Evaluation of the Bioavailability of a Solid Dispersion of Phenytoin in Polyethylene Glycol 6000 and a Commercial Phenytoin Sodium Capsule in the Dog

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Abstract □ In this study a solid dispersion of phenytoin in polyethylene glycol 6000 was prepared by the melt method. The content uniformity of the dispersion indicated a range of 98.7–102.0%. X-ray crystallographic data showed no changes in the crystalline structure of the phenytoin in the dispersion when compared with that in the bulk reagent. Scanning electron micrographs of the dispersion show a reduction in the size of the crystals when compared with the bulk reagent. The commercial product and the dispersion were compared using the USP criteria for the prompt phenytoin sodium dissolution test. The salt dissolved at a rate four times greater than the dispersion. Bioequivalency comparisons between the dispersion containing phenytoin and the commercial prompt phenytoin sodium were studied in six mixed-breed dogs. All samples were analyzed using a commercial enzyme immunoassay system. Statistical analysis of the plasma levels obtained from the animal studies indicate no significant differences ($p > 0.05$) between area under the curve, maximum plasma concentration, and time to peak for the commercial salt and the acid formulation.

Keyphrases □ Phenytoin—bioavailability of the acid and sodium salt in dogs, comparison with *in vitro* dissolution data □ Dissolution—phenytoin and phenytoin sodium *in vitro*, comparison to the *in vivo* bioavailability in dogs

The use of finely subdivided or micronized particles as a means of increasing the rate of dissolution has been considered (1–4). Theoretically, if the dissolution rate is enhanced, the oral absorption rate should be increased if the absorption process is dissolution rate limited. Sekiguchi *et al.* proposed using an inert water-soluble material as a vehicle for a poorly water-soluble drug to enhance the rate and extent of the absorption of the drug (5, 6). Several carriers have been employed to prepare solid dispersions. The most successful include polyethylene glycols (7–11), urea (12), dextrose (12), citric acid (12), succinic acid (13), and polyvinylpyrrolidone (14, 15). Polyethylene glycols have been used to increase the dissolution rate of griseofulvin (7), coumarin (8), dicumarol (9), indomethacin (10), and glyburide (11).

Phenytoin is practically insoluble in water (≅14 μg/mL) at room temperature (16), whereas the solubility of phenytoin sodium is ~66 g/dL in water (17). This difference in solubility results in higher bioavailability for the salt (18–20). If the poor solubility of phenytoin in GI fluids is partially responsible for the great variability observed in phenytoin dosing, then enhancing the dissolution step may result in a more uniform absorption and improved bioavailability.

The purpose of this study is to evaluate the bioavailability in dogs of a phenytoin solid dispersion in polyethylene glycol 6000 using a commercial prompt phenytoin sodium capsule as the reference formulation. Data are presented which indicate that the phenytoin dispersion is bioequivalent to the commercial prompt phenytoin sodium, which is contrary to what one would expect based on the *in vitro* data obtained from the USP dissolution test for phenytoin sodium. The designation "prompt" refers to the fast dissolution category of phenytoin sodium set forth in the USP XX.

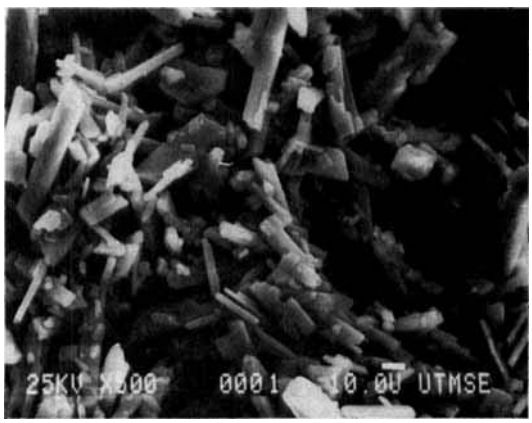

Figure 1—*Photomicrograph of the reagent-grade phenytoin (5,5-diphenylhydantoin). The 10-μm calibration bar is located in the lower right corner.*

