

## THE PHARMACOLOGY OF FUROSEMIDE IN THE HORSE

### V. Pharmacokinetics and Blood Levels of Furosemide after Intravenous Administration

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#### ABSTRACT:

Studies were undertaken to determine blood levels of furosemide in horses after 0.5- and 1.0-mg/kg doses administered iv. Analyses indicated that the pharmacokinetic parameters were dose independent and best described by a three-compartment open model. The  $\alpha$ -,  $\beta$ -, and  $\gamma$ -phase half-lives of 5.8, 22.3, and 158.5 min, respectively, were observed after the 0.5-mg/kg dose. Similarly, the respective half-lives after the 1.0-mg/kg dose were 5.8, 24.1, and 177.2 min. After a 0.5-mg/kg dose of furosemide, population frequency distributions were evaluated at 1 hr and 4 hr post-drug administration. At 1 hr after dosing, the blood levels of furosemide in 30 horses were normally distributed. The mean plasma level was

97.9 ng/ml with a range of 41.9 ng/ml to 155.8 ng/ml and a SD of 25.0 ng/ml. Analyses of blood levels of furosemide in 47 horses at 4 hr after drug administration indicated that the population distribution was better fit by a normal curve after log transformation of the values. The mean plasma level at 4 hr post-dosing was 9.6 ng/ml with a range of 4.0 ng/ml to 19.4 ng/ml and a SD of 3.1 ng/ml. Based on this population distribution of the plasma levels, the probability of finding furosemide plasma concentrations above 24.6 ng/ml 4 hr after anti-epistaxis dose was estimated at less than 1 in 100.

Furosemide (Lasix), a potent, high ceiling diuretic, is currently the drug of choice for the prophylactic treatment of epistaxis or EIPH<sup>2</sup> in the horse (1). Historically, the incidence of EIPH in horses has been reported as low as 2.5 to 5% (2). However, recent surveys using fiberoptic endoscopy have indicated that at least 43% of racing horses have some degree of this condition (3). Occurrence of EIPH during a race can cause the affected horse to slow or stop abruptly, posing a serious threat to horses and jockeys in a tightly packed field. In an effort to control this condition during races, many racing commissions have permitted the pre-race use of furosemide.

The principal objection to the pre-race use of furosemide is associated with the diuretic response. This diuresis may result in the dilution of illegal drugs and drug metabolites in post-race urine samples, rendering their detection more difficult. Approval of furosemide without controls on its use may therefore make the task of the racing chemist more difficult.

One approach to obviating furosemide's interference with drug screening is to avoid urine collection during the period of diuresis.

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<sup>2</sup> Abbreviations used are: EIPH, exercise-induced pulmonary hemorrhage; AIC, Akaike's Information Criterion.

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Studies have shown that furosemide-induced diuresis has a rapid onset and decline (4). After prophylactic doses of furosemide (0.5 mg/kg) administered iv, the diluting effects of furosemide on the levels of drugs and glucuronide metabolites in the urine are essentially complete within 3 hr of dosing (5). Therefore, furosemide administration at prophylactic doses to within 4 hr of post-race urine samples would result in no effective dilution of post-race urine samples. This procedure has also been suggested by the Veterinary Chemist's Advisory Committee to the National Association of State Racing Commissioners (6).

The currently accepted method of enforcing time rules for furosemide is by means of a detention barn system. In this system, horses to be treated with furosemide are stabled in a secure barn about 5 hr before race time. Furosemide is administered at the approved dose and time pre-race under close and maintained supervision. Although highly visible and effective, such systems are expensive and may not be justified by the magnitude of the drug-diluting effect observed in practice (1). An alternate and less expensive approach to enforcing compliance with a "4-hr" furosemide rule could be to designate a plasma "tolerance level" of furosemide, above which there would be a substantial probability of violation of the time rule. The frequency distribution of furosemide plasma levels at 4 hr after administration of the anti-epistaxis dose can be used as the basis for determining this tolerance level.

#### Materials and Methods

Horses. Thoroughbred and half-thoroughbred mares, geldings, colts, and fillies were used in these experiments. The mares and geldings were broodmares, while the colts and fillies were in active training. The horses were brought into individual box stalls before all experimental procedures. Six horses were used for the 1.0-mg/kg furosemide iv kinetic study. Four horses were used to study the kinetics after a 0.5-mg/kg dose. After iv administration of 0.5 mg/kg furosemide, 30 horses were sampled at 1-hr post-dosing and 47 horses were sampled at 4 hr post-dosing.

