THE PHARMACOKINETICS AND BLOOD LEVELS OF FUROSEMIDE AFTER INTRAVENOUS ADMINISTRATION.

S. Chay, BS, W.E. Woods, MS, and T. Tobin, MRCVS, PhD, Dept. of Veterinary Science, University of Kentucky, Lexington, Kentucky.

Epistaxis or bleeding from the nose seen in horses during or after exercise is a condition known to Thoroughbred racing for 300 years. Work done by Pascoe and co-workers using a fiberoptic endoscope has shown that the indidence of epistaxis in race horses is much more extensive than had been previously thought.[1,2] Historically the reported incidence of epistaxis in the horse was 1-2%, but the endoscopic survey revealed that actually close to 50% of racing horses have some degree of this condition. In efforts to control this condition a variety of treatments have been recommended. In the early 1970's equine practitioners and horsemen began recommending the use of furosemide for the prophylactic treatment of epistaxis or exercise induced pulmonary hemorrhage.

Furosemide is a potent high-ceiling diuretic commonly prescribed in human medicine. The technical problem with the approval of the pre-race use of furosemide is its ability to dilute certain drugs and drug-metabolites in equine urine. Research published at the University of Kentucky and research recently reported by Drs. Sams and Maylin of the NASRC Quality Assurance Program has demonstrated the conditions under which furosemide does not interfere with drug detection.[3,4] These conditions are that the drug be administered four hours prior to post-time and at a dose not in excess of 0.5 mg/Kg. Under these conditions no interference with drug detection is observed and there is some evidence for enhancement of detection of some drugs.[5]

Ensuring compliance with these restraints on dose and time prior to

post-time for drug administration is a regulatory problem. One approach to this problem is a detention barn in which horses are isolated and the dose administered and time of administration are carefully monitored. Although highly visible and effective, such systems are expensive and may not be justified by the magnitude of the drug-diluting effect likely to be observed in practice. An alternative means of enforcing compliance with a four hour medication rule is to designate an acceptable plasma tolerance level of furosemide above which there would be a substantial probability of violation. Studies were undertaken to determine plasma levels of furosemide following 1.0 and 0.5 mg/Kg doses administered intravenously. Population distributions following the anti-epistaxis dose of 0.5 mg/Kg were then used as a basis for determining plasma tolerance levels.

Figure 1 shows the structure of furosemide. Because of its polarity it is not detectable by gas chromatography without prior derivatization. However, furosemide will undergo an electrophilic attack at the carboxylic and sulfonamide moieties by the methyl radical of methyl iodide in the presence of heat. The resulting derivative trimethyl furosemide is then suitable for analysis by gas chromatography.

The analytical procedure used for this study is an extractive alkylation method modified from Roberts and co-workers and based on one developed by Lindstrom and Molander.[6,7] The acidified plasma sample was extracted into dichloromethane and concentrated under a stream of nitrogen. Following adjustment to a basic pH furosemide was temporarily paired with tetra-hexylammonium hydrogen sulfate allowing it to enter the organic phase where it undergoes electrophilic attack by the methyl radical of methyl iodide. This method yielded approximately 87% recovery with a lower limit of detection of 2.0 ng/ml using a 63 Ni electron capture detector.

Gas chromatograms obtained from methylated furosemide in spiked plasma show a linear increase in detector response with the addition of increasing amounts of furosemide (Fig 3). A plasma sample obtained 30 minutes after a 1.0 mg/Kg dose detected by electron capture detection is shown in the far left panel. The chromatogram of blank plasma shows the lack of interfering peaks at 4 minutes 25 seconds, which was the retention time of furosemide represented by the solid peaks.

Figure 4 shows the mass spectrum of the methyl derivative of furosemide. The molecular ion at 372, base peak at 81, and fragments at 53, 69 and 96 were consistent with that of trimethyl furosemide.

Figure 5 is a standard curve of furosemide extracted from horse plasma. The solid circles represent the detector response measured in peak area to the added amounts of furosemide expressed as the mean of five experiments. The vertical bars represent the standard error of the mean. The standard curves were linear within our working range with a least squares regression line fitted by the solid line with a correlation coefficient of 0.990 and a slope of 5.7.