EXPERIMENTAL

Preparation of Formulation—A 40% (w/w) solid dispersion of phenytoin[1] in polyethylene glycol 6000[2] was prepared using a melt method. The polymer was melted in a beaker on a thermostatically controlled hot plate at ~65–70°C. The reagent phenytoin was added with constant stirring until a clear liquid was observed. This liquid was poured into a glass mortar which was encased in ice. Solidification of the melt occurred on contact. The mass was dried in an oven at ~40°C for at least 1 h to remove excess moisture. The melt was triturated and then dried for at least 6 h. The powder was sieved through a 60-mesh sieve, and the batch was divided into 750-mg units.

Content Uniformity—Five of the 750-mg units of the phenytoin–polyethylene glycol 6000 dispersion were each dissolved in 1000 mL of 0.1 M NaOH, pH 12.5, in volumetric flasks. Duplicate samples were assayed using a commercial enzyme immunoassay[3]. The five determinations resulted in 98.7–102.0% of the theoretical amount.

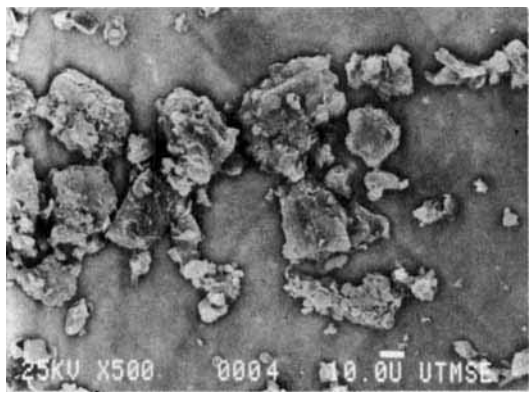

Figure 2—*Photomicrograph of the 40% phenytoin solid dispersion. The 10-μm calibration bar is located in the lower right corner.*

[1] 5,5-Diphenylhydantoin, 99+%, Gold Label; Aldrich Chemical Co., Milwaukee, Wis.
[2] Lot B-586; Alcon Laboratories, Fort Worth, Tex.
[3] EMIT Systems; Syva Co., Palo Alto, Calif.

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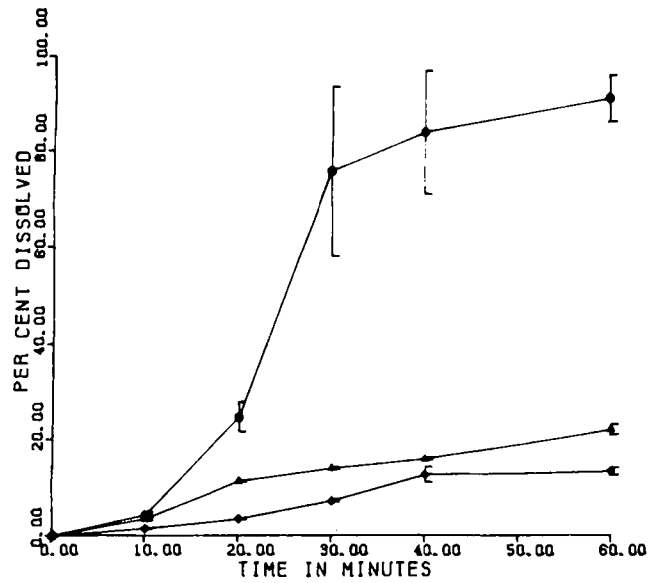

Figure 3—*Dissolution of the test preparations. Each point represents six capsules sampled and assayed in duplicate. All doses are equivalent to 92 mg of phenytoin. Key: (●) 100 mg of prompt phenytoin sodium; (▲) 230 mg of 40% solid dispersion of phenytoin in polyethylene glycol 6000; (◆) 92 mg of the reagent phenytoin. Bars represent 1 SD.*

Assay Procedure—A commercial enzyme immunoassay technique was utilized to assay all samples. This technique has been shown to be comparable in specificity and reproducibility (21, 22) to RIA (21, 23) and GC (21–24), and superior to spectrophotometry (21, 23). The range recommended was expanded by adding or deleting one dilution. Calibrated solutions provided by the manufacturers were used to check the effect of dilutions of samples on accuracy. A coefficient of variation of <10% was observed in each procedure.

