

# Disposition, elimination, and bioavailability of phenytoin and its major metabolite in horses

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**Objective**—To determine pharmacokinetics and excretion of phenytoin in horses.

**Animals**—6 adult horses.

**Procedure**—Using a crossover design, phenytoin was administered (8.8 mg/kg of body weight, IV and PO) to 6 horses to determine bioavailability (F). Phenytoin also was administered orally twice daily for 5 days to those same 6 horses to determine steady-state concentrations and excretion patterns. Blood and urine samples were collected for analysis.

**Results**—Mean ( $\pm$  SD) elimination half-life following a single IV or PO administration was  $12.6 \pm 2.8$  and  $13.9 \pm 6.3$  hours, respectively, and was  $11.2 \pm 4.0$  hours following twice-daily administration for 5 days. Values for F ranged from 14.5 to 84.7%. Mean peak plasma concentration ( $C_{max}$ ) following single oral administration was  $1.8 \pm 0.68$   $\mu$ g/ml. Steady-state plasma concentrations following twice-daily administration for 5 days was  $4.0 \pm 1.8$   $\mu$ g/ml. Of the  $12.0 \pm 5.4\%$  of the drug excreted during the 36-hour collection period,  $0.78 \pm 0.39\%$  was the parent drug phenytoin, and  $11.2 \pm 5.3\%$  was 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (p-HPPH). Following twice-daily administration for 5 days, phenytoin was quantified in plasma and urine for up to 72 and 96 hours, respectively, and p-HPPH was quantified in urine for up to 144 hours after administration. This excretion pattern was not consistent in all horses.

**Conclusions and Clinical Relevance**—Variability in F, terminal elimination-phase half-life, and  $C_{max}$  following single or multiple oral administration of phenytoin was considerable. This variability makes it difficult to predict plasma concentrations in horses after phenytoin administration. (*Am J Vet Res* 2001;62:483–489)

Phenytoin (5,5'-diphenylhydantoin) is used primarily as an anticonvulsant but can be effective for treating animals with various skeletal muscle disorders.<sup>1,3</sup> It also may be helpful in the management of horses with clinical signs of chronic intermittent rhabdomyolysis, myotonia, stringhalt, and hyperkalemic paralysis.<sup>1,4,8</sup> Information is limited about the range of effective plasma concentrations used in the treatment of these disorders in horses. Effective concentrations in the blood for the treatment of seizures in humans are

between 5 and 20  $\mu$ g/ml.<sup>9–11</sup> Phenytoin has a low therapeutic index, and when it is used in humans, monitoring of plasma concentrations is helpful in guiding treatment.<sup>12,13</sup> Phenytoin has low aqueous solubility and a low rate of absorption from the gastrointestinal tract. The rate of absorption of phenytoin is inconsistent and slow. After oral administration in humans, half the total absorption of phenytoin is during a 2-hour period, but the remainder is absorbed slowly during a 30-hour period. Total absorption of phenytoin in humans during a 33-hour period was 90%.<sup>14</sup> In contrast, bioavailability (F) of phenytoin in horses is reportedly approximately 34% of the total dose administered.<sup>15</sup> Binding of phenytoin to plasma protein in horses is approximately 78% at a concentration of 2.5  $\mu$ g/ml of plasma. Plasma protein binding is highest in humans and lowest in horses and ponies.<sup>16</sup> Interaction of phenytoin with many other compounds has clinical implications. There are a number of drugs such as valproic, salicylic acids, phenylbutazone, and some sulfonamides that displace phenytoin from plasma albumin.<sup>12</sup> Thus, clinical implications of concurrent administration of other drugs and phenytoin must be considered during treatment.

Following a single injection (8.8 mg/kg of body weight, IV) in horses, the terminal elimination-phase half-life ( $t_{1/2\beta}$ ) ranged from 5.8 to 13 hours.<sup>15</sup> Following a 14-day course of treatment (15 mg of phenytoin/kg, q 24 h), peak concentrations in some horses exceeded 15  $\mu$ g/ml. Peak plasma concentrations were detected between 1 and 8 hours after administration. Traces of phenytoin were still evident in blood samples collected 3 days after treatment had been discontinued.<sup>8</sup> Doses (15 to 25 mg/kg) administered daily were required to maintain plasma concentrations between 6 to 10  $\mu$ g/ml in a group of horses with chronic intermittent rhabdomyolysis and myotonia, as measured by a clinical analyzer.<sup>4</sup>

Two metabolites of phenytoin have been detected in horse urine. These include a major metabolite, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) and a minor metabolite, 5-(*m*-hydroxyphenyl)-5-phenylhydantoin.<sup>17</sup> With increased use of phenytoin in the management of racehorses with chronic intermittent rhabdomyolysis, there is a corresponding increase in the number of positive results when postrace urine samples are tested for phenytoin or its metabolites. This problem is a result of limited information on the pharmacokinetics, disposition, and elimination of phenytoin in horses; thus, a reasonable withdrawal time from administration prior to a race has not been established. To address the issue of withdrawal time for administration of phenytoin prior to a race, the study reported

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here was conducted to determine the pharmacokinetics, disposition, and elimination of phenytoin and its major metabolite in horses.