In Figure 6, the plasma concentration-time curve of furosemide following a 1.0 mg/Kg dose in 6 horses is shown. The solid circles represent the mean and the vertical vars are the standard error of the mean. The mean level was approximately 7500 ng/ml at 3 minutes after dosing and fell to a mean level of 4 ng/ml at 8 hours after dosing. We found these results to be in good agreement with those of Roberts and co-workers whose data are shown as open circles expressed as the mean of 5 horses.[3] The principal discrepancies are at the later time points which may have been influenced by increased assay sensitivity.

The 1.0 mg/Kg plasma concentration data replotted are shown in Figure 7.

The open circles show the calculated plasma levels when the curve was fit by a non-linear regression analysis with a 1/y2 weighting.[8] Points represented by a single solid circle represent an overlap in real and calculated values. The model which best described the data was chosen based on the application of the Akaike's Information Criterion.[9] Analysis of this data showed the curve was best described by the tri-exponential equation shown at the top of Figure 7.

The plasma-concentration time curve following a 0.5 mg/Kg dose of furosemide is shown in Figure 8. The solid circles represent the mean plasma levels of 4 horses, while the vertical bars again represent the standard error of the mean. At 3 minutes after dosing, the mean furosemide plasma level was 5400 ng/ml which fell to 2.2 ng/ml at 8 hours. At 4 hours following this anti-epistaxis dose of furosemide, there was a mean plasma level of 7.3 ng/ml. This data indicated that by 4 hours post-dosing there was a residual of furosemide detectable in the plasma upon which a plasma tolerance level could be based. The non-linear regression analysis again confirmed a tri-exponential fit as described by the equation at the top of the graph.

The pharmacokinetic parameters of furosemide following 1.0 and 0.5 mg/Kg doses were calculated based on experimental constants obtained by the non-linear regression analyses of the plasma concentration-time curves.[10] The kinetic parameters at both doses of furosemide are shown in Figure 9. A paired t-test indicated that these parameters were not statistically significantly different at $p < 0.05$. This work suggests that furosemide does not show dose-dependent kinetics in the horse.

Furosemide plasma levels at 1 hour after the anti-epistaxis dose of 0.05 mg/Kg were studied in 30 horses (Fig 10). Furosemide levels were measured

as ng/ml and the height of the vertical bars represent the number of horses included in the indicated range. We found that the range at 1 hour after dosing was 41.9 to 155.8 ng/ml, with a mean of 97.9 ng/ml. The distribution of furosemide plasma levels was normal with a Shapiro-Wilke's statistic of $p < 0.989$.

By 4 hours after dosing, plasma levels in 47 horses ranged from 4.0 to 19.4 ng/ml, with a mean of 9.56 ng/ml (Fig 11). Furosemide levels had diminished by 4 hours enough to be clearly distinguishable from those found at 1 hour. The population distribution appeared skewed at 4 hours and the Shapiro-Wilke's statistic of $p < 0.01$ confirmed a non-normal distribution. Figure 12 shows the 4-hour post-dosing data replotted. Following log-transformation of the plasma concentration of furosemide, the Shapiro-Wilke's statistic was $p < 0.53$.

Based on the assmumption that the data were fit by a log-normal distribution, a population curve was generated using the mean plasma level and standard deviation. This analysis suggested that the probability of a plasma level of 24.54 ng/ml at 4 hours following a prophylactic dose of furosemide is less than one in one thousand. 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 one in one thousand.

SUMMARY

Blood levels of furosemide in horses following 0.5 and 1.0 mg/Kg doses administered intravenously were determined. The trimethyl derivative of furosemide was quantitated using a gas chromatograph equipped with a 63Ni electron capture detector and a 2% OV-101 column. The individual and mean plasma concentration-time curves were analyzed by non-linear regression analyses. These studies indicated that the pharmacokinetic parameters were independent of the dose administered. A 3-compartment open model was found to best describe the data based on the Akaike's Information Criteria.

Following a 0.5 mg/Kg dose of furosemide, population frequency distributions were evaluated at 1 and 4 hrs following administration. At 1 hr after dosing blood levels of furosemide in 30 horses were normally distributed with a mean plasma level of 97.9 ng/ml. Analyses of blood levels of furosemide in 47 horses at 4 hrs following drug administration indicated that the population distribution was better fit by a normal curve after log transformation of the values with a mean plasma level of 9.6 ng/ml. The log transformed values were used to determine the probability of finding furosemide plasma concentrations above certain levels based on the method of means and moments. Our studies suggest that the probability of finding a plasma concentration of 24.6 ng/ml at 4 hrs was less than 1 in 1000.