Electron Microscopy—The sizes of the various particles were estimated by observation under a scanning electron microscope. Figure 1 shows a sample from the reagent phenytoin. A great variability in dimensions is observed. Figure 2 is a micrograph of the dispersion particles. The large particles are ~10 × 40 μm with several smaller fragments.

Dissolution Studies—Six 100-mg capsules of the commercial prompt phenytoin sodium were compared with six 92-mg samples of the reagent phenytoin and six 230-mg samples of the 40% (w/w) solid dispersion. All

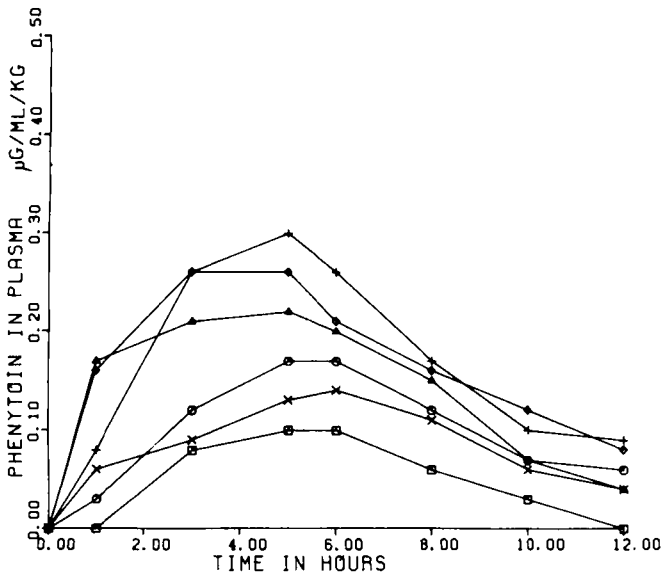

Figure 4—*Observed plasma concentrations of phenytoin obtained from six dogs (13–24 kg). Each animal received 300 mg of the prompt phenytoin sodium.*

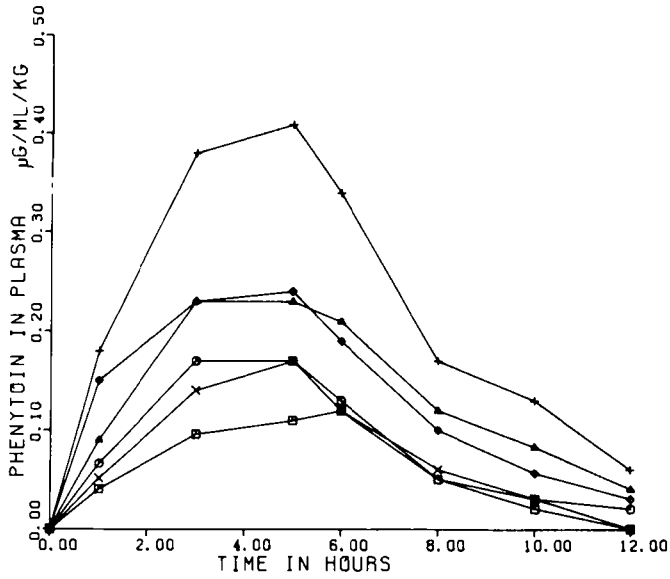

Figure 5—*Observed plasma concentrations of phenytoin obtained from six dogs (13–24 kg). Each animal received 750 mg of the 40% phenytoin solid dispersion.*

samples, equivalent to 92 mg of the phenytoin, were placed in size 1 gelatin capsules. The dissolution criteria were those stipulated for the oral phenytoin sodium in the USP XX. Duplicate samples were drawn and filtered through 0.45-μm filters[4], and were assayed at least twice. An additional study of six intact commercial prompt phenytoin sodium capsules using the same criteria was also performed.

