376 Part 2

CORRUPT HORSERACING PRACTICES

HEARINGS

BEFORE THE

SUBCOMMITTEE ON CRIMINAL JUSTICE

OF THE

COMMITTEE ON THE JUDICIARY

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ON

CORRUPT HORSERACING PRACTICES

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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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Furosemide in Horses: A Review

Albert A. Gabel, D.V.M., M.S.
Thomas Tobin, D.V.M., Ph.D.
Richard S. Ray, D.V.M., M.S., Ph.D.
George A. Maylin, D.V.M., M.S., Ph.D.

From the Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by: Gary P. Carlson, University of California; Harold E. Garner, University of Missouri; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; William W. Muir, Ohio State University, and David Snow, University of Glasgow, and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Finley, A.V. Villalico, Robert E. Vessey, Dr. Francis Osog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Bierhaus, and General Wayne Kester.

1. Injectable furosemide[a] is a potent, effective, safe diuretic which at the usual dose has its peak diuretic effect in about 20 minutes in horses, at which time it increases urine output up to 40-fold for a short time. Most of its diuretic effect is within the first 2 hours after injection.

2. During the first 2 hours, it decreases the pressure in the right side of the heart, decreases left atrial pressure, decreases cardiac output, and concurrently it increases peripheral (systemic) blood vessel resistance and heart rate.

3. It reduces edema in the lungs and airway of horses with certain respiratory diseases by 1 or more mechanisms, perhaps by lowering the pressure of the left atrium and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.

4. The mechanism by which furosemide helps prevent epistaxis may result from the beneficial effect outlined in (3) above since most horses bleed from the lungs. Lasix[a] also appears to affect the platelets of horses during exercise.

5. The blood (plasma) level of furosemide declines rapidly. It can be detected in the urine by screening methods for the first 24 hours and by more sophisticated methods for up to 48 hours after injection.

6. Furosemide does not affect the blood levels of other drugs.

7. Furosemide has little effect on the concentration of certain drugs, such as procaine, in the urine. However, during its peak diuretic effect, within 2 hours after injection, furosemide causes up to 40-fold dilution of certain other drugs such as phenylbutazone. However, furosemide administered more than 4 hours before race time does not significantly reduce the ability to detect those drugs studied to date.

[a] "Lasix", National Laboratories, Inc., Somerville, NJ 08876

8. There is no evidence that furosemide directly or indirectly affects the behavior of the horse.

9. Furosemide cannot make horses race faster or perform better than their innate ability, but in many cases it restores normal performance of horses which bleed.

Introduction

There is no scientific evidence that furosemide will increase the speed or improve performance of a horse beyond its innate capability. In a recently completed double-blind study there was no significant difference in the times of time-trial miles of clinically normal Standardbreds after intravenous furosemide injection compared to saline injection, 2 hours and 10 minutes before the trials.[19] A mechanical prompter was used for uniform psychological stimulation. However, clinical observations indicate that the drug may help a horse to more nearly reach his racing capacity if he is a bleeder, or has certain other conditions, including perhaps early obstructive lung disease "heaves" or diseases in which pulmonary edema occurs, even transiently, during exercise.[12,16]

Mechanism of Action (Diuretic Effect)

The primary effect and use of injectable furosemide has been as a potent diuretic. It has a high degree of efficacy, low-inherent toxicity, and a high therapeutic index in horses.[13] Furosemide produces the increase in urine by inhibiting active electrolyte (inorganic ions such as chloride) transport in that part of the kidney known as the diluting segment of the ascending limb of the loop of Henle.[1,8,21,25] Furosemide produces increased losses of chloride, sodium, other electrolytes, and water.[13] There is little potassium loss in horses.[13] In normal horses, no dehydration or electrolyte imbalance occurs if they have access to water and salt before and after the injection.[22] Its onset of action is rapid.[11,13,20,21,25] The diuretic effect of furosemide peaks within 15 to 30 minutes, when there is up to a 40-fold increase in urine production for a short time.[13,26] Dosed at 1 mg/kg body weight, most of its diuretic effect occurs within 2 hours.[24–26] Doses between 0.01 and 1.0 mg/kg (the dose recommended by the manufacturer) produce a graded increase in urine volume and decrease in its specific gravity.[13,26] The average urine production during a 2-hour period after injection of the usual dose of furosemide is 3 to 12 liters and is dependent on the horse's state of hydration when the drug is given.[20,21,26] Only 1 liter of urine is produced during an average 2-hour control period.[20] Due to the loss of fluid during the period of increased urine production, horses which are dehydrated before the injection make less urine during several hours which follow; therefore, it is sometimes difficult to get a urine sample during this period.[13] Race Commission veterinarians do not find this to be a significant problem.[13]

