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The Pharmacology of Furosemide in the Horse. III. Dose and Time Response Relationships, Effects of Repeated Dosing, and Performance Effects

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Furosemide was administered intravenously (IV) and intramuscularly (IM) to Thoroughbred horses at various doses. After rapid IV injection, furosemide produced up to a 40-fold increase in the rate of urine formation with a concomitant decrease in its specific gravity. This effect peaked between 15 and 30 minutes after dosing and then declined rapidly.

At doses of up to 1 mg/kg IV, the bulk of the diuretic effect occurred within 1 hour. A second dose at 1 or 2 hours again produced substantial diuresis (70% of the initial response). Larger doses or intramuscular (IM) administration prolonged and enhanced the diuretic effect. The diuretic effect was closely related to plasma levels of the drug, and the transient response after IV administration was apparently due to the short plasma half-life of the drug. Increasing doses of furosemide produced a graded increase in urine volume and a fivefold increase in its Na^+ concentration at all doses above 0.01 mg/kg. In contrast, increasing doses produced a graded decrease in urinary K^+ concentrations. The net effect on K^+ excretion was that all doses tested produced a small and transient increase in urinary K^+ output.

At 1 mg/kg IV, furosemide increased packed cell volume (PCV) about 5%, total plasma solids by about 10%, and reduced plasma K^+ concentrations. These effects peaked between 10 and 30 minutes postdosing, then returned rapidly to control values.

Repeated doses of furosemide resulted in loss of the diuretic effect. Dosing every hour at 1 mg/kg, a third dose produced 37% of the diuresis of the first dose, while a fourth dose was about one-quarter as effective as the first dose.

This loss of diuretic effect was associated with no further changes in the blood picture. These repeated dosing experiments suggested that about 25 liters of fluid can be withdrawn from a 435 kg (1,000 lb) horse by furosemide. Marked reductions in plasma K^+ concentrations were seen after repeated doses of furosemide, but no clinically significant changes in blood enzymes were observed. Similarly, daily dosing with 1 mg/kg furosemide IV for 4 days produced no clinically significant cumulative changes in any of the blood values tested.

Time trials over a 1-mile course showed no statistically significant changes in the performance of Standardbred horses treated with 0.55 mg/kg furosemide IV 30 minutes prior to the test. Similarly, a study of the track times of 58 Standardbred horses running with and without furosemide at Louisville Downs showed no significant difference between times on and off furosemide.

Introduction

Furosemide (Lasix®)[a] is a member of the "high ceiling" group of diuretics and is the member of this group most widely used in equine medicine.[7] It is used in the treatment of various forms of edema, in azoturia, to reduce space-filling lesions, and more recently, in the prophylaxis of epistaxis in racing horses.[7] As well as its actions against epistaxis, it is suspected in racing circles of "diluting out" prohibited drugs in the urine of racing horses and also improving their racing performance.[17] In recent years its use for the prophylaxis of epistaxis in horses during races has been approved by some racing authorities; as a result, its use in horses racing in these jurisdictions has increased up to 80%, a frequency of use far beyond that which might be expected on the basis of the relatively small (<5%) incidence of epistaxis in Thoroughbred horses.[13]

Because of this widespread use of furosemide in racing horses, many questions concerning its actions and use in the horse have arisen. Surprisingly, even the diuretic effect of this drug in the horse is not well characterized,[8] in particular, with respect to dose and time response relationships. Beyond recent work from this laboratory,[14,15] no data whatsoever were available on its pharmacokinetics and clearance times from urine in the horse. Preliminary reports on the effect of furosemide on the disposition of other drugs in the equine have been published from this laboratory.[15,13,14] No studies on the efficacy of furosemide in the prophylaxis of nosebleed nor on the effect of furosemide on racing performance in the horse are available. Further, concern exists among horsemen regarding the effects of repeated doses of furosemide in the horse because of the possibility of producing hypokalemia, dehydration, or other forms of toxicity.[8]

In this study, we report on dose and time response relationships for the natruretic, kaluretic, and diuretic responses of horses to furosemide after its IV and IM injection. We further report on the performance effects and the effects of repeated doses of furosemide in the horse. Companion papers on the detection and pharmacokinetics of furosemide and its effects on the disposition of other drugs in the horse have been published,[14,17] and preliminary reports of this work have been presented.[15]

Materials and Methods

Mature Thoroughbred and half-Thoroughbred mares (375–550 kg), kept on pasture, were housed in individual box stalls with hay and water *ad libitum* unless otherwise noted on the days of an experiment. The horses were administered agents either intravenously into the left jugular vein or by intramuscular injection into the deep muscles of the neck. Blood samples were collected in 15-ml heparinized Vacutainer®[b] tubes from the opposite jugular vein. Urine samples were collected by bladder catheterization. Urine samples for analysis were collected "midstream" after catheterization. Bladders were drained completely at each time period, and volumes were recorded to calculate the quantity of drug or electrolytes excreted over a time period. Because the diuretic effect of furosemide administered IV was both very marked and very short lived (Fig 1), complete draining of the bladder at up to 15-minute intervals was important to accurately monitor the time course of the diuretic effect. Mean urinary flow rates were calculated by dividing urine volume for each period by the collection time in minutes. All samples were held on ice until they could be taken to the laboratory for analysis or for further storage at −40°C until the samples were analyzed.

Sodium and potassium concentrations in plasma and urine were analyzed by flame photometry.[c] Urine samples were centrifuged at 10,000 X g for 10 minutes to prevent clogging of the aspirator line. Sodium and potassium concentrations were recorded as milliequivalents/liter (meq/liter). Urine specific gravity was determined by means of a urinometer float. Total plasma solids were

[a] Lasix®, 50 ng/ml Hoechst-Roussel Pharmaceuticals, Somerville, NJ.

[b] Becton-Dickinson Co., Rutherford, NJ.

[c] Model 143, Instrumentation Laboratories, Inc., Lexington, MA.