X-ray Diffraction—Spectra were obtained from the reagent phenytoin, polyethylene glycol 6000, and the dispersion. A scanning rate of 3°/min was made from 6° to 70°. The minimum peak height was 2% of the reference peak. Spectra indicate that the phenytoin in the dispersion is the same crystalline form as that in the reagent. Also, no change was noted in the polyethylene glycol 6000 spectra.

Bioequivalence Studies—Six mixed-breed dogs (three of each sex), ~13–24 kg in weight, were used in this study. The animals were housed at the Animal Resources Center, The University of Texas. The dogs were fasted overnight prior to drug administration; water was allowed *ad libitum*. Each animal received 30 mL of water prior to and immediately after oral administration. The dogs were observed for 30 min and then returned to their cages for blood sampling.

The experimental design in the study is a balance of preparations over weeks and each animal received each preparation. The design was repeated twice for a total of two Latin squares. Two dogs received either 300 mg of the commercial prompt phenytoin sodium, 300 mg of phenytoin in the 40% dispersion, or 300 mg of a commercial intravenous phenytoin sodium on each study day. Studies were separated by 1-week wash-out periods to allow for drug elimination as well as to allow for stabilization of the animals' blood volume.

Blood samples were drawn by serial venipuncture. Sampling times after oral administration were at 0, 1, 3, 5, 6, 8, 10, 12, 24, 72, and 96 h. Samples were drawn at 0, 10, 20, 30, 45, and 60 min, and 2, 4, 12, 24, 48, 72, and 96 h after intravenous infusion at 50 mg/min. All blood samples were collected in heparinized evacuated tubes[5] which were gently inverted to allow for complete mixing. The samples were centrifuged at 2500 rpm for 15 min. The plasma was transferred into polyethylene tubes and frozen until assayed.

Statistical Analysis—A three-way ANOVA was performed on the *in vivo* data. The F ratio, as expected, was significant ($\alpha = 0.05$) for the intravenous data for $AUC_{0\rightarrow12}$ when compared with either of the oral preparations. The F ratio was not significant ($\alpha = 0.05$) when the two oral formulations were compared. Paired t tests compared the 40% phenytoin solid dispersion and the commercial prompt phenytoin sodium; this resulted in $p > 0.2$ for C_{max}, t_{peak}, and $AUC_{0\rightarrow12}$.

RESULTS AND DISCUSSION

***In Vitro* Studies**—Figure 1 is the electron micrograph of the reagent phenytoin. It shows a great variation in crystal size; many of the crystals exceed 40 μm in length and 10 μm in width. The micrograph of the dispersion par-

[4] Millipore Corp., Bedford, Mass.

[5] Vacutainer; B. D. Dickenson & Co.

Table I—Pharmacokinetic Parameters Obtained in Plasma after Oral Administration of Phenytoin

Parameter	Treatment A[a] Observed Mean (*SD*)	Treatment A[a] Calculated Mean[c]	Treatment B[b] Observed Mean (*SD*)	Treatment B[b] Calculated Mean[c]
C_{max} (μg/mL/kg)				
All subjects	0.20 (0.076)	0.20	0.22 (0.10)	0.24
Excluding dog 3	0.18 (0.063)	0.18	0.19 (0.049)	0.19
T_{peak} (h)				
All subjects	5.1 (0.41)	3.8	4.5 (1.2)	3.6
Excluding dog 3	5.2 (0.45)	4.0	4.4 (1.3)	3.6
$AUC_{0\rightarrow12}$ (μg/mL/h)				
All subjects	1.49 (0.602)	1.48	1.60 (0.808)	1.52
Excluding dog 3	1.37 (0.585)	1.33	1.22 (0.470)	1.22

[a] 300 mg of commercial prompt phenytoin sodium. [b] 750 mg of the 40% phenytoin solid dispersion. [c] Calculated values were obtained using Eq. 2.

ticles (Fig. 2) shows no large, discrete crystals in the formulation. The majority of the dispersion particles were ≤30 μm. A very small percentage exceeded 50 μm. An accurate estimate of the size of the phenytoin crystals in the dispersion could not be made, although some crystalline shapes could be discerned in the particles. Since none of the large rectangular crystals (>40 μm) observed in the reagent were noted in the dispersion or the residue, the formulation method used in this study must have reduced the particle size of the crystals. This probably occurred during the rapid solidification of the melt and during the trituration steps.