**Drug Administration.** Pre-drug blood samples were drawn from all horses after which they were dosed with furosemide (Lasix, National Laboratories Corp., subsidiary of American Hoechst Corp., Somerville, N. J.) by rapid iv injection *via* the right jugular vein. Care was taken to insure that the whole dose was injected iv. All subsequent blood samples were drawn from the left jugular vein into 15-ml evacuated heparinized tubes (Becton-Dickinson, Rutherford, N. J.). After centrifugation at 1150 g, 5°C, for 15 min, the plasma was removed and stored in 10-ml aliquots at -10°C until assayed. To determine the frequency distributions of plasma levels after 0.5 mg/kg furosemide, blood samples were drawn from the horses at grass at 1 and 4 hr post-dosing. Horses in training were exercised at about 3 hr after dosing and blood samples were drawn at 4 hr after dosing. For the pharmacokinetic studies, doses of 1.0 mg/kg and 0.5 mg/kg furosemide were administered on separate occasions. Blood samples were then collected at 3, 6, 9, and 20 min and at 1, 2, 3, 4, 5, 6, 7, and 8 hr after dosing.

**Analytical Method.** The analytical method was modified from that of Roberts *et al.* (7) and is based on the method of Lindstrom and Molander (8) with a recovery of approximately 80% when spiked plasma was compared to standards in methanol added directly to the methylating system.

All samples were analyzed in duplicate. Standards were prepared by using dilutions of a 0.1 mg/ml furosemide stock solution dissolved in distilled water with the pH adjusted to 9.5 with concentrated ammonium hydroxide. Appropriate dilutions of furosemide were added to 1-ml aliquots of blank plasma to achieve a concentration range of 2.0 to 50 ng/ml. Standard curves to cover our working range were run with each assay. Early plasma samples were diluted with blank plasma to reduce their concentration to within the linear portion of our working range and to maintain a consistent background. Control plasmas from individual horses, standards, and plasma samples were extracted by using 1 N HCl and 5.0 ml dichloromethane. All samples were mixed at room temperature for 3 min, then centrifuged at 1150g, 5°C, for 30 min to achieve phase separation. After centrifugation, the aqueous layer was aspirated and discarded. The proteinaceous plug was dislodged by using a glass pipette and the dichloromethane phase transferred to a clean screw-cap tube. The organic phase was then evaporated to dryness under a stream of prepurified N<sub>2</sub>. After evaporation, 2 ml of 0.2 M NaOH, 5 ml of 0.5 M methyl iodide (Fisher Scientific Company, Fair Lawn, N. J.) in dichloromethane, and 50 µl tetrahexylammonium hydrogen sulfate in dichloromethane were added to the residue in each tube. The samples were then incubated by shaking horizontally in a shaker-waterbath at 50°C for 25 min. After removal of the aqueous phase, the dichloromethane layer was transferred to clean screw-cap tubes and evaporated to dryness under a stream of prepurified N<sub>2</sub>. Hexane (2.0 ml) and 2.0 ml of 0.5 M NaOH were added to the residue in each tube. The derivatized samples were then mixed at room temperature for 5 min and allowed to stand overnight at 10°C. One milliliter of the hexane layer was transferred to a clean screw-cap tube. Before analysis on the gas chromatograph, each sample was evaporated to dryness under a stream of prepurified N<sub>2</sub> and redissolved in 50 µl of hexane. After vortexing for 15 sec, a 2-µl sample was injected on a Varian 2700 gas chromatograph equipped with a 6-foot column packed with 3% OV-101, and a <sup>63</sup>Ni electron capture detector. The operating conditions included: column temperature of 265°C, detector temperature of 310°C, injector temperature 300°C, and a nitrogen flow of 31 ml/min.

**Statistical Analysis.** The frequency distributions at 1 and 4 hr after a 0.5-mg/kg dose of furosemide were analyzed for normalcy by using the Shapiro-Wilk's statistic. After the best-fit transformation was determined, the curve was estimated by using the methods of moments based on the calculated mean and SD. This distribution curve was then used to determine the plasma levels below which 99.9% of the population was included. This data then served as the basis for determination of plasma tolerance levels for compliance with a "4-hr furosemide rule."