Furosemide is used by many veterinarians in the treatment of acute tieing-up and azoturia to increase urinary output, since high concentrations of the pigment from damaged muscles can cause kidney shutdown.

Cardiovascular Effects

In resting horses, in addition to increased urine production, the following changes were found to be statistically significant ($P<0.05$)[20] from 15 minutes to 110 minutes after intravenous injection of the usual dose (1 mg/kg) of furosemide:

1. Decreased blood pressure in the right side of the heart, pulmonary artery, and left atrium. This may be due to hypovolemia and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.
2. Decreased venous return and therefore decreased cardiac output.
3. No significant change in systemic arterial blood pressure, although there was a significant increase in peripheral (systemic) vascular resistance and reflex increase in heart rate.
4. Hemoconcentration. Packed cell volume and total protein percentage in the blood increased.

In a recent double-blind study intravenous furosemide injection was compared to saline injection under simulated race conditions using Standardbreds.[19] Hemodynamic and biochemical data were recorded before and during the first "warm-up miles" 15 minutes after injection and before, during, and after the third (time-trial) mile 2 hours and 10 minutes after injection. After furosemide, compared to saline, pulmonary artery pressure was lower during the first warm-up mile, 15 minutes after injection, but not during the time-trial mile 2 hours and 10 minutes after injection. There was no difference in cardiac output immediately after and 10 minutes after each of the miles, nor in the pulse rate during or after each of the miles.

These experiments suggest that, if given during the 2-hour period prior to a race, furosemide might have a negative effect on performance because of a decreased cardiac output.

Respiratory Effects

A clinically important action of furosemide in both man and small domestic animals is its rapid action against pulmonary edema. Since this effect also occurs in patients in which this drug does not produce diuresis, and in patients which are kept hydrated, the volume change is not totally due to diuresis.[1,12]

Recent observations suggest that the pulmonary effects of furosemide may be mediated by prostaglandins released from the kidneys.[1,12] Another theory is that there may be a tendency for lung edema to develop when mean pulmonary artery pressure exceeds 40 mm Hg by very much.[1,12,27] Average mean pulmonary artery pressures of approximately 70 mm Hg have been measured in Standardbreds during time trials.[16] Edema (which may occur transiently) has a negative effect on breathing reflexly through the J-fibers of the vagus nerve.[12] The more severe the exercise, the greater the tendency for pulmonary edema to occur. This may explain why furosemide is used more in Thoroughbreds, which work harder, than in Standardbreds.[13]

The experience of race track veterinarians indicates that the drug appears to have an effect of producing a more nearly optimal performance in Thoroughbreds which have heavy or labored breathing while training or racing, in those which have nasal exudate after racing, and in some which become excited in the paddock.[12]

It is thought that horses with edema and narrowing of the upper airway, especially the pharynx by such conditions as follicular pharyngitis, may race more normally following the use of furosemide, because tissue fluid is removed, enlarging the airway toward normal size.[13,27]

Epistaxis

It is thought that in most cases the blood comes from rupture of small vessels in the lungs, and sometimes the upper airway, when horses have epistaxis following work.[8,16] The experience of many veterinarians shows that furosemide injection approximately 4 hours before exercise is effective in preventing epistaxis in a high percentage of Thoroughbred and Standardbred horses which bleed during or after racing or hard workouts.[13]

In a double-blind study under simulated racing conditions, Standardbred horses were injected with 1 mg/kg of furosemide or saline 15 minutes before the first warm-up mile.[19] Hemostatic function including the blood clotting mechanism and platelets were evaluated prior to injection, immediately following the first warm-up mile, and after the time-trial mile 2 hours and 10 minutes after the injection. There was a slight increase in the retention of platelets in glass bead columns, but this platelet function change could not be substantiated in a follow-up study using furosemide injections in nonexercised horses. No significant changes were found in screening tests of the blood clotting mechanism.

