1,4-Bis[2-(3,4,5-trimethoxyphenyl)ethyl]piperazine (IV)—Compound IV was prepared in the same manner as described for III, but with piperazine[6]. After evaporation of the solvents, the residue crystallized from 1:1 diethyl ether–petroleum ether: 2.3 g, 32% yield, mp 120–122°; NMR (chloroform-*d*): δ 2.65 (s, 16H, CH_2), 3.87 (s, 18H, OCH_3), and 6.41 (s, 4H, aromatic). IR (KBr) 3040, 1620, 1500, and 1160 cm^{-1}. The dihydrochloride was prepared as described for I: mp 259–260° dec.

Anal.—Calc. for $C_{26}H_{40}O_6N_2Cl_2$: C, 57.03; H, 7.36; N, 5.12. Found: C, 56.85; H, 7.23; N, 5.01.

Pharmacology—The experiments were performed on male Swiss white mice (18–26 g) and male Wistar rats (150–180 g). The investigated compounds were administered intraperitoneially in aqueous solutions.

The LD_{50} values were determined by a previous method (9).

The effect of I–IV on locomotor activity in normal and amphetamine-treated mice was recorded throughout 30-min sessions in photoresistant cages. The investigated compounds (administered intraperitoneally) and amphetamine (5 mg/kg sec) were administered 60 and 30 min, respectively, before testing.

The effect on the apomorphine-induced stereotypy was investigated in rats. Apomorphine (1.25 mg/kg sec) was injected 60 min after the tested compounds.

For the effect on sleeping time in mice, hexobarbital (70 mg/kg ip) was injected 60 min after the test compounds.

Anticonvulsant activity was investigated by the minimal and maximal pentylenetetrazol shock (pentylenetetrazol, 80 and 50 mg/kg sc, respectively). The number of animals protected against clonic convulsions in minimal shock or tonic extensions of limbs in maximal shock was registered.

The effect on behavioral despair in mice was examined by a previous method (10). The compounds were injected 60 min before examination.

The effect on the central action of 3-(3,4-dihydroxyphenyl)-L-alanine (levodopa) was tested on mice by modifications of a previous method (11). The alanine (100 mg/kg) was injected 4 hr after pargyline (40 mg/kg po) and 1 hr after the investigated compounds.

The effect on the aggressiveness of isolated (3 weeks) mice was examined by a previous method (12). Behavior was tested at 1-hr intervals for 4 hr.

Rectal body temperature in mice was measured with a thermometer for 3 hr after administration of the test compounds.

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Phenytoin I: *In Vitro–In Vivo* Correlation for 100-mg Phenytoin Sodium Capsules

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Abstract □ *Dissolution profiles for 11 brands of phenytoin sodium capsules were carried out by the basket and paddle methods (USP) and the spin-filter method. The results from the dissolution studies have been correlated with observed differences in in vivo parameters (C_{max} and t_{max}). The dissolution by the basket method at 50 rpm in water gave a correlation >0.9. The results suggest the existence of two types of phenytoin sodium products on the market.*

Keyphrases □ Phenytoin—*in vitro–in vivo* correlation for sodium phenytoin capsules, dissolution □ Dissolution—*in vitro–in vivo* correlation for sodium phenytoin capsules □ *In vitro–in vivo* correlation—sodium phenytoin capsules, dissolution

Increasing evidence has been presented in the scientific literature which show correlations between the *in vivo* performance of formulations and their *in vitro* dissolution behavior (1–3). To obtain an *in vitro–in vivo* correlation for any product, two criteria are essential: (*a*) the differences in *in vivo* parameters such as *AUC*, t_{max}, C_{max}, or C_p at a time among different lots tested and (*b*) differences in the *in vitro* dissolution rates of the same products. In cases where differences are observed in *in vivo* behavior, the *in vitro* parameter can be altered to optimize the correlation with *in vivo* data. This is achieved by varying such parameters as dissolution methodology, dissolution medium, rate of agitation, *etc.* In many instances, it has been possible to obtain correlations with *in vivo* data where a discriminating and reproducible *in vitro* test is employed (1–3). *In vitro–in vivo* correlations can generally be achieved with any reproducible method provided the proper selection of medium and the degree of agitation are made so as to permit discrimination among drug products. The key elements are reproducibility of the method, proper choice of medium, and degree of agitation.