## Materials and Methods

**Animals**—Six healthy mares ranging from 8 to 12 years old and weighing (mean  $\pm$  SD)  $564 \pm 34$  kg were used in the study. The study was conducted in 2 parts: an experiment to determine F, which included single oral (PO) and intravenous (IV) administrations of phenytoin to the horses and a 5-day experiment that involved twice-daily oral administration of phenytoin to the same 6 horses. The University of Pennsylvania Institutional Animal Care and Use Committee approved the study protocol.

**Drug administration and collection of samples**—Values of F were determined by IV and PO administration of phenytoin (8.8 mg/kg). Horses were assigned to 3 groups (2 horses/group). In each group, 1 horse was randomly assigned to initially receive PO or IV administration, and the other horse in the group initially was given the drug by the other route of administration. Following a washout period of 3 weeks, phenytoin was given to each horse via the other route of administration.

Phenytoin formulation for IV administration was an aqueous solution containing 100 mg/ml.<sup>a</sup> Solution was administered slowly into a jugular vein during a 30-minute period to prevent ataxia that may result from rapid administration. For oral administration of phenytoin (8.8 mg/kg), capsules (100 mg/capsule<sup>b</sup>) were opened, and the contents were mixed with a small amount of warm water and molasses. This mixture was administered twice daily to horses, using an oral dosing syringe. Horses were allowed ad libitum access to hay and water. Grain was fed to the horses 1 hour before administration of the morning and evening doses.

Blood samples were collected into potassium-oxalate tubes,<sup>c</sup> using a 14-F catheter<sup>d</sup> inserted in the jugular vein contralateral to the jugular vein used for IV administration. Samples were collected immediately before (time 0) and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, and 96 hours after administration. Blood samples were centrifuged ( $2,500 \times g$  for 15 minutes) to obtain plasma. Three test tubes for each horse, each containing a 4-ml aliquot of plasma, were immediately frozen at  $-20$  C. Samples remained frozen until analyzed. The purpose for the multiple aliquots was to eliminate possible effects of freeze-thaw cycles on the concentration of phenytoin in the samples.

Urine was collected continuously by use of an indwelling 24-F self-retaining catheter<sup>e</sup> inserted in the bladder and attached to a drainage bag.<sup>f</sup> At 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 28, 32, and 36 hours after drug administration, urine was aspirated from the bladder, and the total volume collected during each collection period was measured. Urine samples also were collected 48, 72, and 96 hours after administration. Urine samples were separated into 50-ml aliquots and frozen at  $-20$  C until analyzed.

After a 3-week washout period the same horses were orally administered phenytoin (8.8 mg/kg of phenytoin, PO, twice daily for 5 days). Blood samples were collected 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 8, and 12 hours after administration of the first dose and immediately prior to administration of each subsequent dose. Following administration of the last dose (7 AM on day 5), blood samples were collected 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 6, 8, 12, 16, 20, 24, 28, 32, 36, 48, 72, 96, 120, and 144 hours after administration. Urine samples were collected starting 1 hour after the last dose. Blood and urine samples were processed as previously described.

**Analysis of samples**—Phenytoin was extracted from

plasma samples, using solid-phase extraction.<sup>17,g</sup> Extracts were evaporated to dryness at 70 C in a sample concentrator<sup>h</sup> under a slow stream of nitrogen gas and stored at 4 C until analyzed. Urine samples for quantification of phenytoin were extracted, using liquid-liquid extraction with ethyl ether and isopropanol. These extracts also were evaporated to dryness and stored at 4 C until analyzed. Urine samples were hydrolyzed, using  $\beta$ -glucuronidase,<sup>i</sup> for quantification of the phenytoin metabolite p-HPPH. Enzyme-hydrolyzed urine samples were extracted, using liquid-liquid extraction. Extracts were dried and stored at 4 C until analyzed. Dried extracts were reconstituted with 100  $\mu$ l of buffer before analysis.

Analyses of phenytoin and p-HPPH were performed, using a high-performance liquid chromatograph<sup>j</sup> equipped with an autosampler and appropriate software.<sup>k</sup> A commercially available column (ID,  $150 \times 4.6$  mm; particle size, 3.5  $\mu$ m; with a guard column [5  $\mu$ m; ID,  $12.5 \times 4.6$  mm])<sup>l</sup> was used for separation of phenytoin and its metabolites. Detection wavelength was  $210 \pm 4$  nm, injection volume was 20  $\mu$ l, and analysis run time was 20 minutes. The mobile phase comprised 2 previously mixed components: component A (20% acetonitrile plus 80% phosphate buffer solution [0.08M, pH 6.0]) and component B (80% acetonitrile plus 20% phosphate buffer solution [0.08M, pH 6.0]).