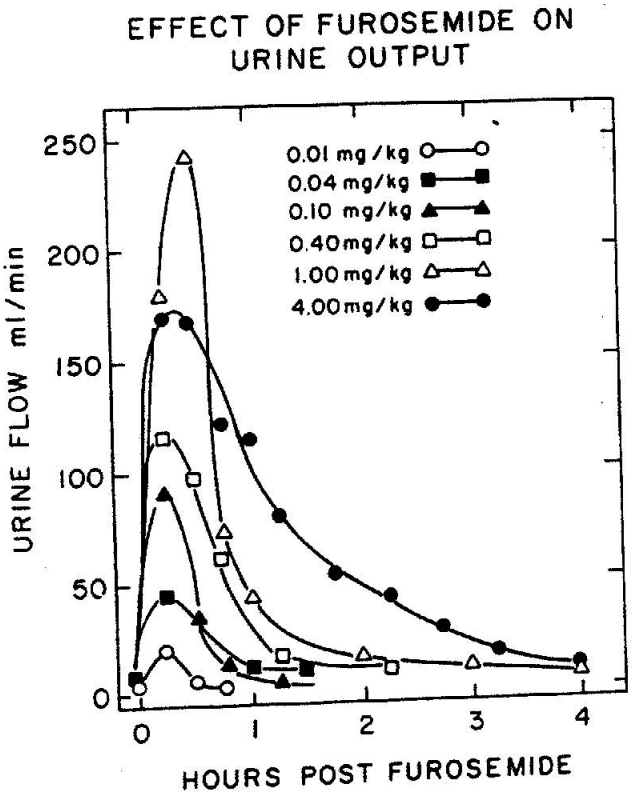

Fig 1—Time course of urinary flow rate response to intravenous furosemide. The symbols show flow rate in ml/minute after the indicated doses of furosemide intravenously. Open circles (○-○), 0.01 mg/kg; solid squares (■-■), 0.04 mg/kg; solid triangles (▲-▲), 0.1 mg/kg; open squares (□-□), 0.4 mg/kg; open triangles (Δ-Δ), 1.0 mg/kg; solid circles (●-●), 4.0 mg/kg. All experiment points except those for 0.01 mg/kg (2 horses) represent means of experiments on 4 different horses.

determined by refractometry.[d] Hematocrits were determined by centrifugation,[e] red blood cell counts on a Coulter counter,[f] and hemoglobin on a hemophotometer.[g] Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and creatine phosphokinase (CP) were determined by the Spin Chem method.[h] Other methods, drugs, and chemicals were as previously outlined.[14,17] Unless otherwise noted, all values are the means ± standard errors of the means of determinations on at least 4 different horses.

The effects of furosemide on performance were determined in Standardbred horses. Standardbred horses in training were purchased locally (commercial value $500–1,000) and entered into the training program. The horses were between 2 and 15 years of age. Training followed the traditional Kentucky pattern for Standardbred horses and consisted of daily jogging for 5 miles, Sundays and weather permitting, with 1 day of "full speed" training each week. When the horses attained what appeared to be their optimal performance, they were entered into the drug trial program. All drug trials were performed double-blind, with an equivalent number of control (saline injection) treatments randomly inserted into the drug sequence. Inasmuch as the diuretic effects of the dose of furosemide were occasionally observable, the driver/trainer was aware of the nature of the drug during the trials. Dosage was 0.55 mg/kg of furosemide (about 5 ml of 50 mg/kg furosemide to a 1,000 lb, 453 kg horse) 30 minutes before the timed run. Times were recorded by the driver by stopwatch. Data were analyzed for statistical significance by the t test and $P < 0.05$ was selected as the criterion for statistical significance.

For analysis of the effects of furosemide on racing performance at Louisville Downs, a list of the horses which elected to go on furosemide during this meet was made available to us. Furosemide was the only permitted medication allowed during this meet, and urine samples taken from these horses were checked for furosemide to monitor compliance with their declared furosemide status. Performance times for these horses obtained prior to going on furosemide were obtained from the track program and compared with those on furosemide. For statistical analysis a randomized block design was used with each horse representing a single block. Statistical significance was determined by an F test.

Results

Furosemide was administered IV to groups of 4 horses at doses of between 0.01 and 4.0 mg/kg. Figure 1 demonstrates changes in urinary flow rates produced by these doses. A base line urine flow of 6.6 ml/minute in the absence of the drug was determined by averaging flow rates for the 30 minutes just prior to injection of drug for all horses tested in this experiment.

The lowest dose tested, 0.01 mg/kg, briefly increased urine flow about threefold over base line, which then returned to the control rate by 30 minutes postdosing. At higher dose rates, urine flow peaked between 15 and 30 minutes after IV injection. Urinary output was largely complete within the first hour and had returned to close to control values by 2 hours postadministration for all but the largest dose, 4.0 mg/kg.

Figure 2 shows the effect of these doses on urinary specific gravity. Mean specific gravity in control urines was 1.031 ± 0.004, with a range of from 1.025 to 1.050. Urinary specific gravity was rapidly reduced after furosemide treatment, with peak reduction occurring be-

[d] Optical Refractometer, Bausch & Lomb, Rochester, NY.
[e] "Autocrit" Centrifuge, Clay-Adams, Inc., NY.
[f] ZBI Coulter Counter, Coulter Electronics, Inc., Columbus, OH.
[g] Hemophotometer, Fisher, Inc., Cincinnati, OH.
[h] Spin Chem, Smith-Kline Instruments, Ltd., Palo Alto, CA.