**Kinetic Analysis.** Individual and mean data were fitted by the NONLIN computer program to a sum of exponentials by using both two and three exponential terms (9). The model which best described the data was chosen based on the application of the AIC (10). The values obtained from the computer analysis were then used to calculate kinetic parameters that were dependent on the number of exponents chosen to best describe the plasma concentration time curve and those independent of such assignment. These

parameters were calculated based on both individual and mean values of A, B, C, and  $\alpha$ ,  $\beta$ ,  $\gamma$  (11, 12).

**Results**

The mass spectrum of plasma sample (3 min after a 1.0-mg/kg dose of furosemide) is shown in fig. 1, confirming the presence of furosemide as the derivative methylated at the carboxylic and sulfonamide moieties. The molecular ion at 372 and the observed fragmentation pattern is consistent with the structural formula for trimethylfurosemide.

Figure 2 shows typical gas chromatograms obtained from methylated furosemide in spiked plasma. The data show a linear increase in detector response with the addition of increasing

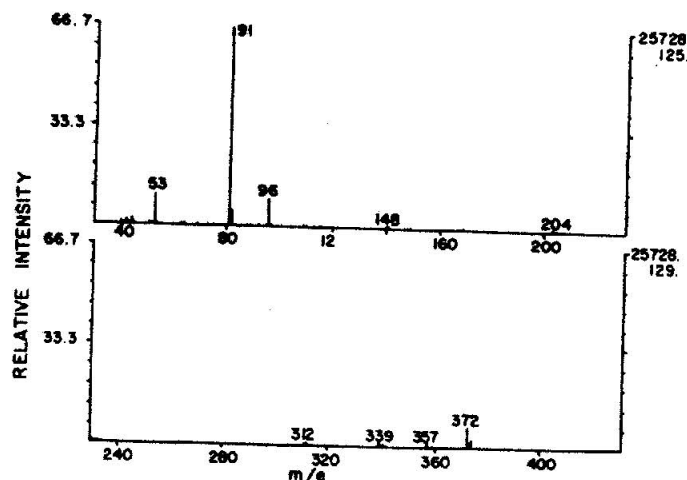


FIG. 1. The mass spectrum of trimethyl furosemide from an equine plasma (3 min after a 1.0-mg/kg dose of furosemide).

The molecular ion present at 372 is consistent with the m.w. of trimethyl furosemide.

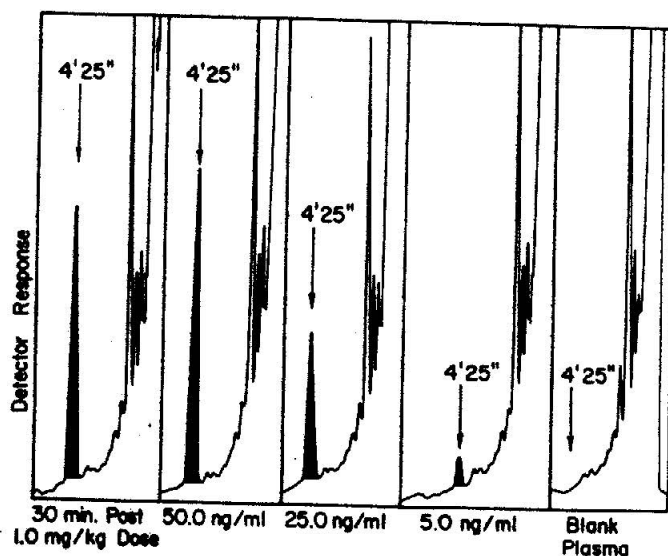


FIG. 2. Detection of furosemide on a gas chromatograph equipped with an electron capture detector.

Blank plasma, plasma spiked with furosemide and plasma from a horse dosed with 1.0 mg/kg furosemide were analyzed for furosemide as described in Materials and Methods. The solid peaks represent furosemide which had retention time of 4 min and 25 sec under these conditions. Increasing the concentrations of spiked furosemide yielded increasing detector responses.

amounts of furosemide. A plasma sample (obtained 30 min after a 1.0-mg/kg dose) detected by electron capture detection is shown in the far left panel.

