 4 Response Value
			Two 10-mg Amitriptyline Tablets					
0	41.7		29.7		15.2		23.5	
0[c]	19.9		14.3		13.2		16.3	
60	14.5	−5.40	11.9	−2.40	10.5	−2.70	14.6	−1.70
120	17.8	−2.10	10.7	−3.63	9.9	−3.30	32.9	16.60
180	10.6	−9.30	10.9	4.80	8.5	−4.70	30.2	13.90
300	18.6	−1.30	24.7	10.40	12.4	−.30	16.9	.60
480	37.0	17.10	33.6	19.30	29.3	16.10	22.8	6.50
0–480 AUC		57.0		3351.0		546.0		2820.0
			One 10-mg amitriptyline tablet and one combination tablet (10 mg amitriptyline, 4 mg perphenazine)					
0	35.4		12.9		16.1		23.5	
0[c]	23.9		9.4		14.3		15.0	
60	17.7	−6.20	8.0	−1.40	11.1	−3.20	13.1	−1.90
120	41.3	17.40	7.0	−2.40	20.2	5.90	49.4	34.40
180	58.3	34.40	25.8	16.40	80.6	66.30	65.3	50.30
300	88.6	64.70	22.3	12.90	79.9	65.60	43.5	28.50
480	50.0	26.10	47.8	38.40	86.5	72.20	39.2	24.20
0–480 AUC		15822.0		6639.0		22467.0		12930.0
			Two combination tablets (10 mg amitriptyline, 4 mg perphenazine each)					
0	37.6		10.4		19.4		22.7	
0[c]	30.6		8.5		14.1		15.1	
60	19.4	−11.20	8.5	0	9.4	−4.70	16.1	1.00
120	72.6	42.00	15.9	7.40	15.6	1.50	80.6	65.50
180	106.2	75.60	26.6	18.10	83.6	69.50	94.8	79.70
300	73.7	43.10	33.1	24.00	101.4	87.30	75.5	60.40
480	75.3	44.70	36.1	27.60	85.1	71.00	48.8	32.90
0–480 AUC		19140.0		8247.0		25549.0		23184.0

[a] Prolactin response = [prolactin in sample] − [predrug prolactin sample]. [b] *AUC* values (60–480 min) for four healthy male subjects given two 10-mg amitriptyline tablets, one 10-mg amitriptyline tablet, one 10-mg amitriptyline and 4-mg perphenazine combination tablet, and two combination tablets. [c] Sample used for prolactin response calculation.

using a 21-gauge thin-wall needle[3] inserted into a 10-ml plain, silicone-coated vacuum blood collection tube[4], 16 × 100 mm. Each sample tube was placed upright in a rack for ~15 min then centrifuged[5] for ~15 min at 3250 rpm. The serum was filtered[6] and transferred to a storage tube[7]. The storage tubes were sealed, placed in a rack, and stored in an upright position in a freezer at −15°.

Assay—The samples were assayed for their prolactin level using a prolactin radioimmunoassay[8] that had a sensitivity of ±2–3 ng/ml of prolactin/sample. The samples were prepared for assay[9] and the prolactin concentration was determined in a gamma well scintillation counter[10].

Calculations and Data Analysis—Several calculations were made once the serum prolactin was determined for each blood sample. First, the mean placebo prolactin concentration over time (60–480 min) for each of the three subjects was determined. Normally, data 0–480 min would have been analyzed. After the blood samples were analyzed it was discovered that the predrug prolactin levels were somewhat elevated. This was attributed to predrug anxiety; therefore, for this study, the predrug (zero) reading was ignored. Data analysis started with the 60-min sample. The second study employed two predrug samples. The prolactin level decreased in every case for the second sample verifying the earlier observation. The appropriate subject placebo mean value was subtracted from each serum prolactin concentration. The resulting value was defined as the prolactin response for each sample. The area under the prolactin response curve (*AUC*) was calculated for the 60–480-min postdrug period. These *AUC* data were used in a statistical analysis of the experiments.