ACKNOWLEDGEMENT

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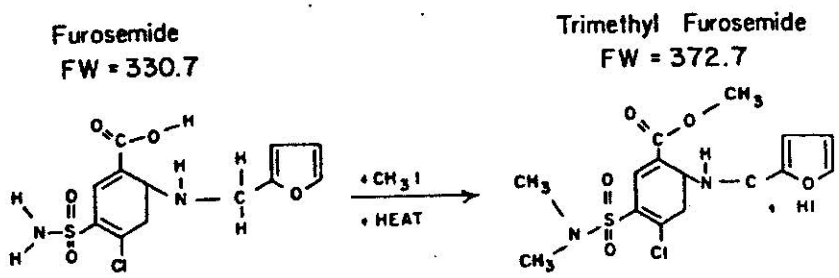

Figure 1. Furosemide reacts with methyl iodide in the presence of heat to form trimethyl furosemide.

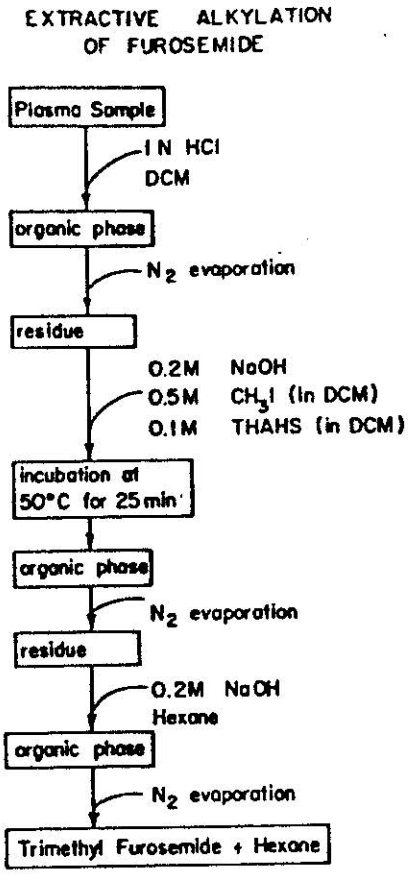

Figure 2. Extractive alkylation of furosemide.

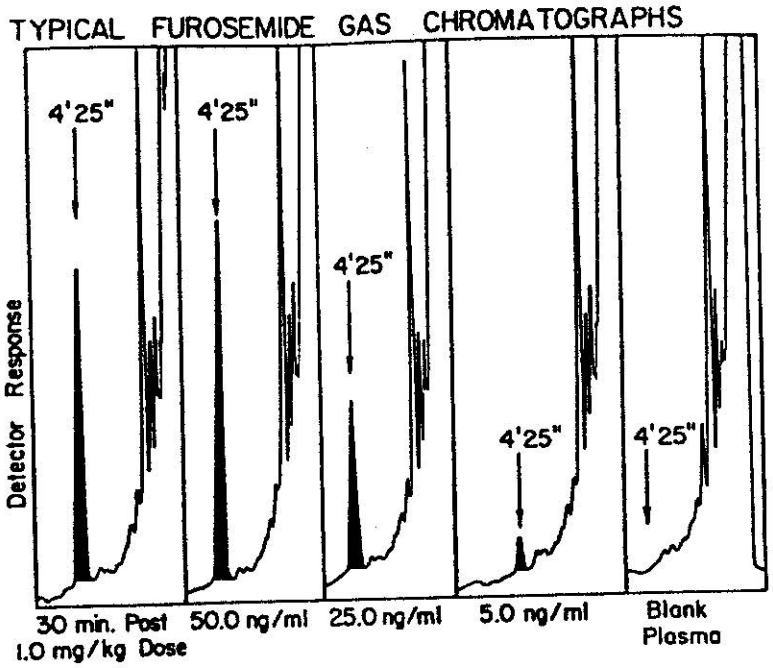

Figure 3. Gas chromatograms of blank plasma, spiked concentrations of furosemide, and plasma sample 30 minutes following a 1.0 mg/Kg dose of furosemide administered intravenously.