Furosemide may be effective in horses which bleed by reducing the blood pressure of the left atrium and increasing capacitance of the lung vessels.[16]

Blood (Plasma) and Urine Levels—Detection

Furosemide is usually administered to horses by intravenous (IV) or intramuscular (IM) injection at doses of 1 mg/kg or somewhat less. After IV administration of 1 mg/kg, its plasma levels decline rapidly, from about 10 μg/ml (parts per million) initially to 500 ng/ml (parts per billion) at 20 minutes.[26] Thereafter, plasma levels fall more slowly with a half-life of about 30 minutes, to less than 20 ng/ml at 4 hours.[26] Urinary levels of the drug start at about 30 μg/ml and fall rapidly over the first 12 hours to about 500 ng/ml.[26] This is about the detection limit of the thin layer techniques used in routine drug testing screens.[26] However, using gas chromatographic (G.C.) methods, urinary levels of the drug can still be detected up to 48 hours postdosing.[26]

Effects of Furosemide on the Concentration of Other Drugs in Equine Blood and Urine

In no instance is furosemide known to significantly reduce the blood or plasma level of any drug.[24,26]

Treatment with furosemide does not appear to significantly affect the concentration of basic lipid soluble drugs in equine urine.[26] Thus, furosemide has no effect on either plasma or urinary concentrations of procaine in horses treated with 10 mg/kg of procaine HCl intramuscularly.[26] Similar results were observed with methylphenidate[9,26] In both cases, urinary output of the drug was increased by the greatly increased urine volume, and the concentration did not decrease enough to cause trouble in detection of the drugs.[26] The same effect probably occurs with amphetamine.[26] Glucuronide and sulfate conjugates of narcotics can be diluted up to several fold.[9]

In contrast, however, urinary concentrations of phenylbutazone are reduced up to 40-fold at peak diuresis.[16] This concentration change can greatly reduce the reliability of routine UV screening for phenylbutazone.[23] Concentrations of phenobarbital also are probably affected, though not quite so severely.[9]

In doses usually used, furosemide can greatly reduce the urinary concentrations of some drugs, but only during the first 2 hours and for a much lesser extent up to 4 hours after it is administered; in other drugs it has little effect. In particular, use of furosemide can interfere with routine screening for phenylbutazone during this period of increased urine production (2 to 4 hours). For this reason, blood testing for phenylbutazone should be recommended where medication rules permit the use of furosemide within 4 hours of racing.

Ritalin Ciba Pharmaceutical Co., 556 Morris Ave., Summit, NJ 07901.

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premature closure of these terminal airways.[10] Since equidae have poor collateral ventilation, restricted movement of a portion of the lung resulting from airway obstruction may result in decreasing intra-alveolar pressure as discussed by Robinson.[11] Strenuous exercise with elevated capillary pressure will further increase the transmural pressure and the possibility of rupture of pulmonary capillaries.

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Chapter 7

BLEEDER MEDICATION: LASIX® (FUROSEMIDE) AND OTHER DRUGS

THE HISTORY OF BLEEDERS is at least as old as Thoroughbred racing itself. Bleeders have been recorded among the earliest Thoroughbred horses and are still with us to this day. Bleeding, known technically as "epistaxis," consists of hemorrhage from the nostrils of a horse, usually either during or after severe exercise.* In racehorses, bleeding is almost always bilateral and from the lungs; this is what is considered the classic "bleeder."

The loss of blood from classic exercise-induced epistaxis is rarely severe, and death in association with exercise-induced epistaxis is essentially unknown. There is apparently no defect in blood coagulation in these horses, and the bleeding stops shortly after the exercise is stopped. The problem with bleeding of this type is that horses that bleed severely may slow down abruptly or stop in the stretch. This can lead to accidents and injury to other horses and jockeys. In addition, the sight of horses pulling up covered with blood and blood-spattered jockeys is not considered good for the image of racing, and for these reasons most racing authorities have regulations and restrictions on the running of bleeders.