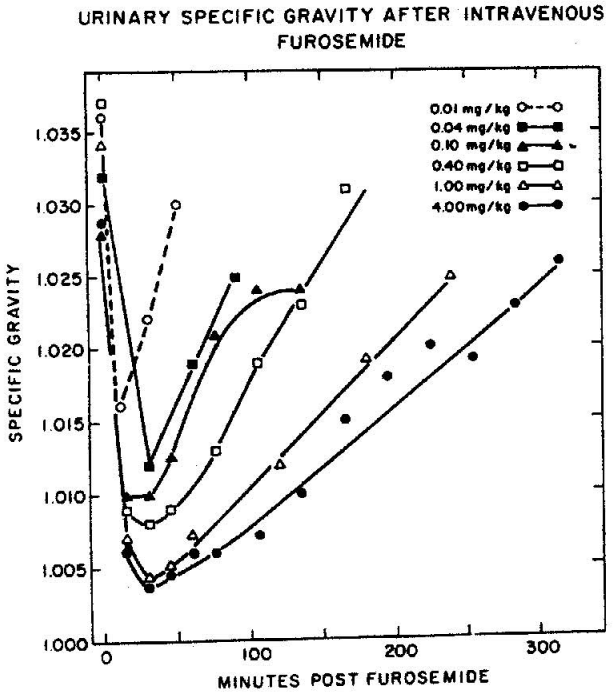

Fig 2—Effect of intravenous furosemide on urinary specific gravity. Furosemide at the indicated dose levels was administered to Thoroughbred horses by rapid IV injection. The symbols show urinary specific gravity after 0.01 mg/kg (open circles, O-O); 0.04 mg/kg (solid squares, ■-■); 0.1 mg/kg (solid triangles, ▲-▲); 0.4 mg/kg (open squares, □-□); 1.0 mg/kg (open triangles, Δ-Δ); 4.0 mg/kg (solid circles, ●-●). All points except those at 0.01 mg/kg (2 horses) are the results of experiments on 4 different horses.

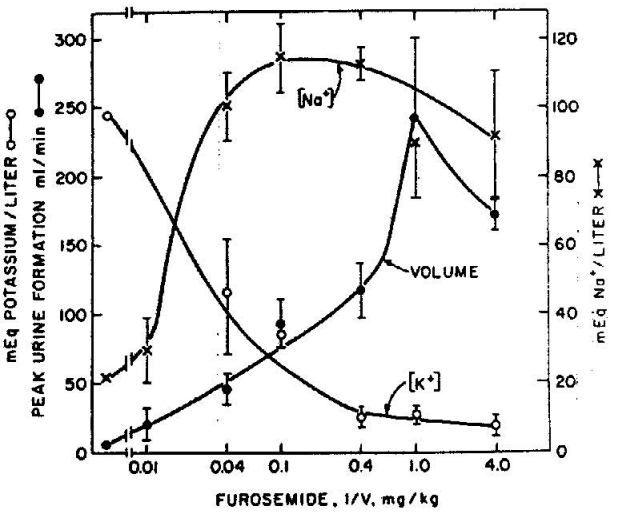

Fig 3—Dose response relationships of urinary output and cation concentrations at peak diuresis due to intravenous furosemide. Furosemide at the indicated concentrations was given by rapid intravenous injection to horses. The solid circles (●-●) show peak urinary output at each of the indicated doses. The crosses (X-X) and open circles (O-O), show urinary concentrations of Na^+ and K^+, respectively, at the indicated dosage levels. All experiments except those at 0.01 mg/kg (2 horses) are the means of experiments on 4 different horses ± standard deviation.

tween 20 and 40 minutes. At higher doses of furosemide, urinary specific gravity dropped to less than 1.005 and then slowly returned toward control values.

Figure 3 shows dose response curves for the indicated doses of furosemide on peak urinary output and the sodium and potassium content of urine at the times of peak flow. The data demonstrate that while urine volume responded with a graded increase to increasing dose, urinary potassium concentration declined in a similar graded manner. In contrast to these graded responses, urinary sodium content was minimally affected by the 0.01 mg/kg dose but was maximally affected by the 0.04 mg/kg dose. Thus, doses of furosemide above 0.04 mg/kg all produced the maximal increase in the concentration of Na^+ in equine urine in contrast with their graded and opposite effects on volume and K^+ concentration.

Figures 4 and 5 show the results of these effects on urinary excretion of Na^+ and K^+. While urinary Na^+ excretion increased with dose in a graded fashion, urinary excretion of K^+ was maximally increased by the smallest dose tested (0.01 mg/kg) and was not further increased by increasing the dose.

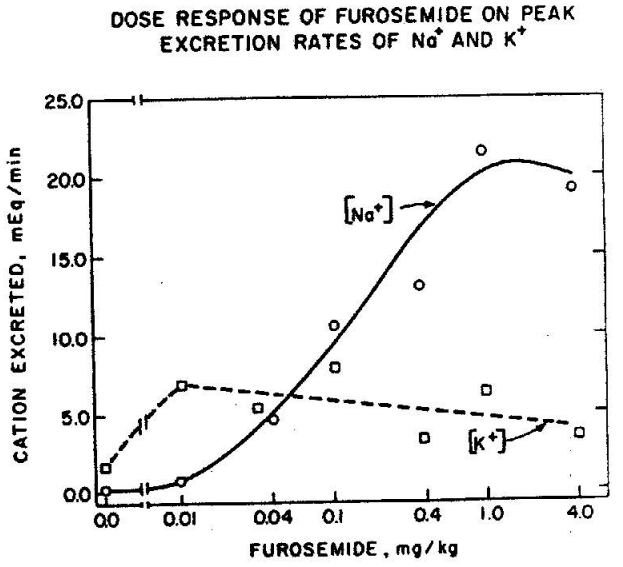

Fig 4—Dose response relationship for intravenous furosemide on cation excretion rates. All data points represent cation excretion rates at peak drug effect. The open circles (O-O) show peak urinary output of Na^+ after the indicated concentrations of furosemide, while the open squares (□-□) show peak urinary output of K^+ after the indicated doses of furosemide. All experiments except those at 0.01 mg/kg (2 horses) are the means of experiments on 4 different horses ± standard deviation.

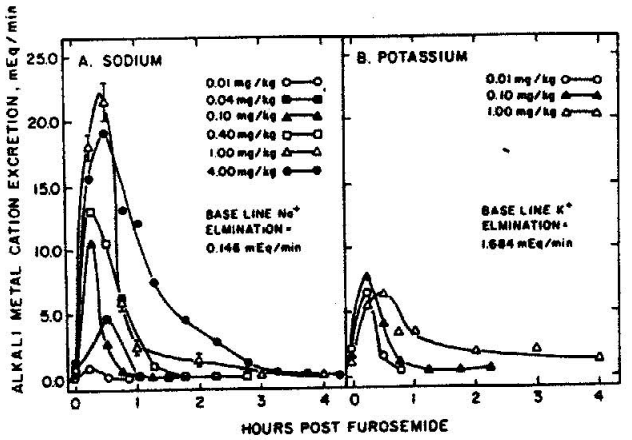

Fig 5—Effect of intravenous furosemide on rates of urinary loss of Na^+ and K^+. The left-hand panel shows the effect of the indicated doses of furosemide on Na^+ output/minute; the right-hand panel the effect of the indicated doses on K^+ loss/minute. All points are means ± standard deviation of determination on 4 different horses.