Statistical Analysis—An ANOVA was performed on the prolactin response *AUC*'s. Pairwise comparisons were made between the treatments employing a least significant difference analysis. Using a method described previously (20), an adequate sample size estimate was calculated for future bioequivalence studies. This estimate was set to determine a sample size large enough to detect a difference of 20% between *AUC*'s, with $\alpha = 0.05$ and $\beta = 0.20$. Also calculated were the best-fitting regression line values for the mean *AUC*'s, and the r^2 value, which expressed the amount of variation in one variable, which could be attributed to the second variable.

A second study was conducted, with several differences in procedures. First, four subjects were employed instead of three. The criteria and methods used to select subjects were not changed.

Second, different dose treatments were selected for study. Again, each subject received the doses in the same order: (*a*) two 10-mg amitriptyline tablets[10], (*b*) one 10-mg amitriptyline tablet and one combination tablet containing 10 mg of amitriptyline and 4 mg of perphenazine[11], (*c*) two tablets each containing 10 mg of amitriptyline and 4 mg of perphenazine.

This dosing provided for a constant level (20 mg) of amitriptyline with increasing doses (0, 4, and 8 mg) of perphenazine.

Third, the prolactin response was defined differently. The prolactin response was defined as the serum prolactin concentration for any sampling time minus the serum prolactin concentration from the second predrug sample.

The data were treated in a manner similar to the data obtained from the first study, but with 0–480 min *AUC*'s being calculated.

RESULTS AND DISCUSSION

The serum prolactin levels, prolactin response values, and the 60–480 min *AUC*'s for the first experiment are presented in Table I. Examination of this table reveals that for each subject the *AUC* value for each experiment increased with the size of the perphenazine dose. The *F* value for the treatment variable was significant in the ANOVA. The least significant difference results indicated that a significantly larger prolactin response ($p < 0.05$) was generated by both the perphenazine dosage forms *versus* the prolactin response produced by the placebo (Table II). There was not a significant difference between the prolactin responses produced by the two perphenazine dosage forms. Given the small sample size, this was not surprising. The mean prolactin response values for the three doses are illustrated in Fig. 1. Despite the lack of a statistically significant

[3] Venoject multisample needles, 21 gauge × 3.81 cm (thin wall), Kimble-Terumo, Inc., Elkton, MD 21921.

[4] Venoject K-T-200 plain, silicon-coated vacuum blood collection tubes, 16 × 100 mm, 10 ml, Elkton, MD 21921.

[5] Spinnette C1370-1 centrifuge, Damon/IEC Division, Needham Heights, MA 02194.

[6] Filter Sampler, Blood Serum Filter, Standard Model, 16 mm × 10.16 cm, Glasrock Products, Inc., Fairburn, GA 30213.

[7] Frozen Serum Transport Containers, Biomedical Laboratories, Inc., Burlington, NC 27215.

[8] Prolactin RIA Diagnostic Kit, Abbott Laboratories, Diagnostics Division, North Chicago, IL 60064.

[9] Tracor 1285 Automatic Gamma System, Tracor Instruments, Austin, TX 78721.

[10] Elavil tablets, 10 mg, Merck Sharp & Dohme, West Point, PA 19486.

[11] Triavil tablets 4–10 mg, Merck Sharp & Dohme, West Point, PA 19486.

Table IV—ANOVA for Postdrug *AUC* Values [a]

Analysis of Variance for Dependent Variable 1

Source	Error Term	*F*	Sum of Squares	Degrees of Freedom	Mean Square	Expected Mean Square	
(1) Mean	*S*	30.9393	1.6509037E + 09	1	1.6509037E + 09	12.000(1)	3.000(3)
(2) *D*	*DS*	12.6304	6.4598068E + 08	2	3.2299034E + 08	4.000(2)	1.000(4)
(3) *S*			1.6007820E + 08	3	5.3359401E + 07	3.000(3)	
(4) *DS*			1.5343418E + 08	6	2.5572363E + 07	1.000(4)	

Estimates of Variance Components

(1) 1.3312869E + 08
(2) 7.4354495E + 07
(3) 1.7786467E + 07
(4) 2.5572363E + 07
\Mean 11729.25000