The true bleeder bleeds not from the head area but from the lungs. Because of the horizontal layout of the horse's respiratory tract, the blood comes up from the lungs as a trickle or stream and appears at the nostrils as a stream. This is in contrast to the situation in man, where blood brought up from the lungs comes as a foam, as this is the only way it can be moved up the vertical human trachea. If a horse bleeds from one nostril, the chances are that the blood is coming from the head area and he is not a true or pulmonary bleeder.

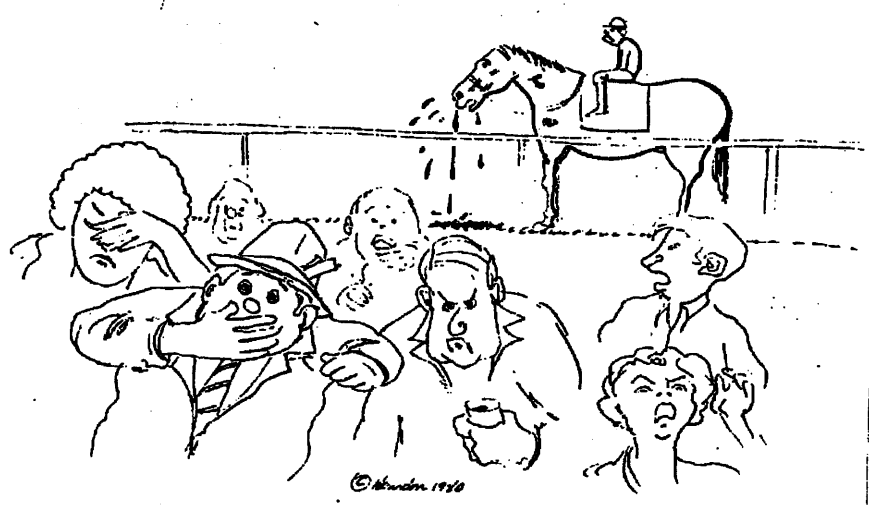
Because a bleeder is unsightly on the track and under some circumstances can rack up a whole field of horses, most racing authorities have strict regulations about bleeders. In New York, a first-time bleeder is put on the "vet's list" for a minimum of two and one half weeks. At the end of this period the horse is worked ⅝ of a mile, and if the horse does not bleed he is permitted to race again. If the horse bleeds a second time he is stopped for three months, and if he bleeds a third time he is stopped for six months. The fourth time a horse bleeds in New York, he is barred as a chronic bleeder.

Because of these stringent regulations for bleeders, there has long been a search for a drug that, when administered pre-race, will prevent bleeding. Among the drugs that have been used for this have been drugs that are thought for various reasons to increase blood coagulability, even though there is no known coagulation defect in bleeders.

Oxalic and malonic acids, which bind calcium ions in the blood, have been used

* Since the classic pattern of pulmonary bleeding occurs after exercise, this syndrome is now known as Exercise-Induced Pulmonary Hemorrhage, or E.I.P.H.

Drugs and the Performance Horse



The sight of blood-spattered horses pulling up is not considered good for the image of racing.

historically for bleeders. The rationale behind this approach was that if one produced a small reduction in free calcium ion in the blood, one could accelerate the clotting process and thus prevent bleeding. While small amounts of oxalic and malonic acid will indeed tie up calcium ions, there is no good reason to think that this will affect the incidence of bleeders in any way, and Cook regards the success reported by some workers with these drugs as surprising. Similarly, the approach of treating horses with Vitamin K would appear to be an exercise in futility, as Vitamin K deficiency is associated with a clotting defect and there is no reason to suppose that there is a clotting defect in these animals.

Other preferred and used therapies have included bloodletting, presumably on the hypothesis that reducing blood volume will tend to reduce blood pressure. Premarin®, which is a preparation of conjugated estrogens (principally oestrone sulfate) from mare's urine, has also been used. This drug has been reported to accelerate blood clotting in dogs and is presumably used in horses for this reason, again despite the fact that there is no evidence for a clotting defect in bleeders.

Another approach to the bleeding problem that has been used for a long time is the withholding of water from the horse for about nine hours before a race. This will have the effect of reducing fluid volume in the horse, and reports from trainers suggest that this approach is successful. It is likely that it is the reported success with this approach that first gave rise to the use of Lasix® in an attempt to control the bleeder problem.

