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Phenytoin Prodrugs V: *In Vivo* Evaluation of Some Water-Soluble Phenytoin Prodrugs in Dogs

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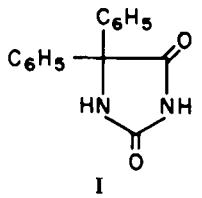
Abstract □ Phenytoin bioavailability was evaluated in beagle dogs after oral and intravenous administrations of sodium phenytoin and two amino acyl esters and a disodium phosphate ester of 3-(hydroxymethyl)phenytoin (three prodrugs of phenytoin). Phenytoin displayed nonlinear pharmacokinetics in the dogs, complicating the determination of the absolute bioavailability of phenytoin from sodium phenytoin and the prodrugs. All three prodrugs essentially released phenytoin after intravenous administration in a quantitative manner, and all gave plasma levels of phenytoin after oral administration greater than those found after administration of sodium phenytoin. Based on the behavior in dogs and the earlier determination of the physicochemical properties of the prodrugs, it was concluded that one of the amino acyl esters, 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylglycine ester methanesulfonate, would be the most useful prodrug for oral administration, while 3-(hydroxymethyl)-5,5-diphenylhydantoin disodium phosphate ester would be the most useful for parenteral administration.

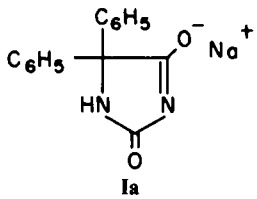
Keyphrases □ Prodrugs—phenytoin, bioavailability, dogs □ Phenytoin—hydroxymethyl esters, prodrugs, bioavailability, dogs □ Bioavailability—phenytoin prodrugs, dogs

Phenytoin (I), because of its weakly acid nature (1–4) and poor aqueous solubility (3–5), shows erratic absorption patterns after oral administration of either the sodium salt (Ia) or the free acid in both humans (6–19) and dogs (20, 21). Sodium phenytoin (Ia) in the parenteral dosage form is hazardous if rapidly injected intravenously (22, 23), and the free acid appears to precipitate at intramuscular injection sites (24–29), leading to prolonged and marginal phenytoin release.

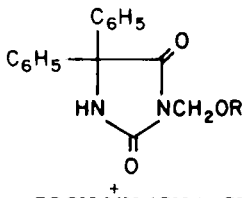
In the previous papers in this series (5, 30), a number of water-soluble prodrugs of phenytoin were evaluated with respect to their physicochemical properties (*e.g.*, solubility and stability), their cleavage to phenytoin in animal tissues, and their anticonvulsant activity in mice. Based on those studies, three of the prodrugs, 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylglycine ester methanesulfonate (II)[1], 3-(hydroxymethyl)-5,5-diphenylhydantoin *N,N*-dimethylaminoethyl carbonate methanesulfonate (III)[1], and 3-(hydroxymethyl)-5,5-diphenylhydantoin disodium phosphate



I



Ia



II: R = $-COCH_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$

III: R = $-CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$

IV: R = $-PO_3^{2-}\ Na_2^+$

[1] Compounds II, III, and IV are equivalent to IV, VI, and VII, respectively, in papers III and IV in this series.

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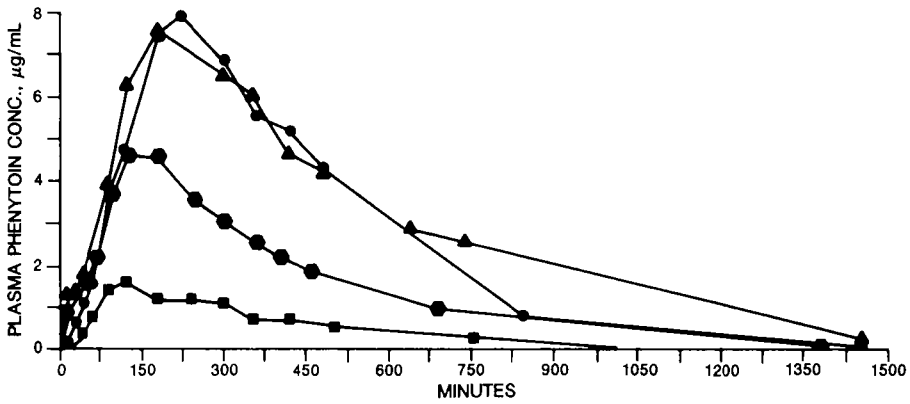


Figure 1—*Plasma levels of phenytoin in dog 20 following oral administration of Ia (■), II (●), III (▲), and IV (●) in capsule form. Doses (phenytoin equivalents) were 15 mg/kg.*

ester (IV)[1] were chosen for further evaluation in the beagle dog.

Compounds II and III were evaluated as possible oral-delivery forms of phenytoin because of their aqueous solubility (~150 mg/mL), pK_a values (6.60 and 8.20, respectively), and because they are readily cleaved enzymatically to phenytoin. However, the chemical stability of II and III indicated that there could be some difficulty in preparing suitably stable injectable dosage forms (30). Ester IV was chosen primarily as a parenteral candidate but was also evaluated for oral delivery of phenytoin, since recent evidence has suggested that dexamethasone phosphate quantitatively releases dexamethasone after oral dosing (31). Beagle dogs were chosen for this study because of their ease of handling and because phenytoin has been shown to be marginally bioavailable in dogs (20, 21), probably because of their short GI transit times (20). Dogs, therefore, may be a good model for pediatric patients, where phenytoin absorption has been shown to be particularly erratic (10, 19).

EXPERIMENTAL SECTION

Prodrugs II–IV were analytically pure and were prepared as previously described (5). Phenytoin and sodium phenytoin were obtained from a commercial source[2].

Gas Chromatographic Analysis of Phenytoin—Venous whole blood obtained from dogs was immediately centrifuged for 15–20 min at 1000×*g* and plasma was collected. In the case of II and III, a 100-μL plasma sample was immediately treated with 100 μL of a 10% metaphosphoric acid solution and assayed for phenytoin by GC according to the method of Stella (32). A second plasma sample was allowed to stand at 37°C for >1–2 h and then was assayed as described above. Any II or III in dog plasma is cleaved to phenytoin during this period (5). This procedure allowed for the analysis of phenytoin (first sample) and phenytoin plus prodrug (second sample), whereby prodrug concentration could be determined by difference. None of the samples (after both oral and parenteral dosings), contained any significant levels of II or III.