There is no compendial dissolution medium recommended for the comparison of the phenytoin and its sodium salt. Dissolution criteria do exist for the prompt and extended phenytoin sodium. These criteria were utilized to compare the dissolution rates of the phenytoin solid dispersion and the commercial prompt phenytoin sodium. Use of this dissolution test would indicate whether the commercial prompt phenytoin sodium samples would meet compendial standards. It would also serve to indicate whether one could use these dissolution criteria to compare two chemically different commercially available phenytoin products: the salt and the acid. The USP dissolution test for phenytoin sodium stipulates the use of 900 mL of water at 37 ± 0.5°C in Apparatus 1[6] (basket assembly) rotating at 50 rpm.

Prior to the dissolution test, all samples were placed in size 1 gelatin capsule. This was done to compare the rates of dissolution from a uniform capsule size. The percent dissolved of the commercial prompt phenytoin sodium at 30 min was 75.9%, which is much greater than the value obtained at 30 min for the solid dispersion (14.1%), or the reagent phenytoin (7.3%). At 60 min, 22.2 and 13.4% of the solid dispersion and the reagent phenytoin, respectively, were dissolved. After 180 min the percent dissolved in both preparations was almost equal: 29.4% for the reagent and 29.2% for the dispersion. The commercial prompt phenytoin sodium was 91.5% dissolved at 60 min.

The faster dissolution rate of the dispersion, when compared with the reagent phenytoin, may be due to the particle size reduction as previously noted in the electron micrographs. The X-ray diffraction data indicate that any change in the solubility of the two phenytoin samples cannot be due to a more-soluble polymorph existing in the dispersion. Comparison of the rate of dissolution of each preparation is presented in Fig. 3. Each point represents six capsules sampled and assayed in duplicate.

The USP states that at least 85% of a commercial prompt phenytoin sodium capsule should be dissolved in 30 min. Two of the size 1 capsules containing the transferred commercial prompt phenytoin sodium did not meet this specification. Thus, an additional study was undertaken. Six intact 100-mg capsules from the same lot were tested using the USP basket dissolution method as specified for phenytoin sodium capsules. At 30 min duplicate samples were taken and filtered through a 0.45-μm filter. Samples were kept in a water bath at ~37°C and assayed within 1 h after sampling. Each sample was assayed in duplicate. The six intact capsules showed an average dissolution of 81.5%, with SD = 14.5 and CV = 17.8%. The large variation noted in the six capsules may be due to two samples that had capsule-shaped masses which remained in the rotating basket apparatus after 30 min. The presence of these masses after a dissolution test has been noted in a previous study done with commercial phenytoin sodium products (25). A Student's t test was done to compare the data obtained from the transferred capsules *versus* the intact capsules. No significant difference was found (α = 0.05).

***In Vivo* Studies**—Each animal received 750 mg of the 40% solid dispersion, 300 mg of the commercial prompt phenytoin sodium, or 300 mg of the commercial intravenous phenytoin sodium. The oral doses were administered in three hard gelatin capsules. The 300-mg dose of each oral formulation was used to represent actual dosage strengths commercially available and dosing practices. The individual plasma levels obtained from these treatments are represented in Figs. 4 and 5. The plasma levels of the phenytoin sodium were normalized according to the phenytoin. It should be noted that dog 3 exhibits the highest blood levels in both Figs. 4 and 5. The difference in the curves demonstrates the effect of dog 3 on the data. Therefore, the t tests on C_{max}, t_{peak}, and $AUC_{0\rightarrow12}$ were done with and without the data obtained from dog 3. These data are presented in Table I. All areas were calculated using the trapezoidal rule. The probability values in the t_{peak}, C_{max}, and AUC were the same regardless of whether or not dog 3 was included. In all cases, $p > 0.2$.