Next to Butazolidin, Lasix® is the most controversial drug in racing. Lasix is a potent diuretic drug, which causes up to a fiftyfold increase in the amount of urine voided in horses and man (Fig. 7-1). It produces this effect within minutes

after its intravenous or intramuscular injection. Lasix is, in general, a safe drug. It is about the fifth most commonly prescribed drug in human medicine, a tribute to safety and efficacy. As will become apparent as this story unfolds, Lasix is just as effective in the horse as in man, and there is no reason to think that it is any less safe in the horse than it is in the human.*

Lasix was introduced into equine medicine by Doctor Marvin Beeman and his colleagues in Colorado. It is useful in edema (excess fluid in the tissues) from all causes. It is particularly useful in cases of pulmonary (lung) edema, because it acts to move this fluid out of the lungs within minutes, even before it causes any increase in the rate of urine formation. Lasix is now standard emergency therapy in the human in cases of pulmonary edema.† Its ability to increase the volume of urine may also be used to dilute substances and prevent kidney damage. Thus, when a horse "ties up," the released muscle pigment may deposit in and damage his kidneys. In situations such as this, the increased urine volume due to Lasix acts to dilute the pigments and greatly reduces the possibility of renal damage. As we will see later, Lasix can produce the same effect with some drugs, diluting them "out" in urine and rendering their detection more difficult.

* NOTE: All the work reported from the Kentucky Equine Drug Research program refers to injectable Lasix®, 50 mg/ml, from National Laboratories Corp., subsidiary of American Hoechst Corp., Somerville, NJ. Most of the research on furosemide reported here was done in my laboratory in cooperation with Brian Roberts, whose MSc thesis was on the pharmacology of furosemide in the horse.

† Pulmonary edema = increased fluid in the lungs

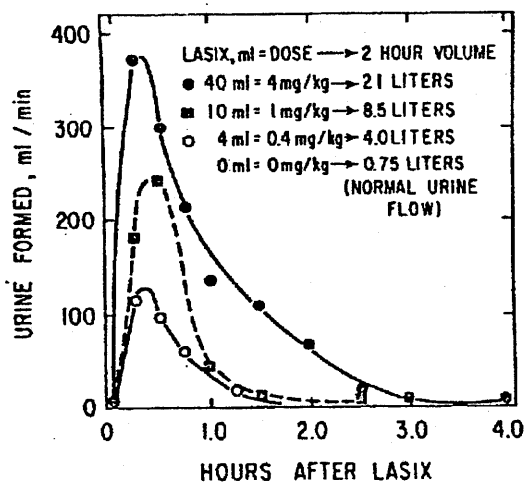
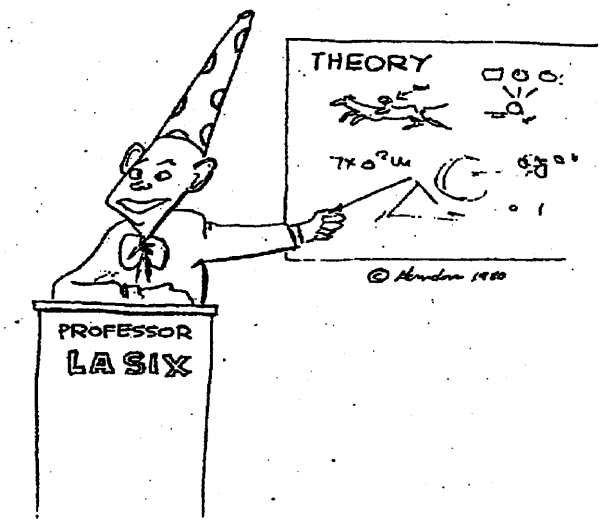


Figure 7-1. Dose and time response relationships for the diuretic response to Lasix®. The symbols show the time course of the diuretic response to an antiepistaxis dose of Lasix (open circles, o—o), a typical clinical dose of Lasix (solid squares, ■—■), and a very large dose of Lasix (solid circles, ●—●). Note that for the antiepistaxis (antibleeding) and the clinical dose, the bulk of the response is over well within one hour of dosing. All doses were administered by intravenous injection in four horses.