The procedure for IV was modified. The first plasma sample was not quenched with metaphosphoric acid, but was extracted into toluene, the first step in preparation for the GC assay. The second plasma sample was diluted with 100 μL of 10% amylase[3], maintained at 37°C overnight, and then assayed for phenytoin as described above. Only in the case of the 5–15-min samples were any levels of IV detectable after intravenous administration of IV; IV could not be detected after oral dosing.

Oral and Intravenous Evaluation Studies in Dogs—Four adult female beagle dogs, ranging in weight from 8 to 13 kg, were used for two crossover studies employing a Latin-square design. The intravenous and oral studies were carried out according to Table I. Each dog received 15-mg/kg (phenytoin equivalent) doses of sodium phenytoin, II, III, and IV orally and intravenously. The dogs were fasted overnight prior to drug administration, but were allowed water *ad libitum*. The dogs were rested for at least 1 week between studies.

For the intravenous study, fresh aqueous solutions of II, III, and IV were used. Phenytoin was administered intravenously as the sodium salt in a solvent system of propylene glycol–alcohol–water (4:1:5). The pH of this solution was adjusted to ~11.5–12.0 with sodium hydroxide. The injection volumes ranged from 2–3.5 mL and were administered into the leg vein over a period of 1–2 min.

In the oral study the compounds were administered in hard gelatin capsules without any excipients. After administering the compound, sufficient water to guarantee that the capsule had been swallowed (100–200 mL) was administered. The dogs were then returned to their respective cages.

After administration of the drug, serial venous blood samples were taken at appropriate times from the neck vein. The samples were withdrawn into 2-ml containers[4] containing 3 mg of EDTA. The blood samples were immediately centrifuged, and the separated plasma samples were analyzed for phenytoin and prodrug as described earlier.

Dose-Dependent Pharmacokinetics in Dogs—The evaluation of plasma concentration time curves of phenytoin, after administration of sodium phenytoin and the prodrugs of phenytoin at 15 mg/kg (phenytoin equivalents), suggested that phenytoin at a dose of 15 mg/kg displayed nonlinear pharmacokinetics. To confirm this, dogs 20 and 21, used in the 15-mg/kg study, were each administered 10-mg/kg and 6.6- or 5.5-mg/kg (phenytoin equivalent) doses of sodium phenytoin intravenously; plasma samples were assayed for phenytoin as described earlier.

RESULTS AND DISCUSSION

Figure 1 is a representative plot of the plasma phenytoin concentration *versus* time obtained after oral administrations of 15-mg/kg (phenytoin equivalent) doses of sodium phenytoin (Ia) and II–IV to dog 20. Dog 22 consistently rejected III, and hence, data for III for that particular dog could not be obtained. The areas under the plasma phenytoin concentration *versus* time curves (AUC_0^∞) after oral administration of Ia and II–IV were evaluated by the trapezoidal method (33) (Table II). Statistical analysis by one-way ANOVA showed that there was significant difference ($p < 0.05$) between the mean AUC_0^∞ values obtained after oral administration of the prodrugs and the mean AUC_0^∞ obtained after oral administration of sodium phenytoin (Ia). However, there was no significant difference ($p > 0.05$) in the mean AUC_0^∞ values between the prodrugs. Figures 2 and 3 show two representative plots of the plasma phenytoin concentration *versus* time after intravenous administration of the prodrugs and sodium phenytoin to dogs 19 and 20. Dogs 21 and 22 were not administered the intravenous injection of III, since the compound was observed to cause pain and toxic manifestations in dogs 19 and 20. Intravenous administration of III to dog 20 appeared to be incomplete, since pain and swelling around the injection site suggested that some of the dose may not have been placed directly in the vein.

Prodrugs II–IV, on oral administration, produced higher plasma phenytoin levels than the levels obtained after oral administration of Ia. The poor availability of phenytoin from Ia was probably due to the precipitation of phenytoin acid in the acidic environment of the stomach as well as the poor dosage form used, *i.e.*, sodium phenytoin in a hard gelatin capsule. Phenytoin has a low aqueous solubility and, thus, the slow and incomplete redissolution probably results in low plasma levels of phenytoin.

The primary purpose of the intravenous and oral administration of the prodrugs to the beagle dogs was to determine (*a*) if the prodrugs quantitatively released phenytoin and (*b*) the extent of absorption of phenytoin from the prodrugs on oral administration. Such an evaluation of the prodrugs of phenytoin is difficult since the elimination kinetics of phenytoin may be

[2] Sigma Chemical Co., St. Louis, Mo.

[3] Diatase or clarase, a phosphatase enzyme preparation, was found to cleave IV with a half-life of <1 h; Fisher Scientific Co., Fair Lawn, N.J.

[4] Vacutainers, No. 6496; Becton, Dickinson and Co., Rutherford, N.J.

Table I—Intravenous and Oral Studies Using a Latin-Square Design Carried out in Beagle Dogs

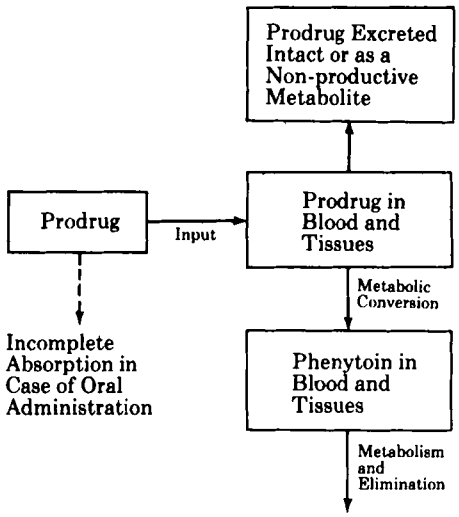
Experiment	Dog 19	Dog 20	Dog 21	Dog 22
Intravenous				
1	III[a]	IV	Ia	II
2	II	III[b]	IV	Ia
3	Ia	II	III[c]	IV
4	IV	Ia	II	III[c]
Oral				
5	Ia	II	IV	III[d]
6	III	Ia	II	IV
7	IV	III	Ia	II
8	II	IV	III	Ia