Cell Means

D =	(1)	(2)	(3)	(4)
	1693.50000	14464.50000	19029.75000	
S =	(1)	(2)	(3)	(4)
	11673.0000	6079.0000	16187.0000	12970.0000

Cell Deviations

X(0.)	− X(. .)			
D =	(1)	(2)	(3)	
	−10035.7500	2735.2500	7300.5000	
X(S.)	− X(. .)			
S =	(1)	(2)	(3)	(4)
	−56.2500	−5650.2500	4457.7500	1240.7500

[a] Analysis of variance for the 0–480 min postdrug *AUC* values for four healthy normal male subjects dosed with (*a*) two 10-mg amitriptyline tablets, (*b*) one 10-mg amitriptyline tablet and one combination tablet containing 10 mg of amitriptyline and 4 mg of perphenazine, and (*c*) two combination tablets, a dose of 20 mg of amitriptyline and 8 mg of perphenazine.

difference between the prolactin responses to the 8- and 16-mg perphenazine doses, the figure demonstrates that an increase in the prolactin response accompanied each increase in the perphenazine dosage level. The best fitting linear regression equation and the mean 60–480-min *AUC*'s are assembled in the dose–effect curve (Fig. 2). More than 99% of the prolactin response (variation) can be explained by the changes in the dose of perphenazine (*i.e.*, $r^2 \geq 0.99$). The subject sample size estimate (20) calculated using these data was 24.

Tables III and IV contain the data and the statistical analysis for the second pilot study using amitriptyline tablets and amitriptyline–perphenazine combination tablets. Again, each increase in the dose level of perphenazine was accompanied by an increase in the size of the 0–480-min *AUC*. Also, the *AUC*'s for both perphenazine treatments were significantly larger than the *AUC*'s calculated for the experiments where amitriptyline alone was given. Figure 3 portrays the mean prolactin response values over time for the three dose treatments. Figure 4 illustrates the mean 0–480 dose–effect curve minute *AUC* values for the three doses. Also, the best fitting linear regression equation is listed. A 93% variation in the *AUC* values can be accounted for by the changes in the perphenazine dosage levels. The subject sample size estimate calculated using these data was 30.

The results of these two studies support the findings of previous investigations demonstrating increased serum prolactin following the administration of various phenothiazine dosage forms. The present studies, however, employed oral phenothiazine dosage forms, in contrast with the parenteral dosage forms used earlier. This is an important addition, since the solid oral dosage forms are generally the least bioavailable but most heavily used in treatment. The present studies demonstrated that these oral dosage forms generated a statistically significant prolactin response.

It can be assumed that the mechanism by which the perphenazine generated the increase in serum prolactin is the same as proposed earlier, *i.e.*, a blockade of the dopaminergic pathways tonically inhibiting prolactin release. In this regard, less concern need be given to the active *versus* inactive metabolites of the drug. If a product of the metabolic breakdown of perphenazine also blockades the prolactin inhibitory pathway, it should also exert an antipsychotic action and contribute to the overall clinical effect.

Meaningful comparisons between the two studies are difficult to make. On the average, the 60–480 postdose *AUC*'s for the amitriptyline–perphenazine study were larger. Amitriptyline, however, should not have a significant effect on dopaminergic pathways (21). A *t* test was performed between the placebo *AUC*'s and the *AUC*'s calculated from the amitriptyline tablet dose treatment experiments. No significant difference was found between these data sets. Hence, the presence of the amitriptyline appears not to add to or detract from the prolactin response produced by the perphenazine, despite possible effect on noradrenergic sites.