**Pharmacokinetic analysis**—Estimates of pharmacokinetic values after IV or PO administration of phenytoin were made by use of a nonlinear least-square regression. One or 2 exponential equations were modeled for the data, using a pharmacokinetic data analysis computer program.<sup>m</sup> Appearance of the curve, sum of squares, and the model selection criterion, which is a modification of the Akaike information criterion, were used to select the best fit for each compartment model.<sup>18,19</sup> Data points from each horse were modeled separately.

Data for oral administration were fitted, using an absorption and 1-compartment elimination model. The rate constant of appearance ( $\dot{A}$ ) of phenytoin in plasma from the gastrointestinal tract and its elimination from plasma ( $\dot{A}$ ) were calculated by use of the following equation:

$$C_p^t = -Ae^{-\dot{A}t} + Ee^{-\dot{A}t}$$

where A and E were the intercepts,  $\dot{A}$  and  $\dot{A}$  were the appearance and elimination phase rate constants, and  $C_p^t$  was the plasma concentration of phenytoin at specific time points (t).

Following IV administration of phenytoin, data points in each horse were fitted to a 2-compartment primary and secondary disposition model with a 0.5-hour IV infusion lag time, using the following equation:

$$C_p^t = Be^{-\alpha t} + Ce^{-\beta t}$$

where B was the primary disposition intercept, C was the secondary disposition intercept,  $\alpha$  was the primary disposition rate constant,  $\beta$  was the secondary disposition rate constant, and  $C_p^t$  was the plasma concentration of phenytoin at specific times (t). Half-lives were calculated as the natural log (base<sub>e</sub>) divided by the appropriate rate constant.

From 0 to 36 hours after administration, area under the curve (AUC) for IV ( $AUC_{IV}$ ) and PO ( $AUC_{PO}$ ) administration were calculated by use of the trapezoidal rule. The AUC from 0 to 36 hours was used to estimate the F of drug absorbed following oral administration, using the following equation:

$$F = \frac{AUC_{PO}}{AUC_{IV}}$$

Following IV administration, total body clearance ( $CL_T$ ) was calculated by use of the following equation:

$$CL_T = \frac{\text{dose}}{AUC}$$

and the specific volume of distribution ( $V_d$ ) was calculated by use of the following formula:

$$V_d = \frac{\text{dose}}{AUC \cdot \beta}$$

The maximum ( $C_{max}$ ) and minimum ( $C_{min}$ ) plasma concentrations and maximum plasma appearance time ( $T_{max}$ ) were derived from the fitted curve. Asymptotic regression of the cumulative urinary elimination of phenytoin was calculated, using the following first-order equation<sup>20</sup>:

$$Y = A(1 - e^{-\delta t})$$

where  $Y$  was the predicted cumulative elimination curve,  $A$  was the asymptotic point or the estimated total amount of drug excreted, and  $\delta$  was the first-order rate constant of cumulative urinary excretion.

**Statistical analysis**—The nonparametric Wilcoxon and Kruskal-Wallis rank-sum tests<sup>21</sup> were used for statistical comparisons<sup>n</sup> of various pharmacokinetic values of phenytoin. An ANOVA<sup>22</sup> was used for all other analyses. All values were expressed as mean  $\pm$  SD. Significance was designated at  $P < 0.05$ .

## Results

Mean and range of pharmacokinetic values obtained after a single IV or oral administration of phenytoin were calculated (Table 1). We did not detect significant differences in terminal elimination phase half-life ( $t_{1/2\beta}$ ) following IV administration, compared with the elimination-phase half-life ( $t_{1/2\lambda}$ ) after oral administration. Terminal elimination curves for IV and PO administrations were similar despite the differing routes of administration (Fig 1). There was a significant difference between  $AUC_{PO}$  and  $AUC_{IV}$ . The  $F$  was extremely variable (range, 14.5 to 84.7%; mean, 63.3  $\pm$  26.9%). This range in  $F$  was attributable to data for 1 horse; the  $F$  was only 14.5%, and exclusion of data for that horse resulted in less variability (range, 56.0 to 84.7%; mean, 73.0  $\pm$  14.0%). This range of values for  $F$  resulted in noticeable variation in the plasma concen-

Table 1—Values for pharmacokinetic variables following administration of phenytoin (8.8 mg/kg of body weight, IV and PO) to 6 horses