Phenytoin is a commonly used anticonvulsant drug and has been classified as a drug with high risk potential with

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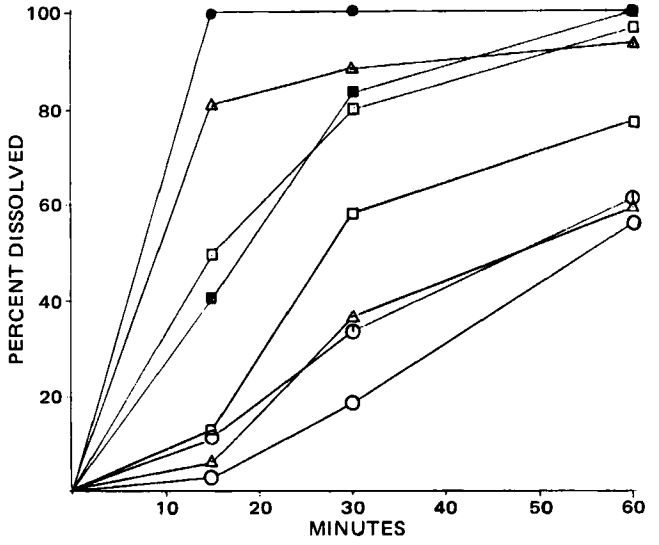

Figure 1—*Dissolution profile of phenytoin sodium products using basket method at 50 rpm in water. Key: (○) Product A; (□) Product B; (●) Product C; (⊡) Product E; (⊙) Product F; (■) Product H; (△) Project J; (▲) Product K.*

respect to bioavailability problems (4). Because of its physicochemical properties, narrow therapeutic range, and dose-dependent kinetics, phenytoin has been identified as a critical drug with a potential bioavailability–bioequivalence problem (5–9). Phenytoin sodium capsules are manufactured by several companies whose formulations have exhibited bioavailability–bioequivalence problems (4, 6, 9). It has been documented that phenytoin products of different manufacturers have pronounced influence on the rate and extent of absorption of the new drug resulting in bioinequivalence (6, 9).

A number of studies, using marketed phenytoin dosage forms were conducted to establish a correlation between the *in vivo* bioavailability and *in vitro* dissolution parameters.

EXPERIMENTAL

All dissolution studies were carried out by USP methods I and II as described in USP XX with an agitation speed of 50 rpm for both methods in distilled water (10) and by the spin-filter method with an agitation speed of 300 rpm in distilled water (11). All samples were analyzed using a spectrophotometer[1].

The studies involving human subjects were carried out after obtaining appropriate clearances and approval[2]. The study design, the analytical method, and the results are published elsewhere (12).

RESULTS AND DISCUSSIONS

The bioequivalence study was conducted in healthy volunteers using 11 lots of marketed 100-mg phenytoin sodium capsules manufactured by eight different companies[3] in two groups (six products each) using the innovator's product[4] as a reference product (12). All products were evaluated with respect to *AUC*, C_{max}, t_{max}, and plasma levels at different time intervals. The results of the previous study (12) show a significant

[1] Beckman spectrophotometer, model 25/7, Beckman Instrument Co., Fullerton, Calif.

[2] From the Risk Involving Human Subjects Committee of Food and Drug Administration, and equivalent committee of the University of Tennessee.

[3] Product A: McKesson capsules, lot no. 7E697. Product B: Westward capsules, lot no. 40981. Product C: Zenith Labs capsules, lot no. 2057-40. Product D: Zenith Labs capsules, lot no. 2057-37A. Product E: Zenith Labs capsules, lot no. 2057-35. Product F: Parke-Davis capsules, lot no. RL288. Product G: Danbury capsules, lot no. 9714. Product H: Danbury capsules, lot no. 9715. Product I: Xttrium Labs capsules, lot no. 403721A-78. Product J: Premo capsules, lot no. 8404. Product K: Kasar Labs capsules, lot no. 280564.