Figure 3 shows a standard curve of furosemide added to 1 ml of horse plasma and analyzed by gas chromatography with electron capture detection. The detector response was quantitated by peak area in  $\text{mm}^2$  with a mean range of 10.6  $\text{mm}^2$  to 288.5  $\text{mm}^2$

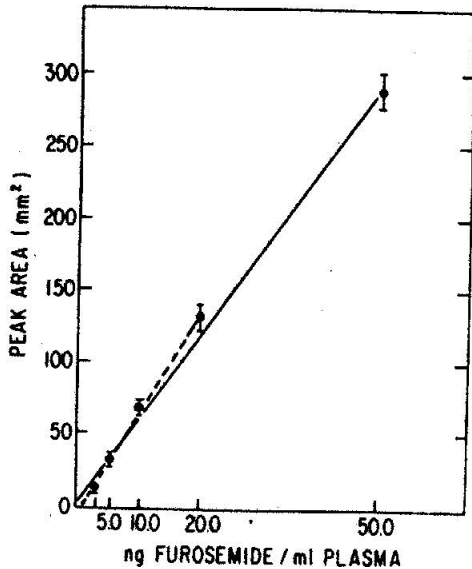


FIG. 3. Standard curve of furosemide extracted from horse plasma.

The indicated amounts of furosemide were added to the plasma. The solid circles (●—●) represent the detector response to the added amounts of furosemide as the mean of five experiments  $\pm$  SE.

( $N = 5$ ). The standard curve is fit by a least squares regression line with a correlation coefficient of 0.990 and a positive intercept on the vertical axis of 4.15. Fitting the curve to the points below 21 ng/ml resulted in a regression line with a correlation coefficient of 0.9980 and a negative intercept on the vertical axis of 1.56. The extrapolated value at the origin was 0.24 ng/ml. To minimize the error when extrapolating the data points, a minimum of four standard points closest in peak area to the unknown were used for determination of concentration. Diluting samples with blank plasma as described in the Materials and Methods section also facilitated control in keeping the concentrations in a linear portion of our curve. There was no significant difference found between the sample duplicates when evaluated by a paired  $t$  test ( $\alpha = 0.01$ ,  $t = 2.21$ ). The accuracy of the technique was also reproducible between standard curves. The SE for the standard points indicated in fig. 3 reflects this, representing less than 5% of the plasma concentration. Variability was 8% of the plasma concentration at the lower levels of detection (2 ng/ml).

Furosemide (1.0 mg/kg) was administered to six horses (415–577 kg) by rapid iv injection. The mean furosemide plasma levels are shown in fig. 4 A. The mean plasma level at 3 min after dosing was 7426 ng/ml and fell in a curvilinear fashion to a mean level of 3.0 ng/ml at 8 hr after dosing. At 4 hours, the mean plasma level of furosemide was  $11.8 \pm 1.4$  ng/ml. These results are in good agreement with those previously reported by Roberts and co-workers (7), whose data are represented in fig. 4 A by the open circles.

In the prophylaxis of epistaxis in race horses, 0.5 mg/kg iv is the dose most commonly used. We therefore dosed four horses with 0.5 mg/kg furosemide iv and followed plasma levels of the drug for 8 hr. As shown in Fig 4 B, plasma levels of furosemide were approximately 5306 ng/ml at 3 min after dosing. Thereafter,