[a] Produced severe toxic reactions in the dog including salivation, dilatation of the pupils, and hypotension. [b] Appeared to cause severe pain on administration, thus resulting in incomplete administration of the dose. [c] Not administered due to toxicity seen in Experiments 1 and 2. [d] Dog 22 rejected III three times. Further attempts to administer III orally to dog 22 were therefore terminated.

nonlinear (34, 35), probably due to the saturation of the enzyme system responsible for the metabolism of phenytoin to 5-*p*-hydroxyphenyl-5-phenylhydantoin (36). Dayton *et al.* (34) and Frey and Loseher (35) have shown that phenytoin exhibits dose-dependent kinetics in the dog. It was reported (35) that after intravenous administration of 20, 10, and 5 mg/kg (phenytoin equivalents) of Ia to dogs, the apparent elimination half-life of phenytoin decreased from 11.2 to 6.1 to 4.3 h, respectively. A plot of the logarithm of the plasma phenytoin concentration *versus* time was curvilinear at the three doses studied. Such a curvilinear plot is typical of drugs with Michaelis–Menten kinetics with a capacity-limited pathway of elimination or for drugs exhibiting product inhibition (37–41). In the present study, the nonlinear behavior of phenytoin in the beagle dog is apparent from the curvilinear shape observed in the plot of the logarithm of the plasma phenytoin concentration *versus* time obtained after intravenous administration of a 15-mg/kg (phenytoin equivalent) dose of Ia (Fig. 4).

In its simplest form, the pharmacokinetic model in Scheme I can be proposed for phenytoin and its prodrugs. Since phenytoin seems to exhibit nonlinear elimination kinetics in the dog (34, 35) at a dose of 15 mg/kg (phenytoin equivalents) of either Ia or the prodrug, quantitation of the extent of conversion of the prodrugs to phenytoin is difficult. This is because the elimination rate and the clearance of phenytoin (and thus the area under the plasma phenytoin concentration *versus* time profiles) depends on its input rate. This, in turn, depends on either the absorption rate of the prodrug after oral dosing and/or the metabolic conversion rate of the prodrug to phenytoin.

To confirm the nonlinear behavior of phenytoin in the beagle dog, a pharmacokinetic study involving variation of the dose of Ia was carried out in dogs 20 and 21. Dog 20 was administered 15-, 10-, and 6.6-mg/kg (phenytoin equivalent) doses of Ia intravenously and dog 21 was administered 15-, 10-, and 5.5-mg/kg (phenytoin equivalent) doses of Ia intravenously. The AUC_0^∞ values obtained after intravenous administration of the different doses of Ia were determined by the trapezoidal method. A plot of the AUC_0^∞ values *versus* the dose is shown in Fig. 5. The plot shows that the relationship between AUC_0^∞ and the dose is nonlinear. In the case of drugs obeying linear pharmacokinetics,



Scheme I

the AUC_0^∞ obtained after intravenous administration of the drug is directly proportional to the intravenous dose:

$$AUC_0^\infty = \frac{D_{iv}}{CL} \qquad \text{(Eq. 1)}$$

where D_{iv} is the intravenous dose and CL is the total body clearance of the drug. Thus, the AUC_0^∞ would increase proportionately with dose if the clearance remains constant. The elimination half-life of the drug should also be independent of the dose for drugs obeying linear pharmacokinetics. Hence, the nonlinear relationship between AUC_0^∞ *versus* dose and the increase in the apparent elimination half-life with increasing dose observed in this study (Table III) confirms the dose dependency and nonlinear behavior of phenytoin in the current study.

The absolute bioavailability of drugs (or prodrugs) exhibiting linear pharmacokinetics is normally determined by calculating the ratio of the AUC_0^∞ of the parent drug obtained after oral administration of the drug (or prodrug) and the AUC_0^∞ obtained after intravenous administration of the parent drug (33). Also, the availability of the parent drug after intravenous administration of a prodrug could be determined by calculating the ratio of the AUC_0^∞ values obtained after intravenous administration of the prodrug and those obtained after intravenous administration of an equivalent dose of the parent drug. Table IV summarizes the AUC_0^∞ values, calculated by the trapezoidal method, of the plasma phenytoin concentration *versus* time profiles obtained after intravenous administration of molar equivalent doses of Ia and II–IV. If phenytoin did exhibit linear pharmacokinetics, then the ratio of the AUC_0^∞ obtained after intravenous administration of the prodrugs and the AUC_0^∞ obtained after intravenous administration of Ia (Table V) would give the extent of conversion of the prodrugs to phenytoin. Similarly, the ratio of the AUC_0^∞ values obtained after oral administration of the prodrug and those obtained

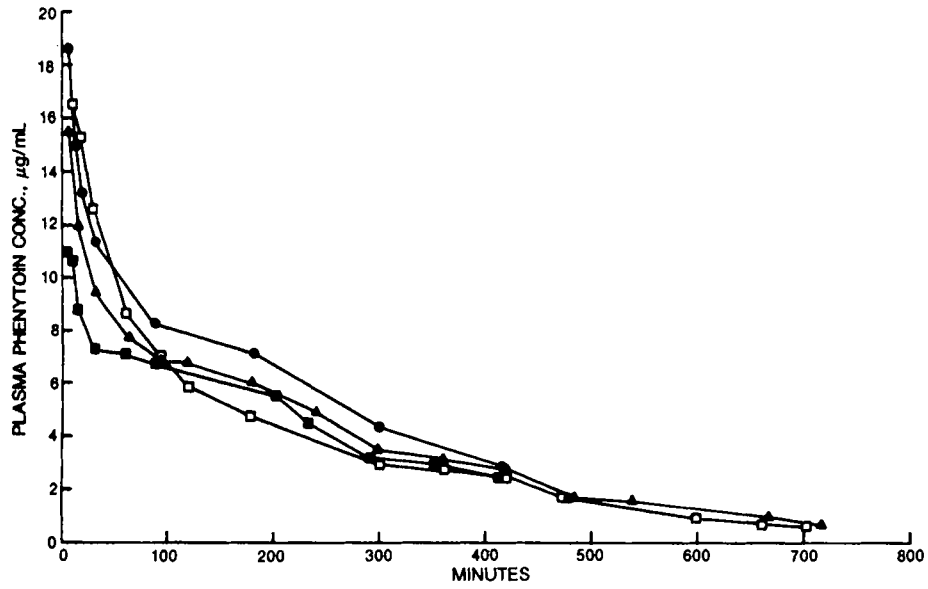


Figure 2—*Plots of the plasma phenytoin concentrations* versus *time obtained after intravenous administration of Ia (●), II (■), III (□), and IV (▲) to dog 19. Doses (phenytoin equivalents) were 15 mg/kg.*