Figure 6 shows the effect of 1 mg/kg furosemide IV on a number of blood values. Packed cell volume increased by about 6%, which effect was accompanied by parallel increases in hemoglobin and red blood cell count (data not presented). Total plasma solids increased by about 10%, while serum K^+ concentrations showed up to a 20% drop. No consistent changes in plasma Na^+ concentrations were observed. All the changes observed were rapidly reversed, and all values had returned to control levels by 120 minutes postdosing.

Figure 7 shows the diuretic response obtained after IM injection of 1 mg/kg of furosemide. Peak flow rate achieved after injection by this route was less than that observed after IV injection, but the diuretic effect was more prolonged. Similarly, the effects on cation concentration in urine were of longer duration than after IV injection and paralleled the prolonged diuretic effect. As shown in Table 1, about 50% more water and 25% more Na^+ were eliminated after 1 mg/kg furosemide was given by the IM rather than by the IV route.

Figure 8 shows the relationship between plasma levels of furosemide and the diuretic response. The diuretic responses to 1 mg/kg furosemide IV or IM were replotted from Figs 1 and 6, while plasma levels of the drug were replotted from Figs 6 and 8 of Roberts *et al.*[14] Baseline urine flow rates were subtracted from all values presented in Figs 1 and 6, so that only the diuretic response to the drug was represented. The data show that in both cases the diuretic response to furosemide declined a little more rapidly than plasma levels of the drug.

Traditionally, the diuretic effect of furosemide has been thought to be limited by the amount of extracellular

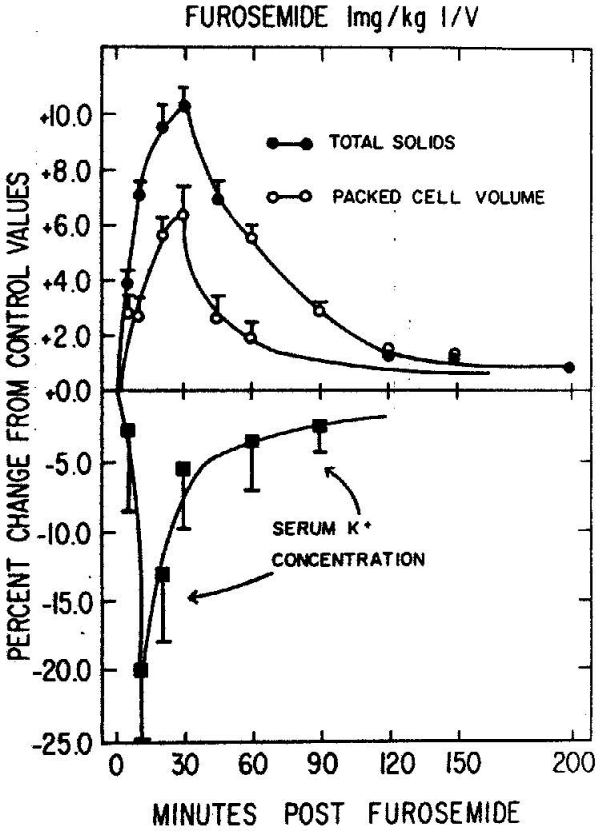

Fig 6—Effect of 1 mg/kg furosemide intravenously on blood values. Horses were administered 1 mg/kg furosemide intravenously and blood samples drawn at the indicated times. The solid circles (●-●) show the percent increase in total plasma solids, while the open circles (O-O) show the percent increase in packed cell volume following furosemide. The solid squares (■-■) show the percent decrease in serum K^+. Control serum K^+ levels were 3.70 ± 0.12 Eq/liter. All data are expressed as percent change from predrug controls and are the means ± standard error of the means of experiments on 6 horses.

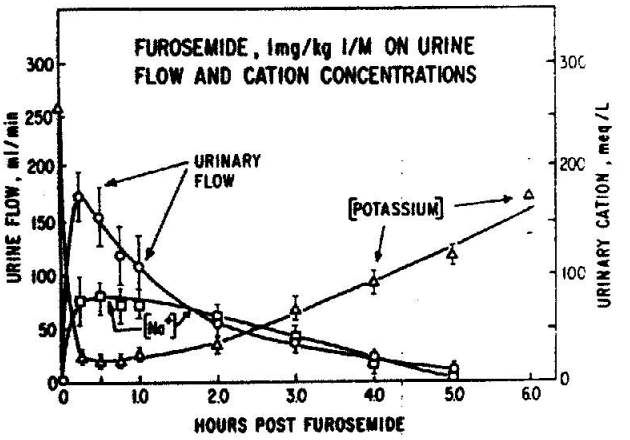

Fig 7—Effect of 1 mg/kg furosemide intramuscularly on urine volume and cation concentration. The open circles (O-O) show urinary output after the indicated dose of furosemide, while the open squares (□-□) and triangles (Δ-Δ) show urinary concentrations of Na^+ and K^+, respectively. All experimental points are means ± standard deviation of experiments on 4 different horses.