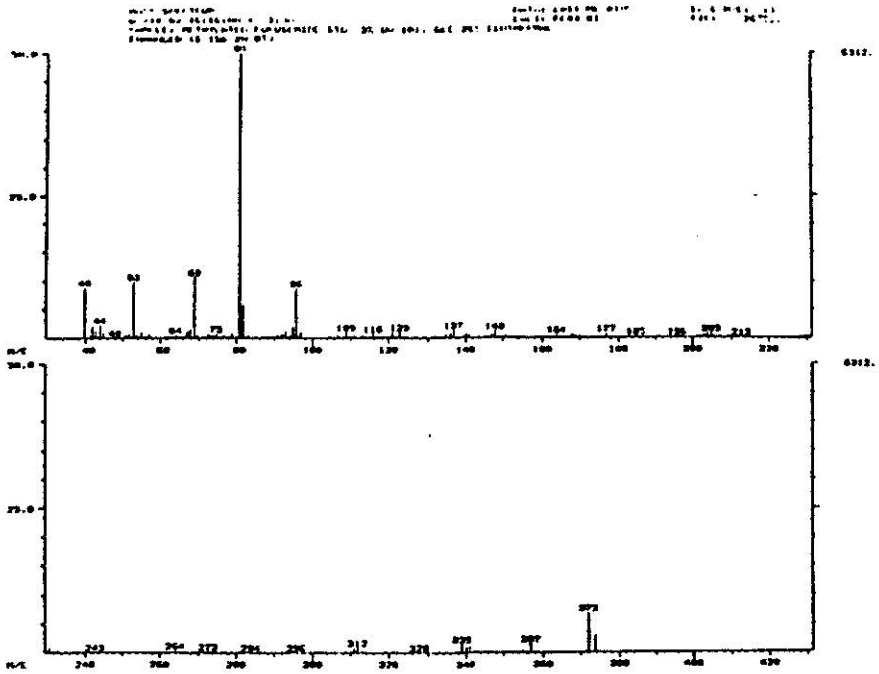

Figure 4. Mass spectrum of methylated furosemide out of plasma.

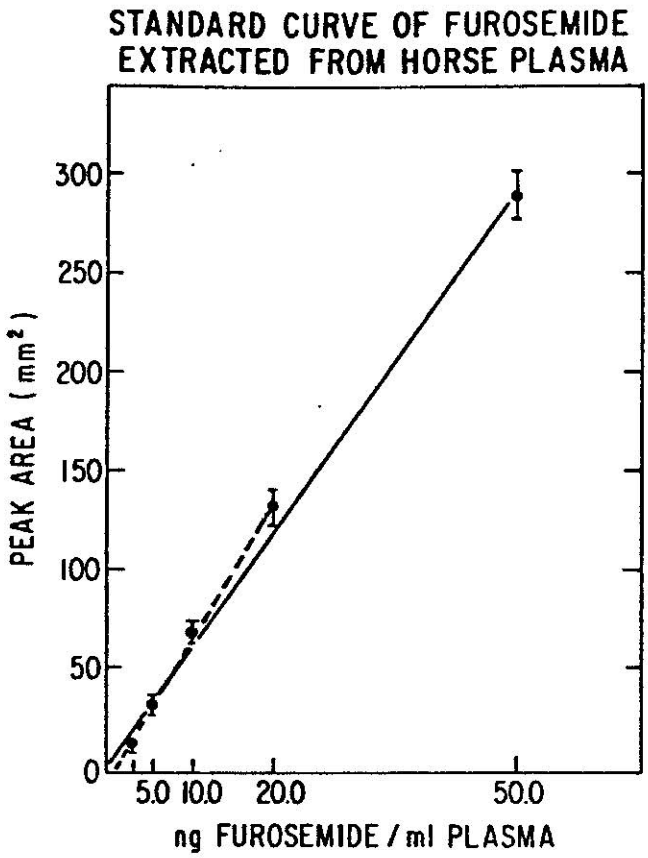

Figure 5. Composite standard curve (n = 5) of furosemide extracted from horse plasma.