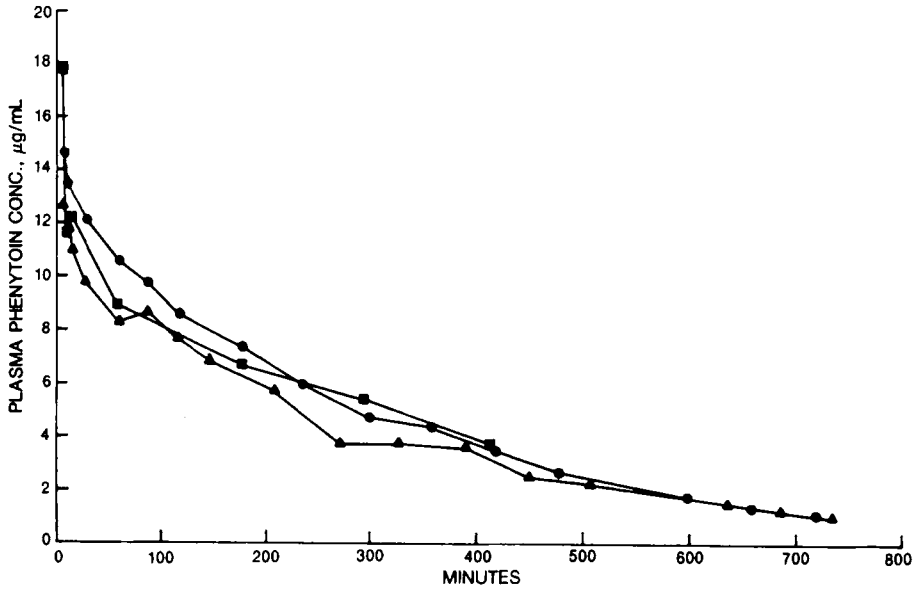
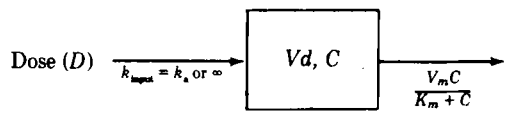


Figure 3—*Plots of the plasma phenytoin concentrations* versus *time obtained after intravenous administration of Ia (●), II (■), and IV (▲) to dog 20. Doses (phenytoin equivalents) were 15 mg/kg.*

after intravenous administration of Ia (Table VI) would give the absolute bioavailability of the prodrugs. However, since phenytoin exhibits nonlinear behavior, the ratios summarized in Tables V and VI are only apparent values of the bioavailabilities of the prodrugs compared with Ia.

To determine the bioavailabilities of the prodrugs, the model shown in Scheme II is proposed, representing a one-compartment model. Although phenytoin does exhibit two-compartmental behavior, the distribution phase in the dog is insignificant; most authors[5] (35, 42, 43), therefore, describe the kinetics of phenytoin according to a one-compartment model[6]. According to the model, elimination of phenytoin, after an intravenous or oral dose D, is assumed to occur by a capacity-limited process. It will be assumed that the capacity-limited process, described by the Michaelis–Menten equation for a one-enzyme reaction, is applicable to phenytoin elimination in the dog.



Scheme II

Following intravenous administration of a dose (D_{iv}) of phenytoin, the differential equation describing the concentration of phenytoin at any time t in the body is:

$$\frac{-dC}{dt} = \frac{V_m C}{K_m + C} \qquad \text{(Eq. 2)}$$

where V_m is the theoretical maximum velocity per unit volume of distribution of the capacity-limited process (and in the present case has the units of μg/mL/min) and K_m represents the Michaelis–Menten constant (and has the units of concentration, μg/mL). Numerical values of V_m and K_m for dogs 20 and 21 were obtained by nonlinear least-squares iteration of the integrated form of Eq. 2, *i.e.*:

$$C_1 - C + K_m \cdot \ln (C_1/C) = V_m(t - t_1) \qquad \text{(Eq. 3)}$$

using the SIMPLEX program; data was obtained at three different intravenous doses of sodium phenytoin. C_1 is the first plasma phenytoin concentration point chosen by inspection in the elimination phase at time t_1.

The volume of distribution, Vd (really Vd extrapolated) was obtained from:

$$Vd = \frac{D_{iv}}{C_0} \qquad \text{(Eq. 4)}$$

where C_0 is the time zero plasma phenytoin concentration, determined by back extrapolation of the elimination phase of the plasma phenytoin concentration *versus* time profile obtained after intravenous administration of Ia.

The values of V_m, K_m, and Vd obtained are shown in Table VII. Figures 6 and 7 show the semilogarithmic plots of the plasma phenytoin concentration *versus* time in dogs 20 and 21, respectively, after administration of the three different doses of Ia; the solid lines were computer-generated from Eq. 2 using the individual V_m and K_m values determined at the different doses. The solid lines in Fig. 4 are the theoretical lines obtained using Eq. 3 and the mean V_m and K_m values shown in Table VII.

To determine the true bioavailability of the prodrugs in dogs 20 and 21, the method described by Martis and Levy (39) and Jusko *et al.* (38) for calculating the bioavailability of drugs exhibiting dose-dependent elimination due to enzyme saturation was employed. The method assumes a one-compartment pharmacokinetic system with a constant volume of distribution and a capacity-limited pathway of elimination. The volume of distribution of phenytoin, shown in Table VII, is practically constant. Following oral administration of