TABLE 1

Cumulative Water and Na^+ Loss After 1 mg/kg Furosemide Intravenously and Intramuscularly

Time after administration	1.0 mg/kg IV		1.0 mg/kg IM	
	Urine Vol. (liter)	Na^+ Excretion (mEq/liter)	Urine Vol. (liter)	Na^+ Excretion (mEq/liter)
1 hour	8.2[a]	732	8.4[a]	621
2 hours	9.0	744	12.6	887
4 hours	10.5	748	16.2	994

[a] n = 4 horses

fluid available to be excreted. However, because of the close correlation between the plasma half-life of furosemide and its pharmacological action (Fig 8), we decided to test this hypothesis by administering repeated IV doses of furosemide to horses. Figure 9 (Experiment 1) shows that a second dose of furosemide within 120 minutes elicited a prompt diuretic response of about 73% of the magnitude of the first response. Similarly, Fig 9 (Experiment 2) shows that about the same response was obtained if a second dose was administered within 60 minutes of the first dose. These experiments show that at the 1 mg/kg dose level considerable quantities of fluid remain available to furosemide at 1 hour after dosing. It thus appears reasonable to assume that the diuretic response to a single IV dose of furosemide at 1 mg/kg is limited primarily by rapid elimination of the drug.[14]

If, however, the 1 mg/kg IV dose of furosemide was repeated, the diuretic response decreased rapidly. Thus, a third dose produced a diuretic response of only 37% of the original response, and a fourth dose produced a response of about 25% of that observed initially. Since these horses were deprived of water during these experiments, it appears likely that the 21.3 liters of fluid withdrawn over the

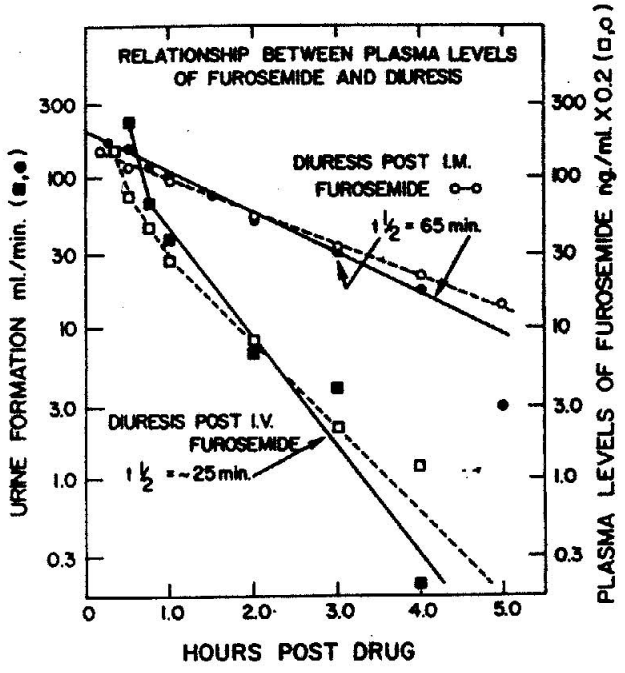

Fig 8—Relationship between plasma levels of furosemide and diuresis. The solid symbols and lines show rates of urine formation in ml/minute after IV injection (solid squares, ■-■, replotted from Fig 1) and after IM injection (solid circles, ●-●, replotted from Fig 6) of 1 mg/kg furosemide. Control rates of urine formation were subtracted from all values so the points represent diuresis due to furosemide only. The open squares (□-□) and circles (○-○) show plasma levels of drug after similar doses of furosemide, replotted from Roberts *et al.*[14] Plasma levels of furosemide were superimposed on urinary flow rates by multiplying all plasma levels by 0.2. The approximate half-lives for the diuretic effect after each route of administration compare with kinetically determined plasma half-lives for furosemide of about 30 and 86 minutes, respectively (Roberts *et al.*[14]).

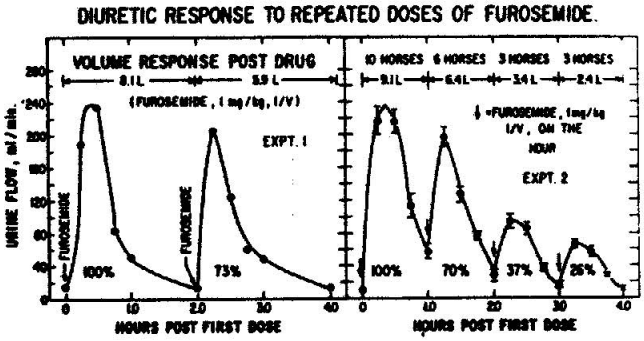

Fig 9—Diuretic response to repeated doses of furosemide. The left panel (Experiment 1) shows the diuretic response to 1 mg/kg of furosemide IV at indicated zero time and 2 hours, respectively. All data points are the means of experimental determinations on 4 different horses. The right-hand panel (Experiment 2) shows the effect of 4 doses of 1 mg/kg furosemide IV. The liter figures refer to the total volumes of urine voided between doses, while the percentage figures express the diuretic response as a percentage of the response to the first dose. No access to water was allowed during these experiments.

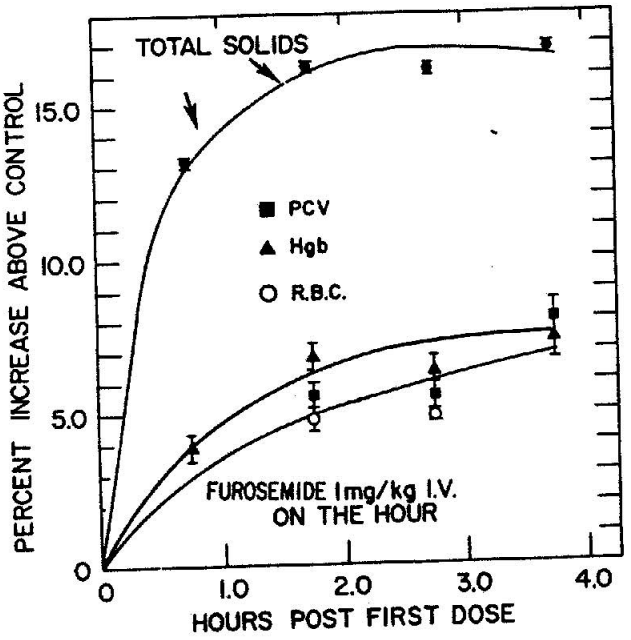

Fig 10—Effect of repeated doses of furosemide on total solids, hematocrit, hemoglobin, and RBC content. Blood samples were drawn from the horses of Experiment 2 (Fig 9) at 45 minutes after each dose of furosemide. The solid circles (●-●) show total plasma solids, the solid squares (■-■) hematocrit, the solid triangles (▲-▲) total hemoglobin, and the open circles (O-O) RBC. All values are plotted as percent increase over control values, ± the standard error of the means.