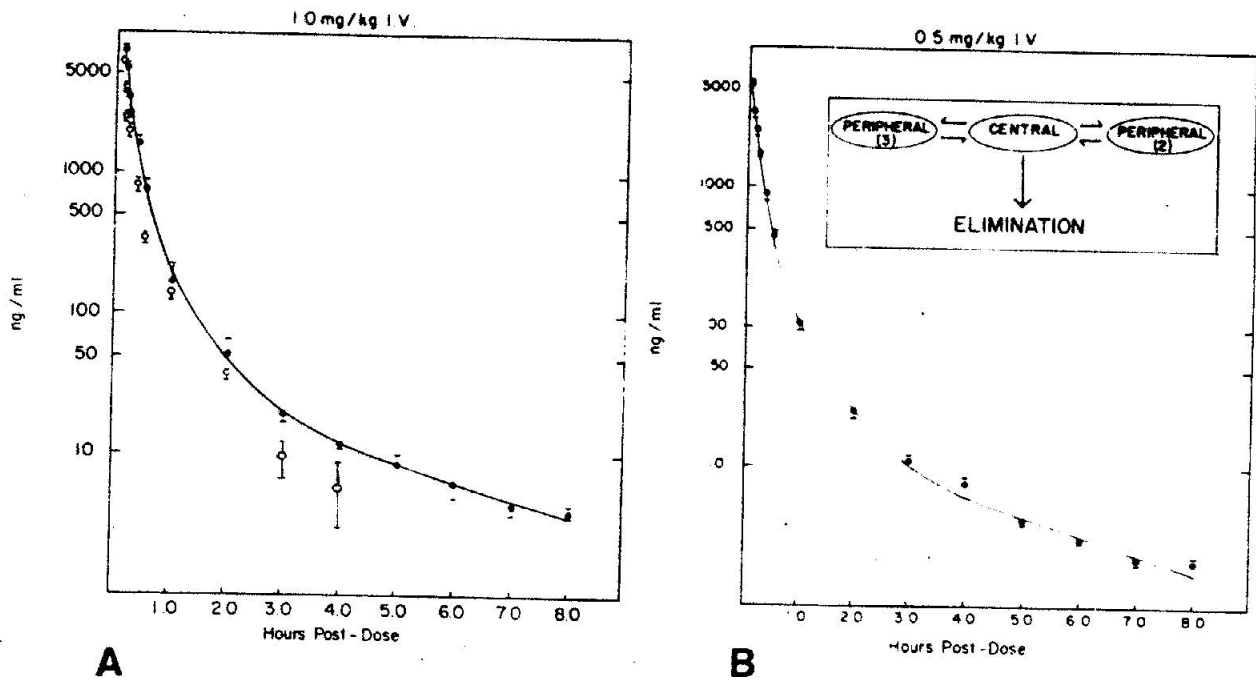


FIG. 4 A. Plasma levels of furosemide after 1 mg/kg iv.

The solid circles (●—●) are plasma levels of furosemide in six horses after rapid iv administration of 1 mg/kg furosemide. The open circles (○—○) show data in five similarly treated horses, as reported by Roberts *et al.* (7). The vertical bars represent the SE of the mean. The solid curve is the best fit curve generated by a nonlinear regression analysis.

FIG. 4 B. Mean plasma levels of furosemide in four horses after a 0.5 mg/kg dose iv.

The solid circles (●—●) are plasma levels of furosemide after 0.5 mg/kg in four horses.

plasma levels of the drug fell in a curvilinear fashion to about 3 ng/ml at 8 hr after dosing (solid circles, ●—●).

The solid curve in fig. 4 A and B represents the best fit curve when these data were assessed by nonlinear regression analyses.

Based on the calculated plasma concentrations from the regression analyses, the values for A, B, C, and  $\alpha$ ,  $\beta$ , and  $\gamma$  (table 1) were derived. Both individual and mean data were evaluated and the mean of the kinetic parameters from individual animals are reported  $\pm$  SE in table 1. Comparisons for statistical significance between the data (0.5 mg/kg and 1.0 mg/kg) were done with a two-sample *t* test ( $\alpha = 0.01$ ). The *t* values are reported as absolute values in table 1.

The data of fig. 5 show the frequency distribution of furosemide plasma levels ( $N = 30$ ) at 1 hr after a 0.5-mg/kg dose. The range was 41.9 to 155.8 ng/ml, with a mean of 97.9 ng/ml. At 1 hr after dosing, the furosemide plasma levels were normally distributed, with a Shapiro-Wilk's statistic of  $p < 0.989$ .

The data of fig. 6 A show plasma levels of furosemide in 47 horses at 4 hr after a 0.5-mg/kg dose. The mean plasma level was 9.6 ng/ml, with a range of from 4.0 to 19.4 ng/ml. These data poorly fit a normal distribution, with a Shapiro-Wilk's statistic of  $p < 0.01$ .

When these data were replotted as frequency against log-concentration of drug in plasma (fig. 6 A), the data much better fit a normal distribution, with a Shapiro-Wilk's statistic of  $p < 0.053$ . These data suggest that at 4 hr after drug administration, the distribution of plasma levels obtained in these horses were log-normal.

Based on the assumption that plasma levels of furosemide are log-normally distributed, the probability of an anti-epistaxis dose of furosemide yielding plasma levels above a certain level can be readily calculated. These data are presented in table 2, and show that the probability of an anti-epistaxis dose of furosemide yielding levels of furosemide above 24.6 ng/ml is less than 1 in 1000. Similarly, the probability of plasma levels above a series of different drug concentrations are also presented (table 2).