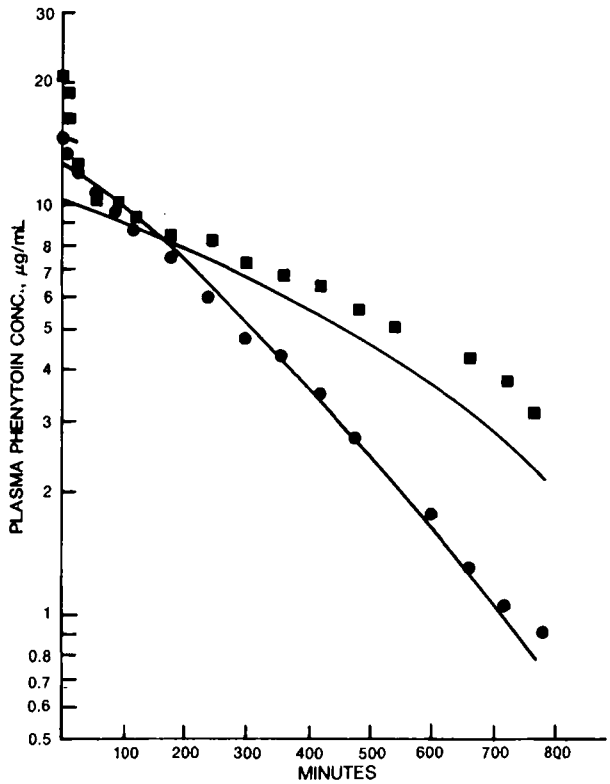


Figure 4—*Semilogarithmic plots of plasma phenytoin concentrations* versus *time after an intravenous dose of 15 mg/kg (phenytoin equivalents) of sodium phenytoin to dog 20 (●) and dog 21 (■). (——) theoretical line using mean* V_m *and* K_m *values.*

[5] D. F. Kowalczyk, unpublished results.

[6] The differences in AUC^{∞}_0 values between assuming a distribution phase and disregarding the distribution phase is only 0.5 and 3.8% in dogs 20 and 21, respectively, for the 15-mg/kg data.

Table II—Area under the Plasma Phenytoin Concentration *versus* Time Curve After Oral Administration of a Dose Equivalent to 15 mg/kg of Phenytoin

Compound	AUC_0^∞, μg·min/mL[a]				
	Dog 19	Dog 20	Dog 21	Dog 22	Mean ± *SD*
Ia	376	668	921	659	656 ± 222
II	2569	3724	2975	2576	2961 ± 543
III	3306	4438	1869	—[b]	3204 ± 1287[c]
IV	1714	2065	4190	2151	2530 ± 1123

[a] Calculated *via* the trapezoidal method. [b] Compound III was rejected three times by dog 22. [c] Mean obtained from results of 3 dogs.

the prodrugs and Ia, the rate of change of phenytoin concentration in the body can be expressed by:

$$\frac{dC}{dt} = \frac{k_a F D_{iv} e^{-K_a t}}{Vd} - \frac{V_m C}{K_m + C} \qquad \text{(Eq. 5)}$$

where k_a is the first-order absorption rate constant and F represents the fraction of the dose absorbed. D_{iv} is the intravenous dose, and this is equal to the oral dose, *i.e.*, 15 mg/kg. The rate of elimination of phenytoin from the body after oral administration is given by:

$$\frac{1}{Vd} \times \frac{dA_e}{dt} = \frac{V_m C}{K_m + C} \qquad \text{(Eq. 6)}$$

where A_e is the amount of phenytoin eliminated at any time t. If the total amount of drug eliminated is equal to the amount absorbed, then:

$$\int_0^\infty \frac{dA_e}{dt} dt = F D_{oral} \qquad \text{(Eq. 7)}$$

where D_{oral} is the oral dose. However, from Eq. 6:

$$\int_0^\infty \frac{dA_e}{dt} dt = Vd \int_0^\infty \frac{V_m C}{K_m + C} dt \qquad \text{(Eq. 8)}$$

Combining Eqs. 7 and 8, the following equality is obtained:

$$Vd \int_0^\infty \frac{V_m C}{K_m + C} dt = F D_{oral} \qquad \text{(Eq. 9)}$$

Dividing Eq. 9 by the intravenous dose, D_{iv}, gives:

$$\frac{\int_0^\infty \frac{V_m C}{K_m + C} dt}{D_{iv}/Vd} = \frac{F D_{oral}}{D_{iv}} \qquad \text{(Eq. 10)}$$

Since equivalent doses are administered intravenously and orally, Eq. 10 reduces to:

$$\frac{\int_0^\infty \frac{V_m C}{K_m + C} dt}{D_{iv}/Vd} = F \qquad \text{(Eq. 11)}$$

where F is the corrected bioavailability. Equation 11 can also be used to determine the availability of phenytoin after an intravenous dose of the prodrug relative to an equivalent intravenous dose of Ia.

To obtain the numerator of Eq. 11, the area under the plot of $V_m C/(K_m + C)$ *versus* time from zero to the last time point (t_L) was determined by the trapezoidal method (33). The concentration at the last time point in the plasma phenytoin concentration *versus* time profile after oral and intravenous administration of the prodrugs was very low. The contribution of the $AUC_{t_L}^\infty$ of a plot of $V_m C/(K_m + C)$ *versus* time would, therefore, be very small. Thus, $AUC_0^{t_L}$ would be approximately equal to AUC_0^∞, which is equal to the numerator of Eq. 11.

The results of the determination of the corrected true bioavailability of intravenously administered prodrugs II and IV in dogs 20 and 21 relative to intravenously administered Ia are summarized in Table VIII, and corrected bioavailabilities of prodrugs II–IV in dogs 20 and 21 after oral dosing are shown in Table IX. Similar calculations could be done to determine the corrected bioavailabilities of oral and intravenously administered prodrugs, respectively, in dogs 19 and 22 if the V_m and K_m values could have been accurately estimated.

Comparison of the AUC_0^∞ values to determine the availability of phenytoin after intravenous administration of its prodrugs relative to Ia can result in an underestimation in the case of drugs like phenytoin, which exhibit nonlinear pharmacokinetics. The extent of underestimation will depend on the rate of conversion of the prodrugs to phenytoin. If the rate of hydrolysis of the prodrugs is slow, the input rate of phenytoin will be slow. This in turn will show a decreased degree of saturation of the enzyme system responsible for metabolism of phenytoin compared with the saturation that would be exhibited by an equivalent intravenous dose of Ia. Similarly, the bioavailability of the prodrugs calculated by determining the ratio of AUC_0^∞ obtained after oral administration of the prodrugs and the AUC_0^∞ obtained after intravenous administration of an equivalent dose of Ia can result in an underestimation. If the input rate of phenytoin (*i.e.*, the absorption and conversion of the prodrug) is slow, the apparent absolute bioavailability is considerably underes-

Table III—Apparent Elimination Half-Lives of Phenytoin after Intravenous Administration of Three Different Doses [a]

Dog	Dose, mg/kg[a]			
	15	10	6.6	5.5
20	3.2 h	2.6 h	2.3 h	—
21	6.7 h	4.4 h	—	2.9 h

[a] Doses of sodium phenytoin, expressed as phenytoin equivalents. Linear kinetics are assumed.