Speculation on the cause of the large difference in the C_{max} found in dog 3 should include the observation that phenytoin exhibits nonlinear kinetics in dogs (26). Dog 3 was also the smallest dog and, therefore, received the largest mg/kg dose since all subjects received the same amount. It is possible that the small increase in the amount of phenytoin in the dispersion, when

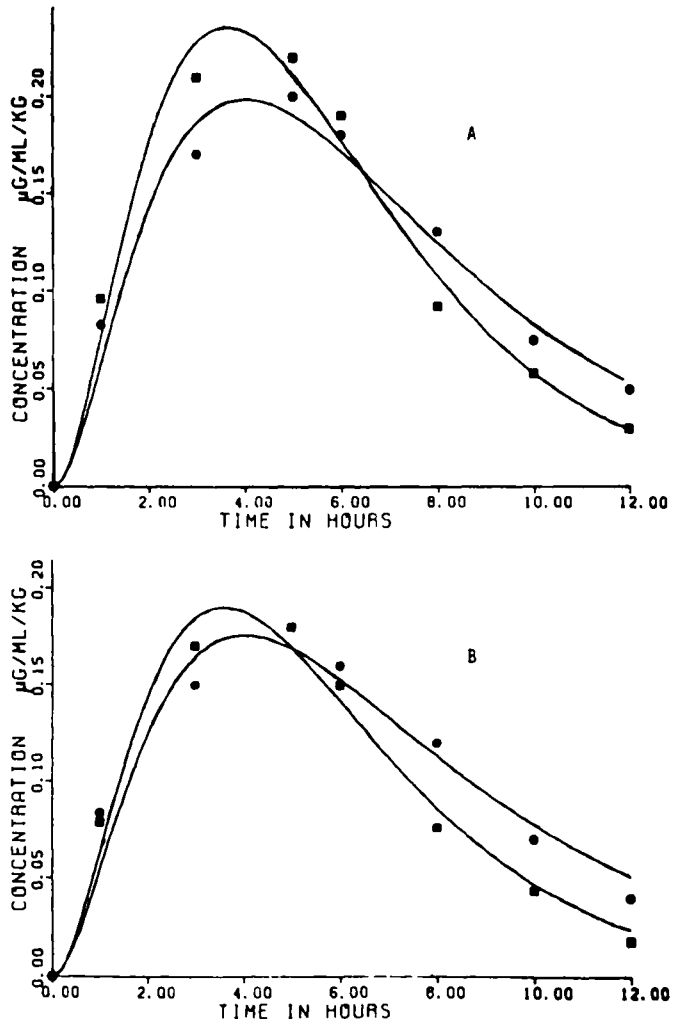

Figure 6— *Plots obtained from fitting averaged data to Eq. 2. Plot A represents the fit of all six animals, plot B represents the fit without the data from dog 3. Key: (■) 40% phenytoin dispersion; (●) commercial prompt phenytoin sodium.*

[6] Easi-Lift Dissolution Test Station; Hanson Research Corp., Nothridge, Calif.

Table II—Estimates of the Parameters Obtained from the Averaged Phenytoin Plasma Concentrations Utilizing Eq. 2

Parameter	Treatment[a] A	B	C	D
k_1 (h^{-1})	0.557	0.434	0.419	0.447
k_2 (h^{-1})	0.721	0.820	1.20	1.19
k_e (h^{-1})	0.414	0.482	0.268	0.241
C_0 ($\mu g/mL/kg$)	0.673	0.632	0.460	0.379

[a] Key: (A) 750 mg of 40% phenytoin solid dispersion, $n = 6$; (B) 750 mg of 40% phenytoin solid dispersion, $n = 5$ (without dog 3); (C) 300 mg of commercial prompt phenytoin sodium, $n = 6$; (D) 300 mg of commercial prompt phenytoin sodium, $n = 5$ (without dog 3).

compared with the same milligram dose of the salt, may have been enough to cause this animal to enter the nonlinear kinetics range.