Discussion

This work confirms and extends the earlier work of Roberts *et al.* (7). Modification of Roberts' method improved the sensitivity of the analytical method about 10-fold and allowed detection of furosemide in equine plasma for up to 8 hr postadministration. The basic characteristics of the detection method, however, are

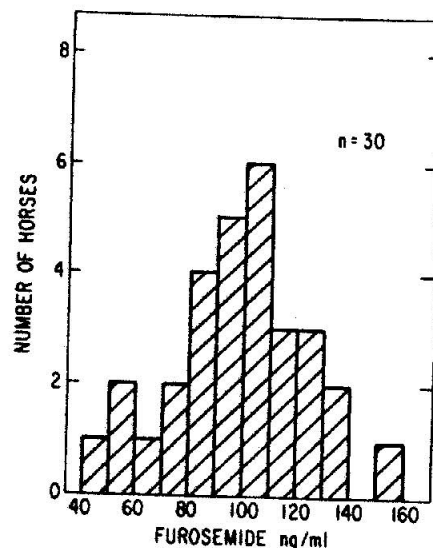


FIG. 5. Furosemide plasma levels in 30 horses 1 hr after iv administration of 0.5 mg/kg furosemide.

The vertical bars represent the number of horses found within the indicated ranges of furosemide plasma levels.

those described by Roberts *et al.* (7) and earlier by Lindstrom and Molander (8). The standard curve is linear over small concentration ranges, and the intercept at the foot of the vertical axis is not significantly different from zero.

Analyzing their data, Roberts *et al.* (7) suggested a two-compartment model for furosemide distribution in the horse after its iv injection. Analyzing our data, we elected to use a nonlinear least squares regression analysis with a  $1/y^2$  weighting (NONLIN Program) to evaluate the plasma concentration-time curve after both the 1.0-mg/kg and 0.5-mg/kg doses of furosemide. Using the AIC, triexponential rather than biexponential equations were found to better fit the data. Elimination in this model is from the central compartment as shown in the inset to fig. 4 B. For the 1.0-mg/kg dose, the following equation best described the data:  $C = 9148.6e^{-0.1330t} + 1016.2e^{-0.0320t} + 32.9e^{-0.0044t}$ . Similarly after the 0.5-mg/kg dose, the following equation best described the data:  $C = 6063.8e^{-0.13t} + 675.7e^{-0.033t} + 21.6e^{-0.0047t}$  where A, B, and G are expressed in nanograms per milliliter and  $\alpha$ ,  $\beta$ , and  $\gamma$  are expressed in minutes<sup>-1</sup>.

The  $\alpha$ - and  $\beta$ -phase half-lives were in good agreement with work previously done in the horse which reported  $\alpha$ - and  $\beta$ -phase half-lives after a 1.0-mg/kg dose of furosemide of 5 min and 31.5 to 38.6 min, respectively (7). Similar  $\beta$ -phase half-lives of 30 min and 26 min have also been reported in human volunteers whose furosemide levels were determined fluorometrically (13, 14). Our studies indicated that there were no statistically significant differences (two-sample *t* test  $\alpha = 0.01$ , see table 1) in any of the kinetic parameters between the two doses administered.

The volume of the central compartment ( $V_c$ ) was 0.074 liter/kg at the 0.5-mg/kg dose and 0.22 liter/kg for the 1.0-mg/kg dose. These findings correlate well with what has previously been reported in the horse (7).

In the triexponential fit, the  $\gamma$ -phase represents less than 6% the total area under the plasma concentration-time curve. For a biexponential fit, the  $\beta$ -phase represents only about 8% the area under the curve. This would indicate that neither the second nor third exponent has much influence on the actual plasma concentration-time curve. The lack of a predominating influence of the terminal phase on the overall curve could be attributed to the