Variable	Mean $\pm$ SD	Range
<b>IV</b>		
$t_{1/2\alpha}$ (h)	0.39 $\pm$ 0.33	0.14–0.98
$t_{1/2\beta}$ (h)	12.6 $\pm$ 2.8	9.7–17.0
$C_{max}$ ( $\mu\text{g/ml}$ )	7.4 $\pm$ 3.5	2.9–12.55
$AUC_{IV}$ ( $\mu\text{g/h per ml}$ )	75.1 $\pm$ 20.3	44.9–91.56
$VD'$ (L/kg)	2.1 $\pm$ 0.38	1.4–2.4
$CL_T$ (ml/h per kg)	102.1 $\pm$ 32.4	86.6–166.1
<b>PO</b>		
$t_{1/2\lambda}$ (h)	2.6 $\pm$ 2.1	1.1–5.9
$t_{1/2\lambda}$ (h)	13.9 $\pm$ 6.3	5.9–21.4
$C_{max}$ ( $\mu\text{g/ml}$ )	1.8 $\pm$ 0.68	0.53–2.4
$T_{max}$ ( $\mu\text{g/ml}$ )	6.3 $\pm$ 2.4	4.3–10.2
$AUC_{PO}$ ( $\mu\text{g/h per ml}$ )	38.1 $\pm$ 14.7	13.3–57.9
$F$	63.3 $\pm$ 26.9	14.5–84.7

$t_{1/2\alpha}$  = Primary elimination-phase half-life.  $t_{1/2\beta}$  = Terminal elimination-phase half-life.  $C_{max}$  = Maximum plasma concentration.  $AUC$  = Area under the plasma concentration-time curve.  $VD'$  = Specific volume of distribution.  $CL_T$  = Total body clearance.  $t_{1/2\lambda}$  = Appearance phase half-life.  $t_{1/2\lambda}$  = Terminal elimination-phase half-life.  $T_{max}$  = Time of maximum plasma concentration.  $F$  = Fractional amount absorbed following oral administration.

tration of phenytoin following a single oral administration (Fig 2). Mean  $C_{max}$  for oral administration was 1.8  $\pm$  0.68  $\mu\text{g/ml}$ .

Oral administration for a 5-day period resulted in considerable variability in peak plasma concentration and time to peak plasma concentration (Fig 3). Peak plasma concentrations estimated from the asymptotic curve ranged from 1.6 to 6.3  $\mu\text{g/ml}$  (mean, 4.0  $\pm$  1.8  $\mu\text{g/ml}$ ). Value of the rate constant for the rate of rise of the asymptotic curve of the plasma concentration of phenytoin ranged from 0.03 to 0.26/h (mean, 0.10  $\pm$  0.10/h). This value represented an appearance-phase half-life ( $t_{1/2\lambda}$ ) that ranged from 2.6 to 22.5 hours (mean, 12.5  $\pm$  8.4 hours). Only data for 5 of the 6 horses could be modeled, using the asymptotic regression. By visually inspecting the 5-day absorption curves, it was evident that the time to steady-state plasma concentration was achieved in approximately 48 hours in the 6 horses. The  $t_{1/2\beta}$  following oral administration of

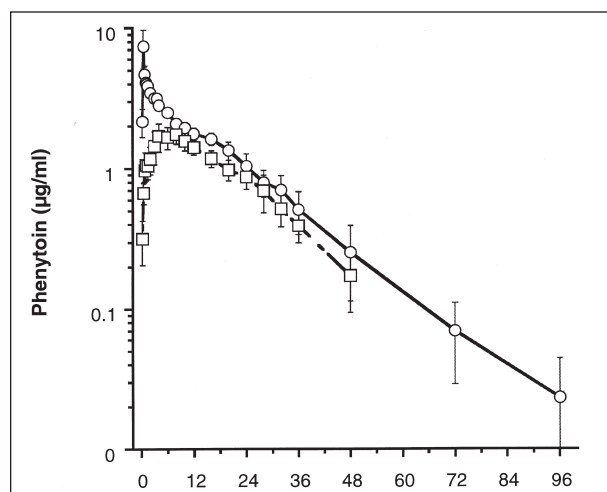


Figure 1—Mean  $\pm$  SD plasma concentrations of phenytoin after IV (O) and PO (□) administration (8.8 mg/kg of body weight) to 6 horses.