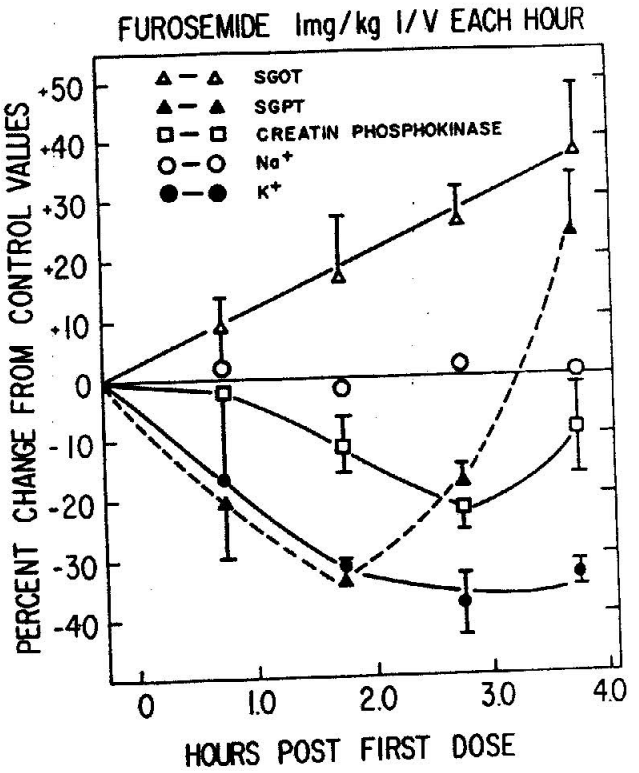

Fig 11—Effect of repeated doses of furosemide on plasma enzymes and monovalent cations. Blood samples drawn from the horses of Fig 9 (Experiment 2) at 15 minutes prior to furosemide and 45 minutes after each dose were analyzed for Na^+ (open circles, C-O), K^+ (solid circles, ●-●), SGOT (open triangles, Δ-Δ), SGPT (solid triangles, ▲-▲), and CP (open squares, □-□). All values represent means ± standard errors of the means on 3 or more horses, and all values are expressed as a percentage of control values at 15 minutes prior to furosemide.

course of experiments of Fig 9 approached the maximal amount of fluid available to this dose level of furosemide in these horses.

Throughout Experiment 2 (Fig 9) blood samples were drawn at 45 minutes after each IV dose of furosemide and analyzed for total protein, Na^+, K^+, SGOT, SGPT, CP, hematocrit, hemoglobin, and total RBC. Figures 10 and 11 show the changes observed. Hematocrit, hemoglobin, and total RBC all increased up to about 8%, whereas about twice this change in total plasma solids were observed. In each case the bulk of the effect was observed with the first dose, and the increases thereafter were considerably smaller.

Figure 11 shows the effect of repeated doses of furosemide on plasma Na^+, K^+, SGOT, SGPT, and CP. Again, no significant change was seen in plasma Na^+ concentration, while plasma K^+ was markedly reduced. No clinically significant changes in any of the plasma enzyme levels were observed.

In another sequence of experiments, 4 horses were dosed daily with 1 mg/kg furosemide IV for 4 days with blood samples being drawn 15 minutes before and 45 minutes after each dose. This experiment was designed to test for any cumulative change in these parameters, either predrug or at peak drug effect (45 minutes postdosing). Again, total protein, plasma Na^+, K^+, SGOT, SGPT, hematocrit, hemoglobin, and total RBC were followed. No clinically significant cumulative effects on any of these values were observed.

The effect of furosemide on the performance of Standardbred horses is presented in Table 2. The dose of 0.55 mg/kg IV or about 5 ml/1,000 lb (453 kg) horse was selected on the advice of Dr. Gene Bierhaus of the Colorado Racing Commission. The test time of 30 minutes after dosing was selected on the basis of the very brief pharmacological action of furosemide and its rapid elimination, demonstrated in this and earlier reports from this laboratory.[14] The data show no statistically significant effects of this dose of furosemide to improve the performance of these horses at 30 minutes after dosing. Table 2 shows the times observed for Standardbred horses racing on furosemide at Louisville Downs this summer. Pre and postfurosemide times were compared for horses which elected to go on furosemide medication during the meet.

TABLE 2

Effect of Furosemide on Time Trials in Standardbred Horses

Trial	Horse	Saline	Furosemide	Δ
1	Noelle	131	141	+10
2		135	134	− 1
3		135	129	− 6
4	Doc Stultz	134	135	+ 1
5		134	132	− 2
6	Beau	142	136	− 6
7		139	141	+ 2
8	Ballards	143	142	− 1
9	Love Bug Wilson	134	134	0
All Horses		136.33	136.00	−0.33

Time trials were performed as described in Methods. All values represent times in seconds required for these horses to pace 1 mile. The Δ symbol refers to the change from the matched control associated with furosemide administration. Applying a t test to all data points, $t = 0.2$, less than one-tenth the value required for significance at the 0.05 level.

The data show no significant difference betwen the times of these horses before and after they went on furosemide.

Discussion

The most surprising result from the present studies has been the apparently fast onset and decline in the diuretic effect of furosemide after IV injection (Fig 1). At all doses and by all routes tested (Figs 1 and 7), the diuretic effect of furosemide peaked between 15 and 30 minutes postinjection. At all IV doses up to 1 mg/kg, the bulk of the diuretic effect was over within 1 hour. Similar results have been reported by Muir *et al.*[12] who observed apparent spontaneous voiding of urine for the first 55 minutes after 1 mg/kg of furosemide IV and no voiding during the following hour. Other work by Garner *et al.*[8] did not report the time course of diuresis due to furosemide.

The rapid peaking and decline in the diuretic effect of furosemide appears to be due to rapid elimination of the drug. Inspection of the data of Roberts *et al.* (Fig 6)[14] shows that, between 10 and 60 minutes postinjection, plasma levels of furosemide fell from 3,000 ng/ml to about 150 ng/ml. This 20-fold drop in plasma concentrations of the drug is sufficient to account for the rapid decline in the pharmacological response to furosemide. The decline in response was not due to any limited availability of fluid to excrete, because a second dose 1 or 2 hours after the first immediately produced a good diuresis (Fig 9). The interpretation that the decay in the diuretic effect of furosemide is primarily due to clearance of the drug is well supported by the data of Fig 8, which shows a close relationship between diuresis due to furosemide and its plasma levels after IV or IM administration.