TABLE 1

Table of kinetic parameters based on the nonlinear regression analyses

	0.5 mg/kg (N = 4)	1.0 mg/kg (N = 6)	t
A (ng/ml)	6063.8 $\pm$ 145.2	9148.6 $\pm$ 666.6	—
B (ng/ml)	675.7 $\pm$ 141.2	1016.2 $\pm$ 174.0	—
C (ng/ml)	21.6 $\pm$ 6.0	32.9 $\pm$ 7.2	—
$\alpha$ (min <sup>-1</sup> )	0.1300 $\pm$ 0.01	0.1330 $\pm$ 0.02	—
$\beta$ (min <sup>-1</sup> )	0.0330 $\pm$ 0.004	0.0320 $\pm$ 0.0049	—
$\gamma$ (min <sup>-1</sup> )	0.0047 $\pm$ 0.0007	0.0044 $\pm$ 0.0006	—
<i>t</i> 1/2 $\alpha$ (min)	5.6 $\pm$ 0.44	5.8 $\pm$ 0.74	0.1341
<i>t</i> 1/2 $\beta$ (min)	22.3 $\pm$ 3.09	24.1 $\pm$ 3.40	0.3674
<i>t</i> 1/2 $\gamma$ (min)	158.5 $\pm$ 24.69	177.2 $\pm$ 28.70	0.4573
$K_{12}$ (min <sup>-1</sup> )	0.0180 $\pm$ 0.0049	0.0230 $\pm$ 0.0047	0.7319
$K_{21}$ (min <sup>-1</sup> )	0.0420 $\pm$ 0.0052	0.0420 $\pm$ 0.0070	0.0730
$K_{13}$ (min <sup>-1</sup> )	0.0079 $\pm$ 0.0016	0.0064 $\pm$ 0.0015	0.6862
$K_{31}$ (min <sup>-1</sup> )	0.0051 $\pm$ 0.0008	0.0100 $\pm$ 0.0070	0.4094
$K_{10}$ (min <sup>-1</sup> )	0.0907 $\pm$ 0.0027	0.0940 $\pm$ 0.0130	0.1930
$V_c$ (liters/kg)	0.0740 $\pm$ 0.0030	0.2200 $\pm$ 0.1100	1.03
$V_{dss}$ (ml/kg)	164.8 $\pm$ 13.82	241.1 $\pm$ 19.29	2.88
Cl (ml/kg/hr)	385.5 $\pm$ 16.31	503.8 $\pm$ 32.88	2.75



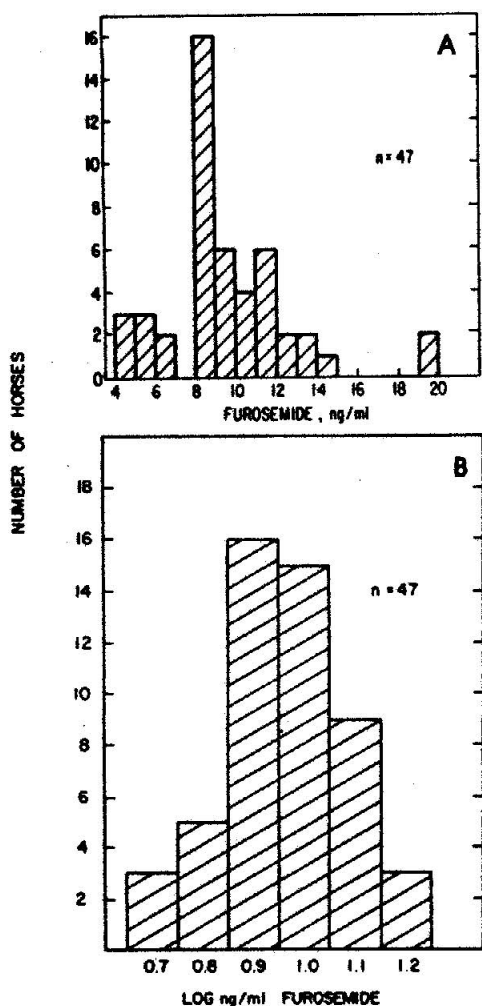


FIG. 6. Furosemide plasma levels in 47 horses 4 hr after iv administration of 0.5 mg/kg furosemide.

A, the vertical bars represent the number of horses found within the indicated ranges of furosemide plasma levels; B, the vertical bars represent the number of horses found within the indicated ranges of the log of furosemide plasma levels.

TABLE 2

Table of plasma levels of furosemide and average levels based on the population distribution of figure 11

Probability of Overage	Plasma Level of Furosemide
%	ng/ml
50	9.10
25	11.30
10	13.74
5	15.44
1	19.24
0.1	24.57
0.05	27.18

rapid decay of drug levels. Based on the AIC the triexponential equation was the simplest description of the data.