Table IV—Area under the Plasma Phenytoin Concentration *versus* Time Curves after Intravenous Administration of a Dose Equivalent to 15 mg/kg of Phenytoin

Compound	AUC_0^∞, μg·min/mL[a]			
	Dog 19	Dog 20	Dog 21	Dog 22
Ia	3697	3889	7225	4720
II	2499	3808	3070	4873
III	2762	—[b]	—[c]	—[c]
IV	3008	3491	4452	3150

[a] Calculated *via* the trapezoidal method. [b] Unable to administer complete dose due to excessive pain on administration. [c] Compound III was not administered.

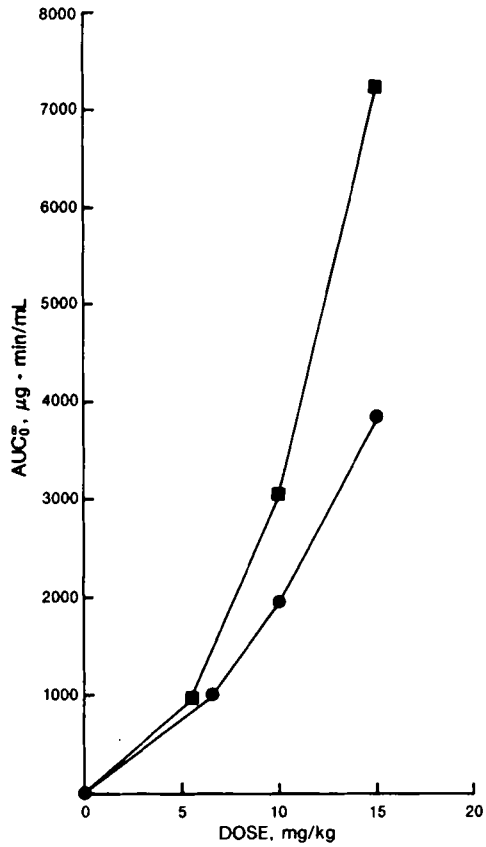


Figure 5—*Plot of the area under the plasma phenytoin concentration* versus *time curve, AUC_0^∞, as a function of the intravenous dose of sodium phenytoin administered to dog 20 (●) and dog 21 (■). Doses are in phenytoin equivalents.*

Table V—Apparent Bioavailability of Phenytoin after Intravenous Administration of Prodrugs II–IV [a]

Compound	F^{abs}_{app}, %[b] Dog 19	Dog 20	Dog 21	Dog 22
II	67	98	42	103
III	75	—[c]	—[d]	—[d]
IV	81	90	62	67

[a] Relative to Ia. [b] Calculated as $[AUC^{\infty}_{0,iv}$ (prodrug)$/AUC^{\infty}_{0,iv}$ (Ia)$] \times 100$. [c] Unable to administer complete dose due to excessive pain on administration. [d] Compound III was not administered.

Table VI—Apparent Bioavailability of Phenytoin after Oral Administration of Ia and II–IV [a]

Compound	F^{abs}_{app}, %[b] Dog 19	Dog 20	Dog 21	Dog 22
Ia	10	17	13	14
II	69	96	41	55
III	89	114	26	—[c]
IV	46	53	58	46

[a] Relative to intravenously administered Ia. [b] Calculated as $[AUC^{\infty}_{0,po}$ (Ia and prodrugs)$/AUC^{\infty}_{0,iv}$ (Ia)$] \times 100$. [c] Compound III was rejected by dog 22.

timated. This is apparent from the results summarized in Tables VIII and IX. The corrected bioavailabilities show that in dog 21, the apparent bioavailabilities are drastic underestimations. This is consistent with the larger change in the AUC^{∞}_{0} and the apparent elimination half-life of phenytoin with dose observed in dog 21 compared with the change observed in dog 20 (Table III),

Table VII—V_m, K_m, and the Apparent Volume of Distribution (Vd) Calculated from the Data Obtained after Intravenous Administration of Sodium Phenytoin

Parameters	Dose mg/kg[a] 15	10	5.5	Mean ± SD
		Dog 20		
K_m, μg/mL	25.3	15.9	7.9	16.4 ± 8.7
V_m, μg/mL/min	0.1	0.087	0.045	0.077 ± 0.03
Vd, L/kg[b]	1.15	1.15	1.28	1.19 ± 0.07
		Dog 21		
K_m, μg/mL	4.5	2.7	4.7	3.99 ± 1.1
V_m, μg/mL/min	0.016	0.016	0.02	0.018 ± 0.003
Vd, L/kg[b]	1.41	1.43	1.77	1.54 ± 0.20

[a] Doses are in phenytoin equivalents. [b] Volume of distribution extrapolated.

suggesting that dog 21 is more susceptible to enzyme saturation than is dog 20 (lower K_m value observed for dog 21).

The corrected bioavailability of both orally and intravenously administered II in dog 20 is ~100%; in dog 21, it is ~60%. This suggests that in dog 20, II undergoes quantitative hydrolysis to phenytoin and is completely absorbed from the GI tract. However, in dog 21, it seems that the complete oral dose of II may be absorbed from the GI tract, but the prodrug may not undergo quantitative conversion to phenytoin, *i.e.*, the ester may be undergoing a nonproductive metabolic pathway such as hydroxylation of the hydantoin phenyl ring followed then by the cleavage of the ester. This pathway may be