Intramuscular administration of furosemide resulted in a more prolonged diuretic effect that after IV injection (Fig 7) and in the excretion of about 50% more urine (Table 1). This prolonged diuretic effect after IM injection correlated well with the more prolonged plasma levels of furosemide observed after IM injection. Therefore, in a situation where a more prolonged or more effective diuretic response to a given dose of furosemide is required, IM injection would appear to be the route of choice.

In these horses, 0.04 mg/kg appeared to be the threshold dose for significant renal effect after IV injection. Below this dose, little effect on urinary Na^+, K^+, or volume was observed. At or above this dose level, urinary Na^+ concentration was maximally increased in what appeared to be an all or none effect (Fig 3). In contrast to this effect on Na^+ concentration, increasing doses of furosemide produced a graded increase in volume and a graded decrease in urinary K^+ concentration (Fig 3). The upshot of these relationships is that while increasing concentrations of furosemide produced an increasing output of urine and also of Na^+, increasing the dose had very little effect on K^+ output. As shown in Figs 4 and 5, loss of K^+ was approximately maximally increased by the lowest dose of furosemide tested and was not further increased by higher doses.

Studying the effects of doses of up to 18 mg/kg furosemide IV in horses, Garner *et al.*[8] reported no significant effects on K^+ excretion. The discrepancy between the results reported here and those of Garner and coworkers[8] is probably due to the fact that these workers pooled all urine samples obtained in the first 6 hours postdrug. As shown in Fig 5B, the effect of furosemide on K^+ output is small and occurs within the first hour. Therefore pooling samples for the first 6 hours would tend to dilute out the effect of furosemide on K^+ excretion and render it more difficult to detect. In addition, the dose response data of Fig 4 show, if anything, a tendency for the effect on K^+ output to remain the same or possibly decrease as the dose was increased. For this reason, even the larger doses used by Garner *et al.*[8] would not tend to overcome this problem.

After intravenous administration of furosemide, the changes in blood values (Fig 6) closely followed the time course of the diuretic effect. Hematocrit values increased about 6%, peaking between 20 and 30 minutes postdosing and then declining. Total plasma solids also increased, but to about 10%, and then declined back to baseline.

Plasma Na^+ levels showed no change, while plasma K^+ levels declined and then turned toward normal.

Muir and co-workers[12] observed similar discrepancies between the effects of furosemide on hematocrit and total solids, and Fregin and co-workers[6] suggested that the effect was due to albumin moving into the circulating compartment. The same effect has also been observed in human plasma, where total colloid osmotic pressure was increased about 15% after a dose of furosemide, compared with a 5% increase in hematocrit.[9] Fregin *et al.*[6] chose to interpret this phenomenon in terms of a furosemide induced net entry of albumin into the vascular compartment but failed to suggest a site or mechanism for such an effect. The reduction in plasma K^+ reported here is similar to that reported by Fregin *et al.*[6] and in good agreement with the observed increase in urinary loss of K^+ after furosemide (Fig 5).

The shape of the curves for the increase in total solids and hematocrit post-IV furosemide reported here (Fig 6) differs considerably from those reported by Muir *et al.*[12] These workers did not observe the sharp peaking of these values at 30 minutes and subsequent rapid return to control values reported here (Fig 6). The reason for these differences is not clear but may relate to the fact that the horses in these experiments had free access to water, while those of Muir *et al.*[12] were likely restrained in stocks and had no access to water.

Another unexpected finding from these studies was the close relationship between plasma levels of furosemide and its diuretic effect. Based on studies on the effects of probenecid on the saluretic effect of low doses of furosemide, Hook and Williamson[10] suggested that either cellular or luminal (urinary) furosemide levels would be most closely related to the natruretic activity of this compound. However, inspection of Fig 9 in the paper of Roberts *et al.*[14] shows that, after IM injection of furosemide, its urinary concentrations rose steeply throughout the diuretic effect, while plasma levels of the drug were falling in parallel with diuretic response. The urinary data are in apparent contradiction with the observations of Burg and Stoner[4] who report that *in vitro* furosemide only acts from the luminal surface of the renal tubule. However, it is difficult to relate bladder concentrations of furosemide (such as were reported by Roberts *et al.*[14]) to concentrations at receptor sites in tubules, especially since urinary output was falling (and therefore drug concentration in urine rising) throughout the experimental period (Fig 9).[14]

In contrast to the apparent lack of relationship between urinary levels of furosemide and the diuretic effect, plasma levels of furosemide were closely related to the diuretic effect (Fig 8). This slightly faster decay of the diuretic effect when compared with plasma levels of the drug probably reflects the fact that we were unable to rehydrate these horses. As shown in Fig 9, the diuretic response to furosemide declined as fluid was eliminated by these animals. It appears probable that a much closer plasma level:diuretic effect relationship could be demonstrated in an experimental situation in which hydration of the test animals could be maintained.

These observations on the correlation between plasma levels of furosemide and its diuretic effect are also in good agreement with some recent work in man. Studying chlorothiazide[12] and more recently furosemide,[†] Brater has observed a probenecid-induced prolongation and enhancement of the diuretic response to both drugs in human subjects. Probenecid apparently inhibited tubular secretion of these drugs, prolonged their plasma half-life, and thus gave rise to a prolonged and enhanced clinical response. In the study reported here, IM administration of furosemide also acted to prolong its plasma half-life and similarly prolonged and enhanced its diuretic response (Fig 7). Similarly, Branch and co-workers[1] have recently reported a linear relationship between plasma levels of furosemide and the rate of Na^+ excretion in man, in good agreement with the results reported here.