Identification of a third pharmacokinetic compartment for furosemide follows a pattern commonly observed when analytical methodology is improved. The ability to analyze for smaller concentrations allows the detection of a slower and slower decay of drug concentration. This pattern suggests that there is no real terminal half-life, but that the apparent terminal slope becomes

less and less. Further enhancement of analytical methodology might presumably demonstrate an even slower terminal plasma half-life than that demonstrated here.

We assessed the clearance and volume of distribution, which are parameters that are independent of dose and modeling of the data (11), at steady state. The clearance at the 0.5-mg/kg dose ranged from 341.9 to 420.9 ml/kg/hr ( $N = 4$ ) with a clearance of 385.49 ml/kg/hr, a mean value based on individual data analyses. At the 1.0-mg/kg dose, the clearance ranged from 420.1 ml/kg/hr to 642.3 ml/kg/hr ( $N = 6$ ) with a mean clearance of 503.8 ml/kg/hr. The volume of distribution at steady state ( $V_{dss}$ ) at the 0.5 mg/kg dose ranged from 126.6 ml/kg to 192.4 ml/kg with a mean  $V_{dss}$  of 164.8 ml/kg. At the 1.0-mg/kg dose, the  $V_{dss}$  ranged from 184.1 ml/kg to 323.2 ml/kg, with a mean  $V_{dss}$  of 241.1 ml/kg. Studying furosemide in human volunteers, similar  $V_{dss}$ 's of 110.0 ml/kg, 176 ml/kg, and 210 ml/kg have been reported by other workers (15-17).

The pharmacokinetics of furosemide in racing horses are of considerable forensic interest to racing authorities. Racing authorities may wish to insure that furosemide is administered at a certain period before race time to prevent its diluting effects on drugs and drug metabolites in equine urine. Although a detention barn can be used to enforce such time rules, a more economical method might be a furosemide tolerance level in equine plasma. Based on this consideration, we elected to develop our pharmacokinetic studies in such a way as to determine what plasma levels of furosemide would suggest compliance with a "4-hr" furosemide rule.

At 1 hr after dosing, blood levels ranged from 41.9 ng/ml to 155.8 ng/ml, with a mean of 97.9 ng/ml and a SD of 25.0 ng/ml. By 4 hr after dosing, the blood levels had fallen to a range of 4.0 to 19.4 ng/ml, with a mean of 9.56 ng/ml and a SD of 3.1 ng/ml. The levels found at 1 hr post-dosing were high enough to be clearly distinguished from those found at 4-hr post-dosing.

At 1 hr the furosemide plasma levels were normally distributed, but by 4 hr the distribution appeared skewed. Based on the assumption that the data were fit by a log-normal distribution, a population curve was generated by using the mean plasma level and the SD. This analysis suggested that the probability of a plasma level of 24.6 ng/ml at 4 hr after an anti-epistaxis dose of furosemide was less than 1 in 1000.

As a practical matter, these experiments suggest that a regulatory level of furosemide in equine plasma can be set and used to control the use of furosemide in racing horses. If this level is set at 30 ng/ml in equine plasma, the probability of a random overage would be substantially less than 1 in 1000. On a 10,000-sample per year jurisdiction, with 10% of the horses running on furosemide, the accidental "overage" rate would not be expected to be more than about one or two a year. Because the great bulk of the diuretic and drug-diluting effects of furosemide are over within 1 hr and are essentially complete by 3 hr after iv administration, the probability of a forensically significant drug-diluting effect in horses with less than 30 ng/ml furosemide in their plasma is minimal.

By 4 hr after furosemide administration, the detection of some drugs by thin-layer chromatography is enhanced (18). Therefore, incorporation of a 30 ng/ml furosemide tolerance into medication rules will obviate the possibility of drug dilution effects and may accentuate the drug detection-enhancing properties of this agent.

The data base developed in this investigation can be used to develop a range of furosemide tolerance levels. For example, a tolerance level of 19.2 ng/ml (20 ppb) of furosemide would set the random "overage" rate at about 1% or 1 in 100. Lower tolerance

levels could also be selected, but would be correspondingly stricter, and are not likely to be as useful as higher tolerance levels.

In applying this furosemide tolerance level, it is important that either the analytical method described here be used, or the method used be compared carefully with this method. This is because the only data on furosemide blood levels in the horse published to date are those described in this and previous papers from this laboratory. How closely alternative methodologies would correlate with this data base is not yet known, and should be checked by investigators who elect to use other methodologies.

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