[4] Parke-Davis.

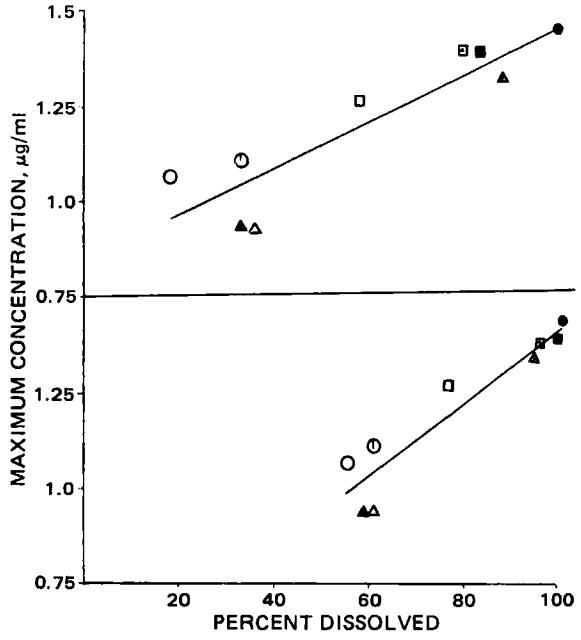

Figure 2—In vitro–in vivo *correlation between* C_{max} *and percent drug dissolved in 30 min (slope = 0.06,* r = 0.902, p < 0.001) *(A); 60 min (slope = 0.10,* r = 0.940, p < 0.001) *(B). Key: (○) Product A; (□) Product B; (●) Product C; (■) Product E; (⊙) Product F; (▲) Product G; (■) Product H; (△) Product J; (▲) Product K.*

difference in rate (C_{max} and t_{max}) and in the extent of bioavailability (*AUC*) in one group, and only a difference in C_{max} in the other group. The bioavailability of the products is defined both with respect to rate and extent. Because of a narrow therapeutic range and dose-dependent metabolism of phenytoin, even small changes in the rate of absorption may result in major changes in serum drug concentration, thus resulting in serious clinical consequences in some patients. Therefore, a clear distinction must be made between slow-absorbing and fast-absorbing phenytoin products.

The *in vitro* dissolution studies for these products were carried out under various conditions in order to obtain the correlation with *in vivo* data. The *in vivo* data obtained are invariant and are dependent on the

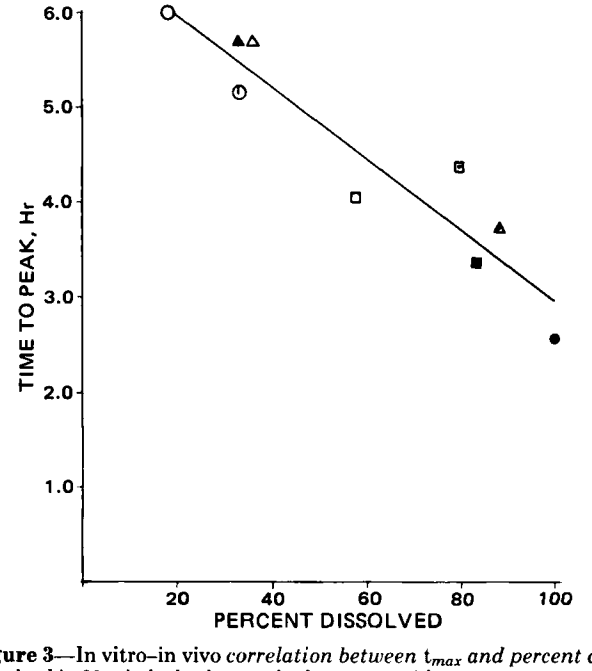

Figure 3—In vitro–in vivo *correlation between* t_{max} *and percent drug dissolved in 30 min by basket method at 50 rpm (slope =* 0.038, r = 0.944, p < 0.001). *Key: (○) Product A; (□) Product B; (●) Product C; (⊡) Product E; (⊙) Product F; (▲) Product G; (■) Product H; (△) Product J; (▲) Product K.*