The data obtained in these studies have implications for screening and bioavailability testing of phenothiazine products. Certainly any potential

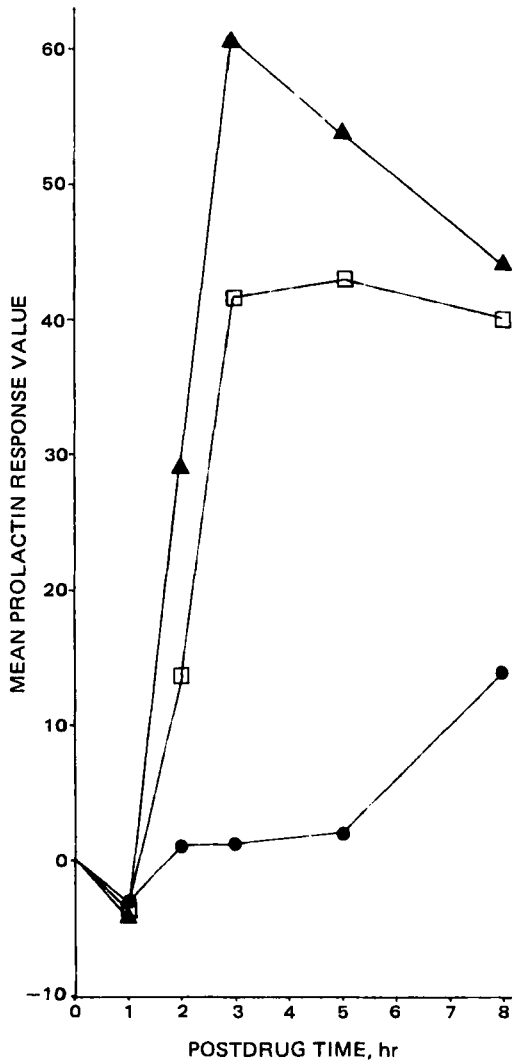

Figure 3—*The mean prolactin response* versus *time over an 8-hr postdrug period for four healthy, normal male subjects dosed with two 10-mg amitriptyline tablets (●); one 10-mg amitriptyline tablet and one 10-mg amitriptyline–4-mg perphenazine combination tablet (□); two combination 10-mg amitriptyline–4-mg perphenazine tablets (▲).*

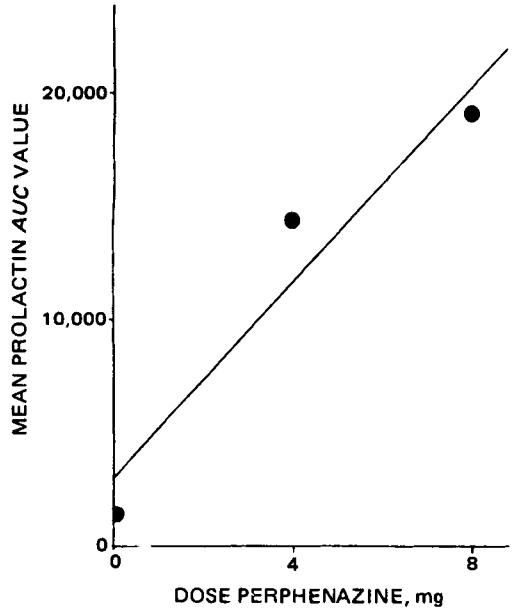

Figure 4—*The mean 60–480 min* AUC's *for the prolactin response for four healthy, normal male subjects dosed with two 10-mg amitriptyline tablets (0 mg of perphenazine); one 10-mg amitriptyline tablet and one combination tablet (4-mg perphenazine and 20-mg amitriptyline); and two combination tablets (total of 8-mg perphenazine and 20-mg amitriptyline) (*$y = a_1x + a_0$; $y = 2167.03x + 3061.13$; $r^2 = 0.931$*).*

phenothiazine product could be screened using the prolactin response. The prolactin response methodology is simpler, more economical, and more sensible; it reflects the proposed clinical effect at a site contiguous with the central nervous system. Therefore, expanded studies of this type, employing a larger subject sample size, could provide the basis for a bioavailability and bioequivalence testing methodology for all phenothiazine dosage forms. These studies could compare the prolactin response to a given product *versus* a placebo and a standard, or one standard product *versus* another, presumably equivalent, product. Some further quantification of the response will be necessary prior to applications of this methodology. It is the prolactin response, not prolactin blood levels, *per se*, that is dose-dependent for the phenothiazines, since intra- and intersubject variability in baseline serum prolactin levels are considerable. The prolactin response, however, is both reproducible and consistent.

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