When our research started there were a multitude of theories about what Lasix did to horses.

Based on clinical experience with the drug, a large number of veterinarians and trainers came to believe that Lasix was an effective drug in the treatment of epistaxis. According to such clinical reports, about 200 mg (4 ml) of Lasix given intravenously or intramuscularly within one to three hours before racing either prevents epistaxis or reduces the risk of epistaxis. Based on this knowledge and the known small incidence of bleeders, many racing commissions approved furosemide for use in racing horses as prophylaxis against epistaxis.

It turned out, however, that while only a small percentage of horses suffer from epistaxis, much larger proportions of horses were being run on Lasix in jurisdictions where Lasix was a permitted medication. Many racing jurisdictions that had approved Lasix for use in bleeders found upwards of 50 percent of their horses were at times being run on Lasix. Therefore, among the questions that we hoped to answer when we studied the pharmacology of Lasix in the horse was the question of why is the use of Lasix so popular in horses that are not bleeders?

Backstretch gossip can suggest an enormous number of reasons for the use of any drug, and it was not short of suggestions about Lasix. Lasix, it was said, could "move horses up"—it would improve their performance, and this was the reason that it was widely used in horses. It was supposed to be very potent in this regard and could make "pulmonary cripples" run like the wind. Lasix also "flushed drugs" out of horses, so that the chemist would not be able to find them in blood. As well as flushing drugs out of horses, it "masked" them and diluted them in urine, so the chemist could not find them in urine. Lasix increased the blood pressure in horses, which, as everyone knows, makes horses run better. Lasix

concentrated the blood in horses, which, as eveyone knows, makes horses run better. Lasix also calmed horses, which helped them to run better. This multitude of suggestions about Lasix indicated the need for a careful study of its pharmacology in the horse.

In our initial studies on Lasix in the horse, Brian Roberts and I elected to simply characterize its diuretic effect in the horse. If you give a 1000 lb horse 4 ml of Lasix (which is about 200 mg), the horse will produce about four liters of urine, mostly within the next forty minutes (see Fig. 7-1). This is about the dose that veterinarians on the track use in horses to prevent bleeding. At this dose, and if the horse is denied access to water, he will run about 8.8 lb lighter than he normally would. Some individuals believe that this is all Lasix does to a horse, and that it will, therefore, both improve his performance and explain the improvement.

A 10 ml dose of furosemide, which is a common clinical dose, produces about 9 liters of urine, mostly within the first hour after dosing. This cannot be considered a large dose of the drug, and it produces only very transient changes in the blood picture. If the dose is increased to 40 ml (4 mg/kg), the horse will lose about 21 liters of urine within two hours after dosing. One finds that if one gives 40 ml of Lasix either as a single dose intravenously (IV) or in repeated doses every hour, one still gets only about the same response (see Fig. 7-4). This and other data suggest that the maximum yield of urine in a normal horse after Lasix is about 20 to 22 liters of fluid and that the diuretic response is self-limiting.

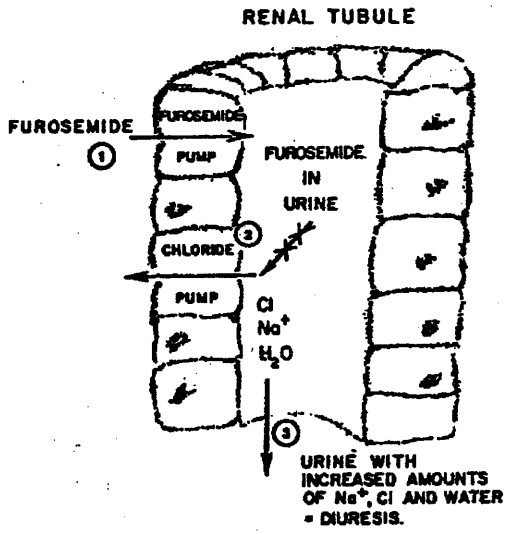


Figure 7-2. Mechanism of action of furosemide. Furosemide is actively pumped (1) into the freshly formed urine in the renal tubules by the organic acid transport system (furosemide pump). Once in the urine, furosemide blocks the reabsorption of chloride from the urine by blocking the chloride pump (2). Since sodium and water reabsorption depends on chloride reabsorption, furosemide administration leads to the elimination of increased amounts of sodium, chloride, and water, or technically, naturesis, chloruresis, and diuresis (3).