Table I—Dissolution Profile of 100-mg Phenytoin Sodium Capsules in Water at 37° Using Paddle, Basket, and Spin-filter Methods

Minutes	Products A	B	C	D	E	F	G	H	I	J	K
	Percent of Drug[b] Dissolved										
	Paddle Method—50 rpm—4.5 cm-900 ml										
15	4.4	24.3	98.5	96.2	58.6	13.8	22.2	19.2	34.8	8.1	28.7
30	38.4	60.0	115.5	114.9	96.4	38.5	52.2	45.8	60.0	33.4	46.3
45	70.1	78.9	—	—	111.5	56.6	70.9	62.9	72.8	51.2	49.7
60	87.7	90.3	—	—	120.9	70.3	84.3	74.6	79.1	63.2	51.9
	Basket Method—50 rpm, 900 ml										
15	2.7	12.9	104.8	—	49.5	11.2	—	40.3	—	6.1	80.8
30	18.4	58.0	111.9	—	79.8	33.4	—	83.5	—	36.2	88.5
60	55.6	77.1	117.0	—	96.5	61.0	—	101.7	—	59.0	93.6
	Spin-filter Method—300 rpm, 1000 ml										
10	0.3	—	55.7	—	13.9	1.1	1.3	0.2	—	12.0[a]	50.9
30	29.4	—	105.0	—	103.1	28.8	95.1	87.9	—	50.0	99.5
60	72.5	—	107.5	—	105.5	62.7	101.8	98.1	—	78.9	99.6

[a] 20 min. [b] Label claim.

behavior of the dosage forms administered. The *in vitro* procedures can be altered such that correlations can be made between *in vitro* dissolution results and *in vivo* pharmacokinetic parameters. Such correlations can be statistically significant with respect to one or more of these *in vivo* parameters. Occasionally, there may be a product that exhibits an anomalous behavior with respect to an *in vitro* or *in vivo* parameter.

The *in vitro* dissolution studies were carried out by using the USP dissolution methods *I and II* (basket and paddle) with an agitation of 50 rpm and by the spin-filter method with an agitation of 300 rpm in water. A marked difference in dissolution profiles of these products was observed, both in terms of rate of extent of dissolution in 1 hr (Fig. 1, Table I). The amount of drug dissolved in 30 min (basket method, 50 rpm) varied between 18 and 100% and between 56 and 100% in 60 min. Based on the dissolution characteristics, the products could be classified in two major groups: products that dissolved slowly and achieved only 50–60% dissolution in 1 hr (*e.g.*, product F, Fig. 1) and products that dissolved rapidly and achieved >80% dissolution in 30 min (*e.g.*, product C, Fig. 1). This observed difference in the dissolution profiles of the phenytoin sodium products manifested itself in the *in vivo* performance of the product, resulting in a significantly different rate, but not the extent of bioavailability.

The correlation between C_{max} and percent drug dissolved in 30 and 60 min, and between t_{max} and percent drug dissolved in 30 and 60 min is shown in Figs. 2 and 3, respectively. Among the methods correlated, the basket method resulted in the best correlation ($r = 0.94$) followed by the spin-filter method ($r = 0.836$) and then the paddle method ($r = 0.694$).

These studies suggest that there are two types of phenytoin sodium products on the market. Because of the wide divergence in the dissolution rate of marketed phenytoin products and because of the very slow dissolution characteristics of the most commonly prescribed reference product, it is difficult to set a single dissolution standard covering all products. Therefore, a more definitive study comparing the slow-dissolving phenytoin sodium reference product and the fast-dissolving phenytoin sodium product with a phenytoin sodium solution as a reference standard was carried out. This study will be described in a subsequent report.

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