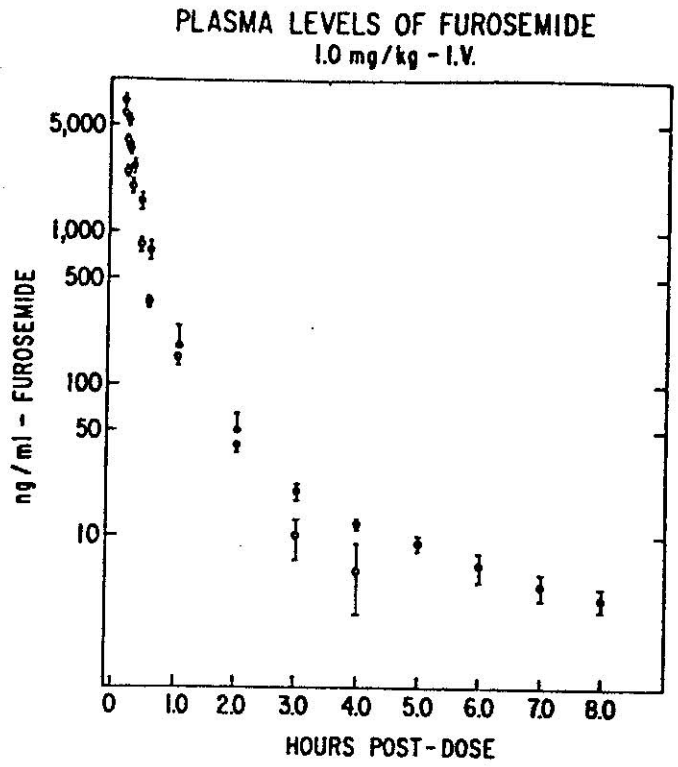

Figure 6. Mean plasma levels of furosemide following 1.0 mg/Kg administered intravenously are represented by the closed circles (●–●) n = 6. The open circles (O–O) represent the mean plasma levels of furosemide in 5 horses previously reported by Roberts and co-workers.[3]

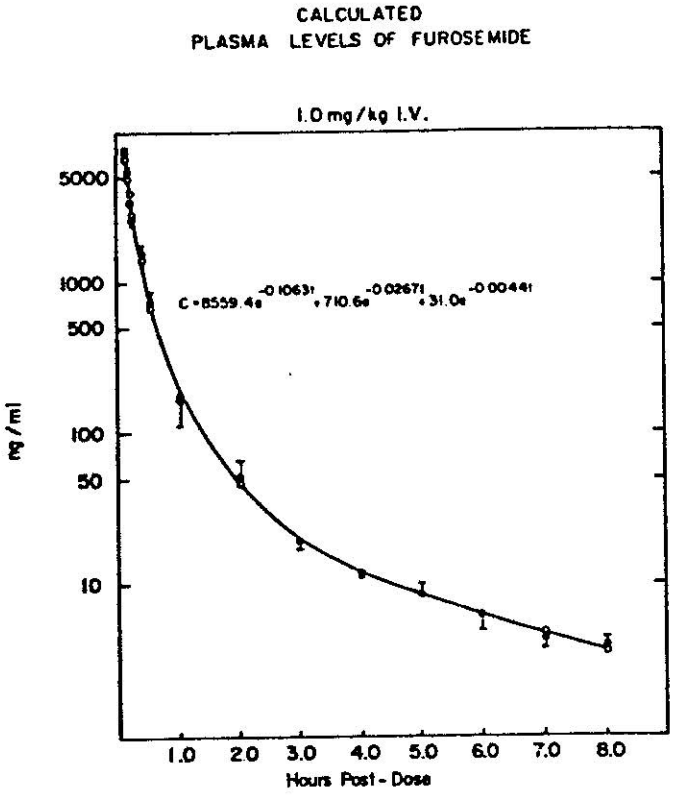

Figure 7. Calculated plasma levels of furosemide (1.0 mg/Kg I.V.) fit by a non-linear regression analysis with a 1/y2 weighting.