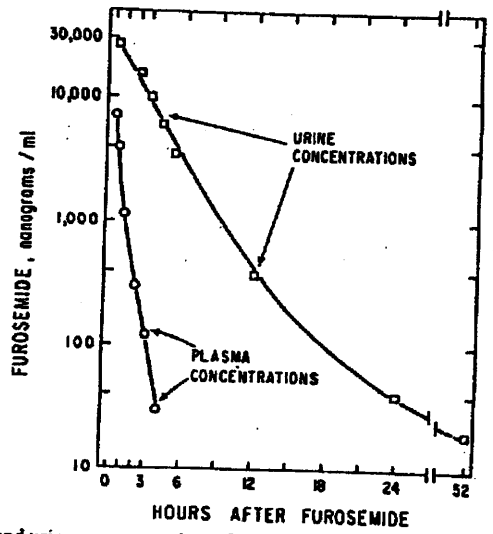


Figure 7-3. Plasma and urinary concentrations of Lasix® after intravenous administration of 1 mg/kg. The open circles (o—o) show plasma levels of Lasix after intravenous administration of 1 mg/kg of Lasix, while the open squares (□—□) show urinary concentrations of the drug. Plasma levels of furosemide fall very rapidly because it is pumped into the urine, where high concentrations of urine are found for long periods of time.

Lasix produces its diuretic effect in the horse directly in the urine by preventing the reabsorption of water in the kidney (Fig. 7-2). Lasix is a strongly acidic drug and is actively pumped out of the blood and into the newly formed urine. In the urine it acts to specifically inhibit another pump, which moves chloride ions from the urine back into the body. Chloride ions (Cl^-) are negatively charged, and positively charged sodium ions (Na^+) and water normally follow the chloride ions back into the body. By blocking the chloride pump, Lasix prevents the kidney from reabsorbing both salt (Na^+Cl^-) and water. Lasix is a particularly potent drug. Whereas normally 99 percent of the water in newly formed urine is reabsorbed during the process of urine formation, in the presence of Lasix only 50 percent may be reabsorbed. Lasix can thus produce, for a short period, a fiftyfold increase in the volume of urine being formed in horses (see Fig. 7-1).

Despite the fact that Lasix is a very potent drug, its action in the horse is very short-lived, especially if it is given by intravenous injection. The most important reason that its action is short-lived is that it is very rapidly pumped into the urine and eliminated. If one looks at the plasma levels of Lasix after it has been injected IV, one finds that they drop very rapidly indeed, with a half-life of a little over thirty minutes. On the other hand, one finds that the concentrations of the drug in urine are high, and that they fall much more slowly than the plasma levels, which one might expect if the drug was being pumped directly into the urine (Fig. 7-3).

The fact that one can get a good response to a second 10 ml dose of furosemide

within one hour of a first dose further supports the idea that it is a lack of blood level of the drug that terminates the diuretic response to Lasix (Fig. 7-4). Further, if you plot the rate of decline of the plasma levels of the drug and compare them with the rate of decline of the diuretic effect, you will find a very good relationship whether the drug is given intravenously or intramuscularly. Based on these observations we concluded that the principal reason for the short action of Lasix after its intravenous injection was that it was rapidly excreted in the urine.

Because furosemide is secreted rapidly and directly into urine, it is found there in quite high concentrations and for a much longer period than it can be detected in blood. Lasix fluoresces strongly under ultraviolet light and under acidic conditions gives a good color reaction in the Bratton-Marshall test. Chemists use both of these reactions to detect Lasix on thin-layer plates. If the Lasix molecule is methylated it runs well on gas-liquid chromatography, and this derivative of the molecule can also be analyzed by mass spectrometry. A good analyst can easily detect furosemide in equine urine for twelve hours after a 10 ml dose, and we were able to detect it in our experiments for up to fifty-two hours (see Fig. 7-3). Because none of the effects of furosemide are likely to last longer than about six hours or be associated with a urinary level of the drug of less than 0.5 µg/ml of furosemide, there appears to be no need for detection of Lasix more than twelve hours after its administration. Racing chemists should bear this in mind when setting the levels of sensitivity of their test systems and not use unduly sensitive tests for this drug.