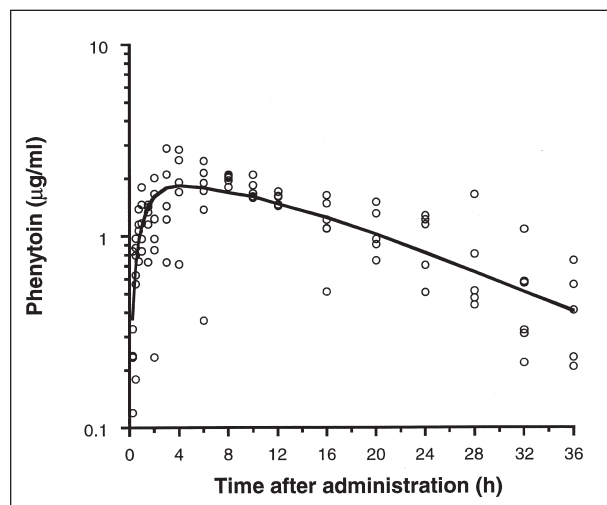


Figure 2—Plasma concentrations of phenytoin following a single oral administration of phenytoin (8.8 mg/kg) to 6 horses. The solid line is the line of best fit and the solution for the absorption and elimination model. The values for each of the 6 horses are indicated.

phenytoin for 5 days ranged from 5.6 to 15.7 hours (mean,  $11.2 \pm 4.0$  hours). This value was not significantly different from the  $t_{1/2\beta}$  and  $t_{1/2A}$  following a single IV or PO administration of phenytoin, respectively.

Times at which the plasma phenytoin, urinary

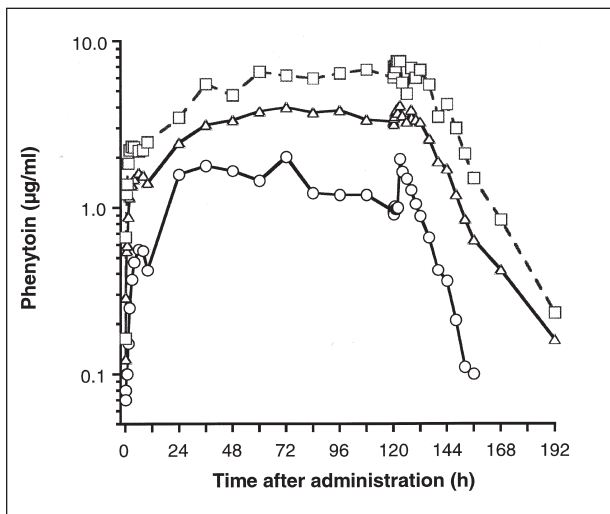


Figure 3—Mean ( $\Delta$ ), minimum (O), and maximum (G) plasma concentrations of phenytoin following administration (8.8 mg/kg, PO, q 12 h for 5 days) to 6 horses. The last dose of phenytoin was administered on the morning of day 5 (120 h).

phenytoin, and urinary p-HPPH for the single IV and PO administrations could be quantified during the 24- to 96-hour excretion period were determined (Table 2). Following the administration of phenytoin (8.8 mg/kg, q 12 h for 5 days), times at which plasma phenytoin, urinary phenytoin, and urinary p-HPPH could be quantified during the 24- to 144-hour excretion period also were determined (Table 3). The limits of quantification for plasma and urinary concentrations of phenytoin and the urinary concentration of p-HPPH were reached at 72, 96, and 144 hours, respectively (Fig 4).

The major product of phenytoin metabolism that was eliminated in the urine was p-HPPH. Of the total dose administered IV ( $4.9 \pm 0.48$  g),  $14.7 \pm 6.2\%$  was accounted for during the 36-hour collection period, of which  $0.89 \pm 0.22\%$  represented the parent drug phenytoin, and  $13.7 \pm 6.1\%$  represented p-HPPH. Accumulative elimination for IV administration was fitted to asymptotic regression to estimate the percentage of the total dose that was excreted. Value of the rate constant for rate of increase in the percentage of accumulation in the urine was 0.01 to 0.04/h (median, 0.013/h). This represented a half-life for the rate constant that ranged from 17.3 to 69.3 hours (median, 53.3 hours). Data for only 5 of the 6 horses could be fitted by use of this model; data for the other horse was fitted to a linear model. Estimated maximum excretion

Table 2—Mean  $\pm$  SD plasma and urinary concentrations of phenytoin and urinary concentrations of metabolite 5-(p-hydroxyphenyl)-5-phenylhydantoin (p-HPPH) from 24 to 96 hours after IV and PO administration of a single dose of phenytoin (8.8 mg/kg) to 6 horses