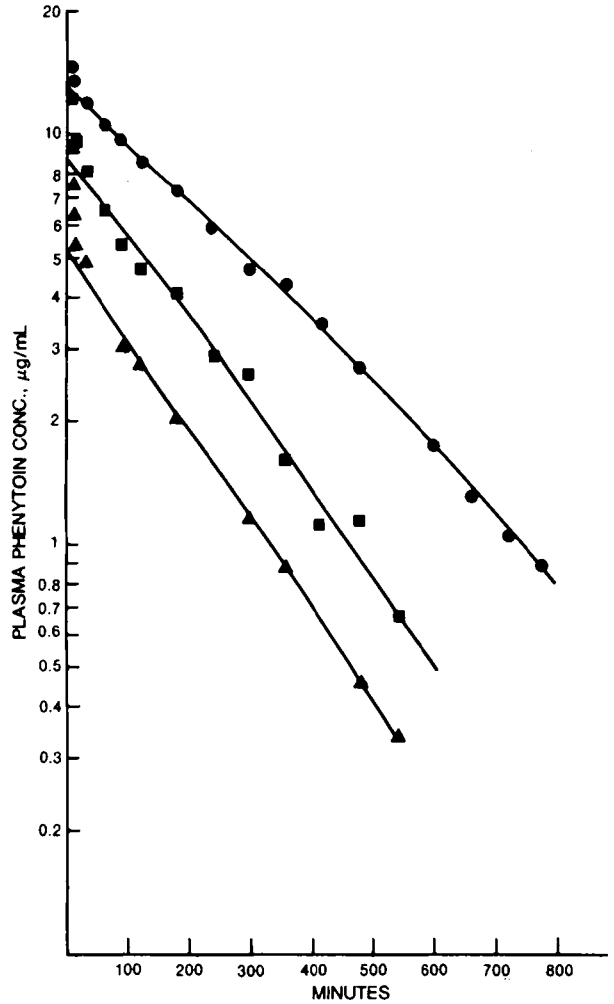


Figure 6—*Semilogarithmic plots of plasma phenytoin concentrations* versus *time following intravenous administration of 15 (●), 10 (■), and 6.6 (▲) mg/kg (phenytoin equivalents) of sodium phenytoin to dog 20; (—) computer-fitted lines.*

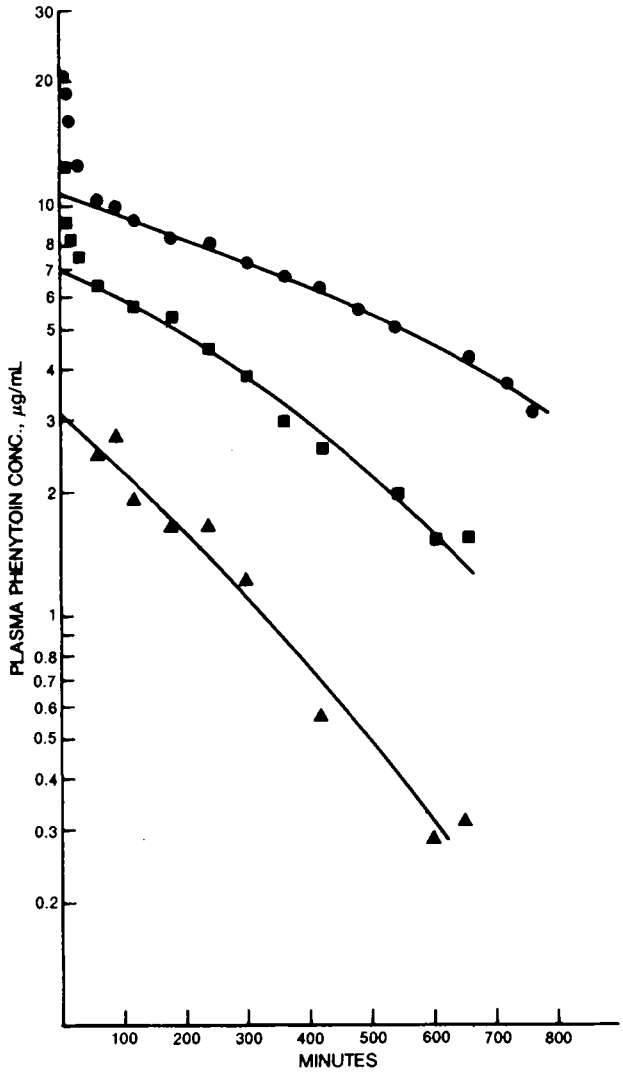


Figure 7—*Semilogarithmic plots of plasma phenytoin concentrations* versus *time following intravenous administration of 15 (●), 10 (■), and 5.5 (▲) mg/kg (phenytoin equivalents) of sodium phenytoin to dog 21; (—) computer-fitted lines.*

Table VIII—Calculation of the Corrected Bioavailability of Phenytoin [a] after Intravenous Administration of II and IV to Two Beagle Dogs

Compound	$\int_0^\infty \frac{V_m C}{K_m + C} dt$		F_{app}^{abs}, %[b]		Corrected F, %[c]	
	Dog 20	Dog 21	Dog 20	Dog 21	Dog 20	Dog 21
II	13.8	6.4	98	42	106	60.2
IV	12.8	10.1	90	62	98.7	95.3

[a] Corrected for enzyme saturation. [b] Calculated by $[AUC_{0,iv}^{\infty}$ (prodrug)$/AUC_{0,iv}^{\infty}$ (Ia)$] \times 100$. [c] Based on Eq. 11.

Table IX—Calculation of the Corrected Bioavailability of Phenytoin [a] after Oral Administration of Ia and II–IV to Two Beagle Dogs

Compound	$\int_0^\infty \frac{V_m C}{K_m + C} dt$		F_{app}^{abs}, %[b]		Corrected F, %[c]	
	Dog 20	Dog 21	Dog 20	Dog 21	Dog 20	Dog 21
Ia	2.5	2.3	17	13	19.5	21.9
II	16.3	6.8	96	41	125.4	64.1
III	16.1	4.5	1114	26	123.8	42.9
IV	8.2	9.2	53	58	63.2	86.8

[a] Corrected for enzyme saturation. [b] Calculated by $[AUC_{0,po}^{\infty}$ (Ia and prodrugs)$/AUC_{0,iv}^{\infty}$ (Ia)$] \times 100$. [c] Based on Eq. 11.

competing with the hydrolysis of the ester to phenytoin. Another possibility is that the esterase activity in dog 21 may be low. Prodrug III also seems to show a bioavailability of only ~45% in dog 21, whereas, in dog 20, the corrected bioavailability of III is ~100%.

The results in Table VIII suggest that IV is quantitatively hydrolyzed to phenytoin on intravenous dosing to dogs 20 and 21. However, the corrected bioavailability of IV on oral dosing in dogs 20 and 21 was ~65 and ~90%, respectively. This suggests that the ester may be incompletely absorbed from the GI tract, probably because of its polar nature.