Studies on the performance effects of furosemide posed the problem of selection of a dose and a suitable time period postdosing to conduct the test. The dose of 0.55 mg/kg (about 5 ml of 50 mg/ml furosemide IV to a 1,000-lb horse) was selected on the advice of Dr. Gene Bierhaus of the Colorado Racing Commission. A time of 30 minutes between dosing and exercise was selected because this was the time at which peak changes in total plasma solids and hematocrit were observed (Fig 6). Other workers[7] have selected doses of furosemide of 1 mg/kg IV and waited 130 minutes before their performance tests. Judging from data reported in this paper and elsewhere,[14] a 130-minute delay would allow 90% of the drug to be eliminated and most or all of the acute cardiovascular and hematological effects to dissipate.[12] Performing trials under these circumstances hampers interpretation of negative data, since the most likely interpretation becomes dissipation of the drug effect. We therefore elected to run the trials reported here as close to peak drug effect as possible. As shown in Table 2, no statistically significant effect of pretreatment with furosemide on the times to pace 1 mile were observed. These data are apparently in good agreement with those

[†] Brater, D.L., Department of Pharmacology, Univ. of Texas Southwestern Medical School, Dallas, TX: Personal communication.

of Gabel and co-workers[7] who reported a small but statistically nonsignificant improvement in the performance of horses pretreated with furosemide in drug trials similar to those reported here.

The principal problem with the drug trial study reported in Table 2 and that of Gabel *et al.*[7] is the small number of animals involved. Thus, if a single trial (trial #1) in which an unusually slow time for furosemide was observed is eliminated, furosemide then improved the performance of 4 out of 5 horses and had no effect in one. Under these circumstances the probability that furosemide improved performance becomes 1 in 16, close to but still less than the 1:20 required for statistical significance. We therefore decided to perform a retrospective study of the effect of furosemide on racing performance of Standardbred horses racing under the furosemide medication rule in Kentucky in 1977.

Through the cooperation of the Kentucky Harness Commission, the list of horses electing to go on furosemide medication during the 1977 Louisville Downs Summer Meet was made available to us. From the names of these horses and the date on which they went on furosemide, their performance times pre- and postfurosemide were obtained from the published race programs. A comparison of these times showed that the mean times to pace 1 mile of the 58 horses selected for study was actually increased by 0.1441 seconds in the horses treated with furosemide. This small slowing in their performance times by furosemide was not statistically significant. The data show that the average horsemen and presumably their veterinary advisors at Louisville Downs were not able to improve the performance of horses during this meet by medication with furosemide. It should be borne in mind that nothing is known about the doses of furosemide used, the times before racing that they were administered, or the conditions which prompted administration of the drug. These observations support the results obtained in the performance trials reported here and those of Gabel and co-workers[7] and suggest that furosemide has no effect on the time to pace 1 mile of Standardbred horses.

Because of the widespread use of furosemide in racing horses, questions arise concerning the safety of repeated doses of furosemide. Studies by the manufacturer indicate that the acute toxic IV dose of furosemide is not less than 300 mg/kg. Irreversible deafness has been reported in human patients receiving massive doses of furosemide.[3] Other workers have reported enhanced tubular necrosis in patients simultaneously receiving furosemide and cephaloridine.[5] Further, very high doses of furosemide in mice have been shown to be metabolized to a chemically reactive metabolite which produces massive hepatic necrosis.[11]

Because of these considerations we studied the effect of repeated dosing with furosemide on a number of blood values. Figure 9 shows that at 1 mg/kg IV the diuretic response to furosemide was self-limiting, and the data suggest that at this dose level about 25 liters of fluid can be obtained from a 1,000-lb (453 kg) horse. Once this amount of fluid was withdrawn, the animal became resistant to further 1 mg/kg doses, and little further diuresis or hemoconcentration was seen. No clinically significant changes in plasma enzymes were seen in this experiment, but plasma K^+ was markedly reduced. Hypokalemia would thus seem to be the most likely acute toxicity problem to be encountered during repeated dosing with furosemide in this horse. However, the horses in these experiments appeared clinically normal at the end of these repeated dosing experiments. Similarly, in the experiments in which 1 mg/kg furosemide was administered IV daily to horses for 4 days, no cumulative deviations

TABLE 3

Effect of Medication with Furosemide on the Performance of Horses Racing at Louisville Downs, Summer, 1976

	# of Horses	# of Trials	Mean Times	S.E.M.	
Prefurosemide	58	160	128·5925	0.2031	F = <0.00
With Furosemide	58	232	128·7366	0.1594	(F for significance should be >3.0)

At this meet furosemide was the only permitted medication, and its use was monitored by urinalysis. Horses could elect to go on furosemide at any time throughout the meet, but once on furosemide had to stay on it. Performance times for horses pre- and postfurosemide treatment were obtained from the meet programs and compared. Only times on good or fast tracks were taken. For the 58 horses selected, 160 prefurosemide times were available and 232 postfurosemide times. A randomized block design was used where each horse represented a block. After adjusting for blocks (i.e., differences between horses), there was no significant difference between treatments (i.e., times on and off furosemide).

from control values were seen in any of the parameters tested.

These results support the conclusions of Gabel and co-workers[7] that, at the commonly used doses, furosemide is a safe drug in the horse. From these studies it is clear that these are relatively small doses (0.5–1 mg/kg) of the drug, and that when administered IV their action is brief and the drug is rapidly eliminated. The 8–9 liters of water eliminated after a 1 mg/kg dose IV is only about 2.8% of the total body water in a 1,000-lb (453 kg) horse. If access to water is restricted, this deficit can presumably be distributed throughout total body water. Given access to water, this deficit is probably readily replaced and accounts for the rapid reversal of the hemoconcentration observed in Fig 6. The sharp decline in plasma K^+ after furosemide (Figs 6 and 11) must acutely reduce the turnover of membrane Na^+K^+-ATPase[16] and increase the net flux of K^+ along the leak pathway to extracellular sites, accounting for the rapid reversal of the decline in plasma K^+ levels observed in Fig 6. Because of the large excess of K^+ in the normal equine diet and the ready availability of intracellular K^+ for the plasma pool, acute hypokalemia would seem an unlikely event in the normal horse after single doses of furosemide. However, the possibility of clinically significant hypokalemia after large or repeated doses of furosemide is very real (Fig 11) and deserves further investigation.

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