Route	Time (h)	Phenytoin		
		Plasma ( $\mu\text{g/ml}$ )	Urine ( $\mu\text{g/ml}$ )	p-HPPH in urine ( $\mu\text{g/ml}$ )
IV	24	$1.10 \pm 0.45$ (6)	$3.1 \pm 1.0$	$77.3 \pm 21.9$
	36	$0.47 \pm 0.30$ (6)	$1.4 \pm 1.2$ (6)	$36.4 \pm 16.1$ (6)
	48	$0.25 \pm 0.24$ (5)	$0.77 \pm 0.72$ (6)	$25.1 \pm 18.5$ (6)
	72	$0.14 \pm 0.01$ (2)	0.27 (1)	$8.5 \pm 9.5$ (5)
	96	0.09 (1)	BLQ	$4.7 \pm 4.9$ (2)
PO	24	$0.87 \pm 0.40$ (6)	$2.4 \pm 1.2$	$72.6 \pm 34.4$
	36	$0.39 \pm 0.23$ (6)	$1.10 \pm 0.80$ (6)	$32.4 \pm 15.6$ (6)
	48	$0.26 \pm 0.18$ (6)	$1.0 \pm 0.93$ (5)	$31.3 \pm 15.2$ (5)
	72	BLQ	$0.34 \pm 0.11$ (3)	$16.6 \pm 15.9$ (4)
	96	BLQ	$0.11 \pm 0.02$ (2)	$6.0 \pm 3.9$ (3)

Numbers in parentheses indicate No. of horses for each time point after administration at which phenytoin and p-HPPH was quantified.  
BLQ = Below limit of quantification.

Table 3—Mean  $\pm$  SD plasma and urinary concentrations of phenytoin and urinary concentrations of p-HPPH from 24 to 144 hours after administration of the last dose of phenytoin (8.8 mg/kg, PO, q 12 h for 5 days) to 6 horses

Time (h)	Phenytoin		
	Plasma ( $\mu\text{g/ml}$ )	Urine ( $\mu\text{g/ml}$ )	p-HPPH in urine ( $\mu\text{g/ml}$ )
24	$1.71 \pm 1.41$ (6)	$3.69 \pm 2.17$ (6)	$95.5 \pm 40.3$ (6)
36	$0.64 \pm 0.60$ (6)	$1.3 \pm 1.2$ (6)	$25.7 \pm 12.2$ (6)
48	$0.42 \pm 0.35$ (4)	$0.90 \pm 1.1$ (6)	$24.9 \pm 13.6$ (6)
72	$0.16 \pm 0.10$ (2)	$0.34 \pm 0.28$ (2)	$6.3 \pm 5.1$ (6)
96	BLQ	$0.10 \pm 0.20$ (2)	$4.6 \pm 1.7$ (3)
120	BLQ	BLQ	$3.7 \pm 0.72$ (3)
144	BLQ	BLQ	2.1 (1)

See Table 2 for key.

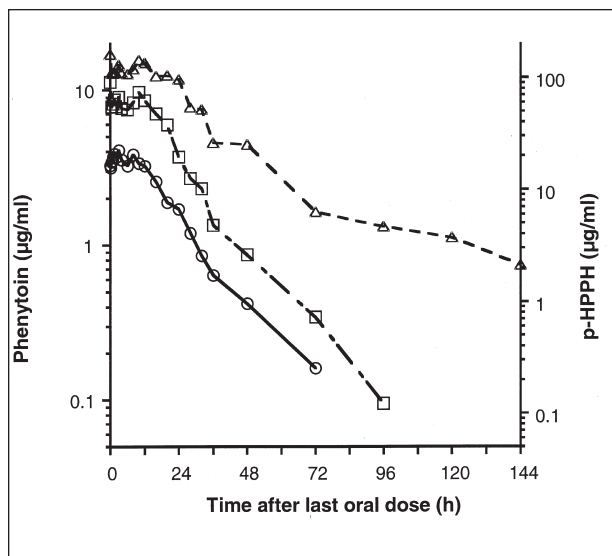


Figure 4—Plasma (O) and urinary (□) concentrations of phenytoin and urinary concentrations of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin metabolite (Δ) following administration of phenytoin (8.8 mg/kg, PO, q 12 h for 5 days). Time 0 is the time of administration of the last dose of phenytoin. Notice the differences in the decrease in plasma and urinary concentrations of phenytoin and urinary concentration of the metabolite.

of the total dose of phenytoin ranged from 21 to 45% (median, 34%). We did not reliably detect the metabolite 5-(*m*-hydroxyphenyl)-5-phenylhydantoin in quantifiable concentrations in the samples.

Of the total PO dose ( $5.0 \pm 0.28$  g) administered,  $12.0 \pm 5.4\%$  was accounted for during the 36-hour collection period. Of the  $12.0 \pm 5.4\%$  excreted,  $0.78 \pm 0.39\%$  was contributed by the parent drug phenytoin and  $11.2 \pm 5.3\%$  was contributed by p-HPPH. The accumulative urinary elimination of oral administration could not be fitted to an asymptotic regression, and, thus, the data were fitted to a linear regression. Based on a linear fit, a plateau was not achieved. For this reason, the total excretion of phenytoin and p-HPPH could not be correctly estimated. The median value of the linear regression slope was 0.33/h with a range of 0.06/h to 0.65/h. The median  $r^2$  was 0.97. Limit of detection for phenytoin, p-HPPH, and m-HPPH was 0.1  $\mu\text{g/ml}$  in plasma and urine and 0.5  $\mu\text{g/ml}$  for p-HPPH in urine.