In all the calculations and discussions to date, it was assumed that phenytoin does not exhibit presystemic clearance in the dog and that orally or intravenously administered prodrugs do not exhibit a sequential first-pass effect (44); *i.e.*, if the prodrugs are cleaved to phenytoin prior to reaching systemic circulation, then the formed phenytoin may be further metabolized prior to release to systemic circulation. For dogs 20 and 21, presaturation clearances could be calculated from:

$$CL = \frac{VdV_m}{K_m} \qquad \text{(Eq. 12)}$$

For dog 20, the approximate phenytoin clearance is 5.6 mL/min/kg, while for dog 21 a clearance of ~6.9 mL/min/kg is calculated. If it is assumed that phenytoin readily equilibrates between red blood cells and plasma, that the metabolism of phenytoin occurs only in the liver, and that liver blood flow in dogs is 40–45 mL/min/kg (45), then it can be estimated that phenytoin at presaturation doses may exhibit a first-pass effect of ~12–14% and 15–17% in dogs 20 and 21, respectively. Obviously, this is not sufficient to account for the poor oral performance of Ia, but it may account for the <100% availability of phenytoin from the prodrugs, even correcting for the nonlinear kinetics.

It can be concluded that II would be a better oral-delivery form of phenytoin than Ia. Compound III caused nausea, vomiting on oral dosing, and pain and hematoma on intravenous dosing. This would make it less favorable as a prodrug candidate for either oral or parenteral use. Compound II would probably be quantitatively hydrolyzed to phenytoin on oral administration to humans, since the esterase activity of the intestinal mucosa of dogs is poor relative to other mammalian species (46). Oral administration of II would help in overcoming the problem of low and erratic bioavailability of phenytoin associated with the oral administration of Ia.

The long shelf life of IV (30) and its high aqueous solubility at physiological pH (5) would render it useful as a parenteral dosage form for the delivery of phenytoin in humans, provided it is quantitatively hydrolyzed to phenytoin (as was observed in the dog). This would aid in overcoming the hazards associated with intravenous administration of sodium phenytoin.

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Phenytoin Prodrugs VI: *In Vivo* Evaluation of a Phosphate Ester Prodrug of Phenytoin after Parenteral Administration to Rats

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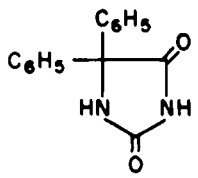
Abstract □ Tissue damage caused by subcutaneous and intramuscular administration of three phenytoin prodrugs to rats was assessed. Since two of the prodrugs caused significant irritation, only 3-(hydroxymethyl)-5,5-diphenylhydantoin disodium phosphate ester might be useful as a nonirritant phenytoin prodrug suitable for parenteral administration. To confirm the release of phenytoin from this prodrug, phenytoin availability after intramuscular and intravenous administrations of the phosphate prodrug was evaluated in rats and compared with sodium phenytoin. The prodrug quantitatively released phenytoin after intravenous administration, and phenytoin levels from intramuscular administration of the prodrug were far superior to those generated from similarly administered sodium phenytoin. Based on this and earlier studies, it was concluded that this prodrug should be further assessed as a parenteral form of phenytoin.

Keyphrases □ Phenytoin—phosphate prodrug, parenteral administration, rats □ Prodrugs—phenytoin, phosphate ester, parenteral administration, rats □ Anticonvulsants—phenytoin, phosphate prodrug, parenteral administration, rats

The parenteral form of the anticonvulsant drug phenytoin (I), *i.e.*, sodium phenytoin (Ia) dissolved in a vehicle consisting of 40% propylene glycol and 10% alcohol at pH ~12, is hazardous if too rapidly injected intravenously (1, 2); intramuscular injection results in delayed release of phenytoin from the precipitated phenytoin acid at the injection site (3–8). We recently evaluated the potential usefulness of a series of phenytoin prodrugs (9–11). Based on those studies, II–IV were thought to be possible parenteral forms of phenytoin because of their superior solubility properties compared with phenytoin (9). In a dog study, these prodrugs quantitatively released phenytoin after intravenous injection (11), although III was observed to cause significant acute toxicity in the dog and II had only marginal chemical stability (10). It was concluded that IV would be the best candidate among prodrugs II–IV (11) for parenteral administration.



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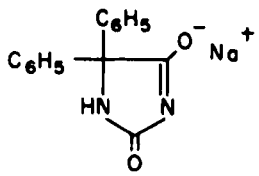

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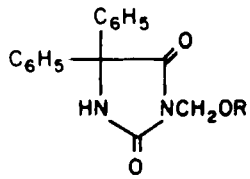

II: R = $-COCH_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$
III: R = $-CO_2(CH_2)_2\overset{+}{N}H(CH_3)_2\ CH_3SO_3^-$
IV: R = $-PO_3^{2-}\ Na_2^+$

Presented here are two further studies, designed to evaluate the possible utility of II–IV as parenteral forms of phenytoin. First, an evaluation of the tissue damage after subcutaneous and intramuscular injection of prodrugs II–IV to rats was initiated. Based on these findings (significant irritation seen with both II and III but not IV) a second study, the intravenous and intramuscular availability of phenytoin from IV in rats, was addressed. The results of the two studies are presented in this paper.

EXPERIMENTAL SECTION

Evaluation of the Tissue Damage Caused by Subcutaneous and Intramuscular Administration of II–IV—Male Sprague-Dawley rats[1], weighing between 250–275 g, were used; twelve rats were used for each ester studied. Fresh aqueous solutions of esters II–IV (9), 25 mg/kg (phenytoin equivalents), were prepared, and each of six groups of six rats received an ester injected intramuscularly in the thigh muscle or an ester injected subcutaneously under the thigh skin. The injection volume did not exceed 200 μL. After administration, the rats were placed in metabolism cages with access to food and water. At 24 h postdose, three rats administered drug intramuscularly and three rats administered drug subcutaneously for each ester were sacrificed, and the extent of tissue damage was evaluated. The extent of tissue damage in the remaining six rats was evaluated after 7 d postdose. Visual observations were made to determine the extent of tissue damage. The rats were observed externally to check for any skin damage after the subcutaneous injection. The animals were then checked for internal damage by exposing the thigh muscle (intramuscular administration) or the muscles underlying the skin at the injection site (subcutaneous injection).

***In Vivo* Evaluation of IV as a Prodrug of Phenytoin in Rats**—The availability of phenytoin after intramuscular administration of IV was studied in

[1] Harlan Sprague-Dawley, Madison, Wis.

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