The averaged data were fitted to a model with two first-order input steps:

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C \xrightarrow{k_e} \quad \text{(Eq. 1)}$$

where k_1 and k_2 are the apparent first-order rate constants for the overall absorption process and k_e is the apparent elimination rate constant. The equation used to calculate the theoretical line is:

$$C_t = k_1 k_2 C_0 \left[\frac{e^{-k_1 t}}{(k_2 - k_1)(k_e - k_1)} + \frac{e^{-k_2 t}}{(k_1 - k_2)(k_e - k_2)} + \frac{e^{-k_e t}}{(k_1 - k_e)(k_2 - k_e)}\right] \quad \text{(Eq. 2)}$$

where C_0 is the fraction of the dose absorbed divided by the volume of distribution.

The estimates for k_1, k_2, k_e, and C_0 were obtained using a nonlinear digital computer program (27) and are presented in Table II. The plots of the average data and the lines generated from Eq. 2 are represented in Fig. 6. Other equations representing biexponential or triexponential oral absorption models, including those that utilized a lag time, were attempted; however, this fit resulted in a smaller sum of squared deviations and larger r and r^2 values. Weights of 1 were used. The parameters obtained were used to calculate the $AUC_{0\to12}$, C_{max}, and t_{peak}. Good agreement is observed between the estimates obtained from the computer fits and the observed data, as seen in Table I. The relative bioavailability of the observed data is 1.01 for all subjects and 0.90 when excluding dog 3.

The data indicate that the dispersion is bioequivalent to the commercial prompt phenytoin sodium whether or not the data from dog 3 are included in the statistical analysis. Since the solubility of the salt and the acid are very different, the formulation method must be partially responsible for the increased acid solubility. The particle size reduction, as verified by the micrographs, of the phenytoin has been shown to increase plasma levels in dogs (18). The polyethylene glycol 6000 may have also affected the solubility of the dispersion. Previous studies have stated possible vehicle effects: a solubilizing effect by the vehicle (13), increased wettability (5), and a decrease in aggregation and agglomeration in hydrophobic drugs (28).

In this study, the combined effects of phenytoin crystal size reduction and any vehicle effects demonstrated bioequivalency with the much more water-soluble salt. This contradicts the *in vitro* dissolution study, which demonstrated a fivefold difference in the percent dissolved at 30 min, and a fourfold difference at 60 min. If the absorption of phenytoin is dissolution rate limited, then a significant difference should exist in the blood levels obtained from these two products. But according to the C_{max}, t_{peak}, and AUC data obtained from the animal studies, no statistical difference exists. This implies that the dispersion method must have increased the bioavailability of phenytoin.

It is possible that the particle size reduction and any vehicle effects on the *in vivo* microenvironment may be responsible for the increased dissolution rate of phenytoin. It is also possible that in an aqueous acidic environment, the relatively high concentration of free phenytoin provided by the dissolution of the salt may result in some precipitation of the free acid, as has been previously postulated (29). If this occurred, then the rate of absorption of the precipitated crystals may be comparable to the rate of absorption of the reduced crystals found in the 40% solid dispersion, and thus, demonstrate bioequivalency. If, indeed, this is a valid consideration, then any conclusions based on the greater dissolution rate of the salt implying better bioavailability must be reexamined.

The results of this study indicate that the phenytoin solid dispersion dissolution profile does not meet the criteria used by the USP XX to define a "prompt" phenytoin sodium, yet the dispersion was found to be bioequivalent to the prompt phenytoin sodium. Unless the vehicle played a very significant part in the phenytoin-microenvironment interaction *in vivo*, the use of a more representative dissolution medium to simulate the gastric fluid should be considered to improve the *in vitro–in vivo* correlation of the different phenytoin products. This study demonstrates once again the difficulties that may be encountered when selecting appropriate dissolution criteria to predict bioavailability.

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