## Discussion

Phenytoin is effective in the treatment of horses with various musculoskeletal disorders including chronic intermittent rhabdomyolysis.<sup>1,4,8</sup> Despite its clinical use in horses, effective plasma concentrations required for treatment of chronic intermittent rhabdomyolysis are not known. The therapeutic dose for treatment of humans with seizures is 15  $\mu\text{g/kg}$ , and serum concentrations reach therapeutic concentrations in approximately 7 hours. The theoretical oral loading dose in humans to achieve a serum concentration of 15  $\mu\text{g/ml}$  is 18.7 mg/kg in males and 24.8 mg/kg in females.<sup>11</sup> A dose of 15  $\mu\text{g/kg}$  administered to horses for 14 days achieved a peak concentration of 15  $\mu\text{g/ml}$ .<sup>8</sup> The dose administered in the study reported here was 8.8 mg/kg, which is approximately 5 g/horse. Follow-

ing the administration of phenytoin at the rate of 8.8 mg/kg twice daily for 5 days, peak plasma concentrations were extremely variable and ranged from a low steady-state value of approximately 1  $\mu\text{g/ml}$  to a high value of 6  $\mu\text{g/ml}$  (Fig 3). This variability in plasma concentrations over a 5-day period reflects the wide range of *F* of the drug (Table 1). We do not have an immediate explanation for the variability in absorption of phenytoin in this group of horses. All horses received the phenytoin 1 hour after being fed grain; horses were allowed ad libitum access to hay and water, and other medications were not administered. Nutritional formulations and medications that can affect absorption from the gastrointestinal tract<sup>12</sup> were not relevant in our study. Similar variability in horses has been reported in another study.<sup>15</sup>

Elimination of phenytoin in urine of horses represents < 1% of the total dose of phenytoin administered. A similar observation was made in humans; only 5% of the phenytoin administered was excreted as the parent compound.<sup>23</sup> On the basis of asymptotic regression, only 34% of the medication was accounted for in the urine of horses in our study, of which only 14.2% was accounted for in the first 36 hours after oral administration. The prolonged elimination phase for phenytoin supports the long withdrawal time required following the therapeutic use of phenytoin in racehorses. The p-HPPH metabolite accounted for most of the urinary excretion of phenytoin as a glucuronide conjugate. Although the minor metabolite identified in humans (ie, 5-[*m*-hydroxyphenyl]-5-phenylhydantoin) also was identified in horse urine, the concentration was too low for reproducible and reliable quantification to be performed.

Excretion of phenytoin in horses was approximately 1%, compared with that of p-HPPH. On the basis of the cross-reactivity between phenytoin and p-HPPH, it is safe to assume that the results of the ELISA used to screen urine samples reflects the detection of p-HPPH more so than of phenytoin. This is in contrast to plasma in which p-HPPH was not quantified, and results of the ELISA reflect detection of the parent drug. In the 6 horses orally administered phenytoin for 5 days, phenytoin was quantified in plasma samples of all 6 horses 36 hours after administration, but it was only detectable in plasma samples of 4 horses 48 hours after administration and 2 horses 72 hours after administration (Table 3). In contrast to phenytoin, p-HPPH was quantified in urine in 1 horse up to 144 hours after administration.

Phenytoin has a low therapeutic index and a narrow therapeutic range; therefore, drug interactions leading to alterations in plasma concentrations may be clinically important because of the effect it may have on plasma concentration and clearance. A number of interactions have been reported in humans, including cases of phenytoin intoxication or decreased effectiveness after administration.<sup>12,13</sup> Phenytoin also reportedly alters the pharmacokinetics of a large number of drugs. The majority of these interactions arise because phenytoin is a potent inducer of cytochrome P450 microsomal enzyme activity.<sup>23</sup> Because of this property, phenytoin may increase the clearance of drugs that are exten-

sively metabolized by the cytochrome P450 pathway. Although studies have not been conducted in horses to document enzyme induction by phenytoin, drugs such as prednisolone, dexamethasone, and theophylline may be affected. In humans, it has been reported that when phenytoin is administered, the dose of dexamethasone has to be increased to maintain a specific plasma concentration.<sup>24</sup>