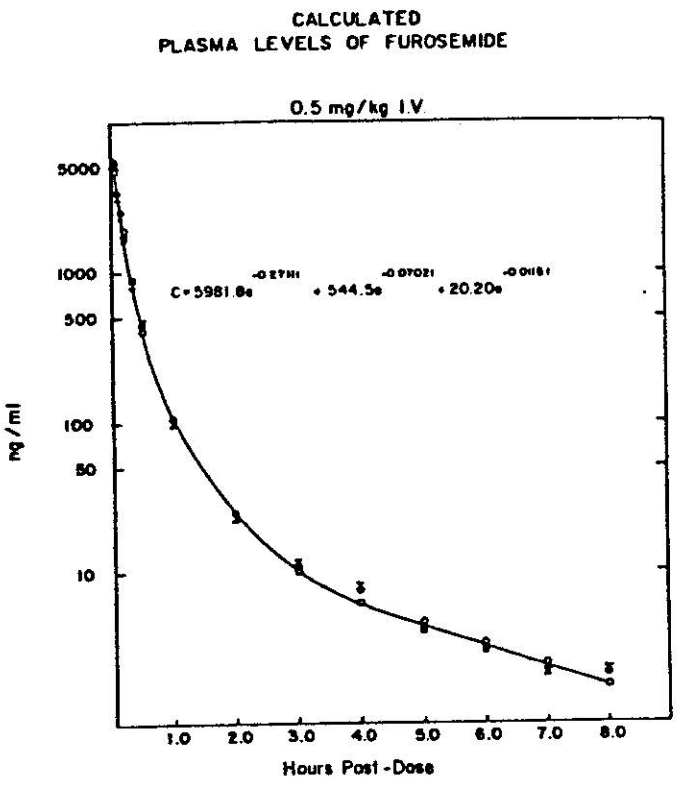

Figure 8. Plasma levels of furosemide following 0.5 mg/Kg I.V. dose are represented by the closed circles (●–●) n = 4. The open circles (O–O) represent the calculated values when fit by a non-linear regression analysis with a 1/y2 weighting.

	0.5 mg/kg (n=4)	1.0mg/kg (n=6)
t1/2 α	5.9 min.	6.5 min.
t1/2 β	22.7 min.	25.9 min.
t1/2 γ	139.2 min.	155.9 min.
K_{12}	0.01554 $min.^{-1}$	0.013831 $min.^{-1}$
K_{21}	0.0378 $min.^{-1}$	0.032887 $min.^{-1}$
K_{13}	0.004312 $min.^{-1}$	0.004517 $min.^{-1}$
K_{31}	0.005234 $min.^{-1}$	0.004713 $min.^{-1}$
K_{10}	0.09027 $min.^{-1}$	0.08155 $min.^{-1}$
Vc	0.08 l/kg	0.10 l/kg
Vd ss	0.1592 l/kg	0.2322 l/kg
Cl	0.3852 l/kg/hr	0.4941 l/kg/hr

Figure 9. Pharmacokinetic parameters of furosemide following 1.0 and 0.5 mg/Kg doses administered intravenously.

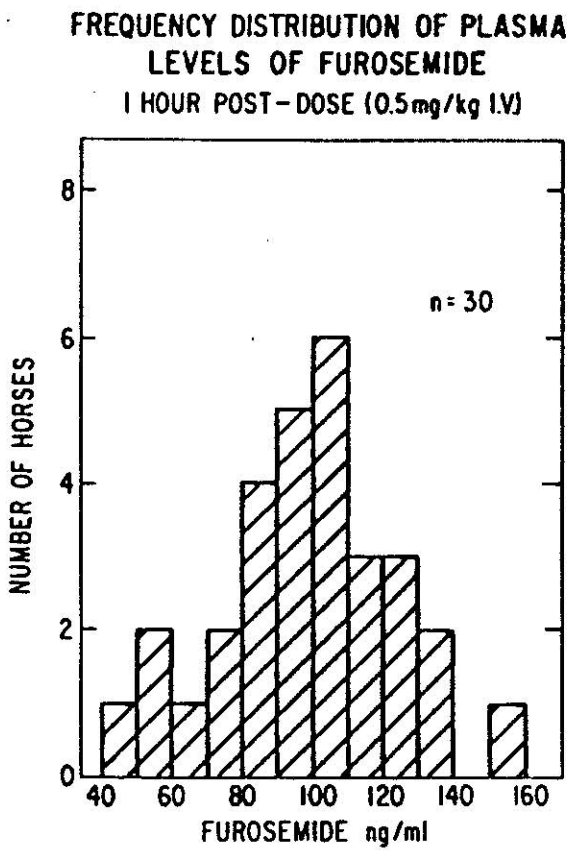

Figure 10. Frequency distribution of plasma levels of furosemide at 1 hour following a 0.5 mg/Kg dose administered intravenously.