There are a number of drugs that may alter the metabolism of phenytoin, and these drugs may be important when considering clearance. The H<sub>2</sub>-antagonist cimetidine can increase plasma concentrations of phenytoin when administered to humans that are orally receiving a stabilized dose of phenytoin.<sup>25,26</sup> A reduction of 12 to 16% in the clearance of phenytoin from plasma has been reported.<sup>27</sup> On the other hand, the H<sub>2</sub>-antagonists ranitidine and famotidine do not affect the steady-state plasma concentrations of phenytoin,<sup>27-29</sup> suggesting that the effect of cimetidine on the half-life of phenytoin is specific for each drug, rather than specific for a class of drugs. In humans, 5 days of treatment with phenylbutazone increased the half-life of a single dose of phenytoin administered IV.<sup>30</sup> Phenylbutazone also increased total unbound serum concentrations of phenytoin. On the other hand, another nonsteroidal anti-inflammatory agent, ibuprofen, did not affect the clearance of phenytoin.<sup>31</sup> Administration of sulfamethizole increased the half-life of phenytoin.<sup>32</sup> Subsequent to this observation, it was found that sulfaphenazole, sulfadiazine, sulfamethazole, and trimethoprim also increased the elimination half-life of phenytoin.<sup>33</sup> Similarly, it was found that chloramphenicol increased the half-life of phenytoin.<sup>34</sup> Thus, chloramphenicol and sulfonamides inhibit the metabolic transformation of phenytoin resulting in decreased clearance and increased half-life. Similar observations were made that indicate the administration of chloramphenicol to patients concurrently receiving phenytoin can result in phenytoin intoxication.<sup>35</sup> Dietary-induced hyperlipidemia can increase the elimination of phenytoin from plasma.<sup>36</sup> Folic acid is a cofactor in phenytoin metabolism, and a depletion of folic acid results in a higher total phenytoin concentration and longer time to reach steady-state concentrations.<sup>37,38</sup> Differences have been documented in F of phenytoin following administration of various forms of the drug. Serum phenytoin concentrations were 22 to 31% lower than expected with the intake of generic phenytoin, compared with concentrations in the same humans that were given a name-brand formulation of the drug.<sup>39</sup>

Analysis of the aforementioned studies indicated that the primary effect of those drugs on phenytoin may be on its metabolism instead of on plasma binding. Although to our knowledge similar studies on the interaction of phenytoin with other medications have not been reported in horses, it should be mentioned that when determining the withdrawal time following the administration of phenytoin in horses concurrently administered phenylbutazone and, possibly, sulfonamides, which are medications commonly used by equine practitioners, effects of interactions with those drugs should be considered.

Rules on use of therapeutic medications for many

racing jurisdictions are based on urine samples as the primary sample for testing. However, concentration of a therapeutic medication in the urine may not be a reflection of its pharmacologic importance. Because of the long elimination phase of phenytoin, which is related to the metabolite p-HPPH and not the parent drug, and the current screening methods that do not distinguish between urinary concentrations of phenytoin and p-HPPH, the use of plasma, rather than urine, for detecting phenytoin should be considered as the basis for determining violations. Information on plasma concentrations and therapeutic efficacy of phenytoin in a large group of horses is not available. For this reason, the plasma concentration at which the medication is no longer pharmacologically effective is not known. On the basis of its low therapeutic index and results of studies in horses,<sup>4,7,8</sup> it is suggested that high plasma concentrations may be necessary to be therapeutically effective. It may be reasonable to assume that lack of detection and confirmation of phenytoin or its metabolites in plasma could serve as an index of an adequate withdrawal time prior to racing for horses receiving phenytoin, despite the detection of p-HPPH or phenytoin in urine samples.

<sup>a</sup>Dilantin (Intravenous), Elkins-Sinn, Cherry Hill, NJ.

<sup>b</sup>Dilantin (Oral), Mylan Pharmaceuticals, Morgantown, WV.

<sup>c</sup>Vacutainer, Becton-Dickinson Co, Rutherford, NJ.

<sup>d</sup>Angiocaths, Becton-Dickinson, Sandy, Utah.

<sup>e</sup>Foley Catheters, CR Bard Inc, Covington, Ga.

<sup>f</sup>Bard center-entry urinary drainage bag, CR Bard Inc, Mullica Hill, NJ.

<sup>g</sup>Benchmate II automated solid phase extraction system, Zymark, Hopkinton, Mass.

<sup>h</sup>Techne Dri-Block, Techne Cambridge Ltd, Cambridge, UK.

<sup>i</sup> $\beta$ -glucuronidase, Sigma Chemical Co, St Louis, Mo.

<sup>j</sup>1090 High-performance liquid chromatograph, Hewlett-Packard Co, San Bernardino, Calif.

<sup>k</sup>ChemStation Software, Hewlett Packard Co, San Bernardino, Calif.

<sup>l</sup>Eclipse XDB-C8, MAC-MOD Analytical, Chadds Ford, Pa.

<sup>m</sup>P-K analyst MicroMath Scientific Software, Salt Lake City, Utah.

<sup>n</sup>JMP version 3 software for statistical visualization, SAS Institute Inc, Cary, NC.

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