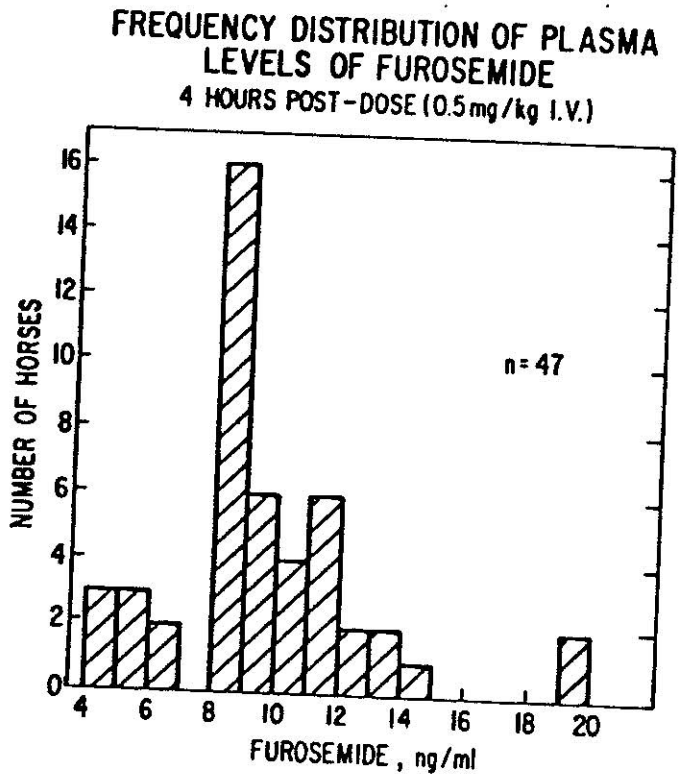

Figure 11. Frequency distribution of plasma levels of furosemide 4 hours following a 0.5 mg/Kg dose administered intravenously.

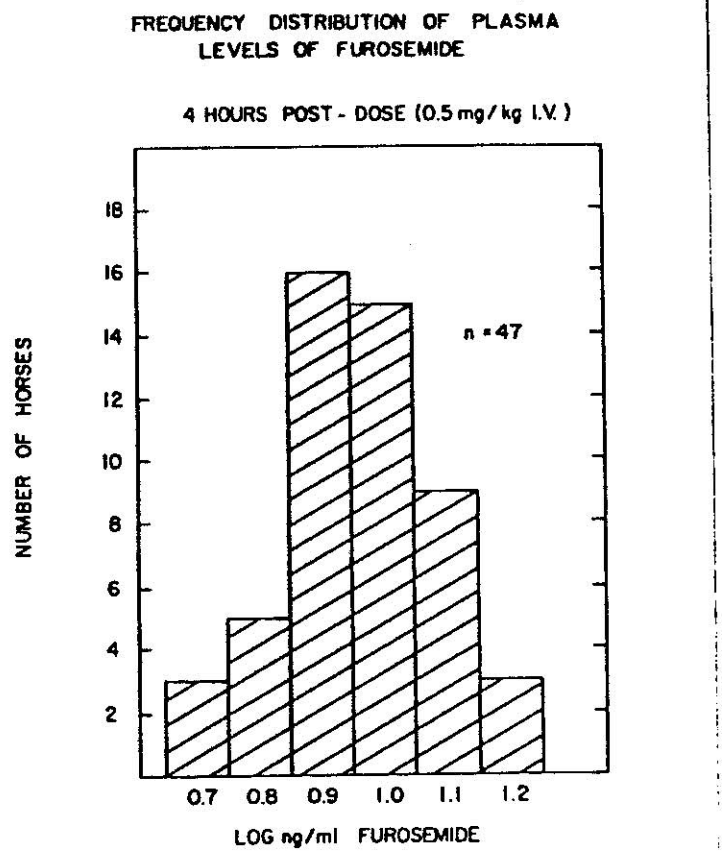

Figure 12. Frequency distribution of the logarithms of plasma levels of furosemide at 4 hours following a 0.5 mg/Kg dose administered intravenously.