This rapid excretion of Lasix also means that a very large proportion of a dose of Lasix is excreted unchanged in the urine. While for most drugs in the horse only a very small (5% or less) proportion of the parent drug is excreted unchanged in the urine, at least 60 percent of a dose of Lasix is excreted in the urine. This means that there is no tendency for Lasix to accumulate or build up in the horse,

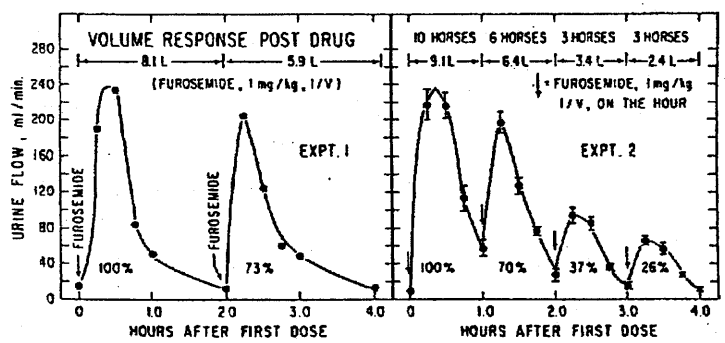


Figure 7-4. Diuretic response to repeated doses of furosemide. To test the hypothesis that it is the rapid elimination of furosemide that terminates its pharmacological effect, repeated doses of the drug were administered. The data show that a second dose within one hour after the first produced a good diuresis, showing that the horse is still able to respond to furosemide. While consistent with the idea that it is drug elimination that terminates the diuretic action after a single dose, the effects of repeated doses show that under some circumstances the availability of fluid to eliminate can be the critical factor. Reproduced with permission from Tobin et al., *J Equine Med Surg*, 2:216, 1978.

TABLE 7-I
CUMULATIVE WATER AND Na^+ LOSS AFTER 1 mg/kg FUROSEMIDE INTRAVENOUSLY AND INTRAMUSCULARLY

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

so the drug is not toxic and doses can be safely repeated at daily intervals. Further, since many drug toxicities are due to drug metabolites rather than to the drugs themselves, the small metabolism and rapid excretion of Lasix makes it that much safer as a drug.

One way of getting partly around the rapid excretion of Lasix is to give it intramuscularly. When given in this way the drug is apparently slowly released from the injection site in muscle into the bloodstream. Its half-life in the blood is therefore about eighty minutes instead of thirty minutes, and the diuretic effect lasts longer. If one gives a 10 ml dose of Lasix by IM injection rather than by IV injection, one gets about a 50 percent greater response and the response is spread over three hours instead of one hour after IV administration. All other things being equal, then, IM administration is probably the most effective way to use Lasix (Table 7-I).

The effects of Lasix on the heart and blood vessels are also transient, peaking within ten minutes and then declining. After IV injection, furosemide reduces blood pressure in the lungs within minutes. This effect occurs independently of the diuretic effect and has been seen in experimental animals in which no urine was being formed. Curiously, however, this effect seems to require furosemide to release a prostaglandin from the kidney. Antiprostaglandin drugs such as phenylbutazone, therefore, may act to block the pulmonary effects of Lasix, a rather surprising finding in view of the frequency with which these two drugs are used together in racing horses.

In the general circulation, Lasix does not affect or reduce blood pressure, despite "off the cuff" comments by people who should know better. Furosemide, however, produces a small increase in heart rate but reduces the actual amount of blood pumped by the heart. After IV injection of the drug at 1 mg/kg (10 ml, more than the usual dose used in epistaxis) the effects of Lasix on the heart and blood vessels are usually over within two hours.

In the bloodstream a dose of Lasix produces an increase in total plasma solids of about 10 percent and an increase in the concentration of red blood cells (hematocrit) of about 5 percent. Despite the fact that sodium is the major cation lost in urine after Lasix, there is never any change in plasma sodium concentrations. Lasix, however, rapidly reduces plasma potassium by about 25 percent. All of these effects occur between ten and thirty minutes after an IV dose, and all values are back to control levels by two hours postdosing (Fig. 7-5).

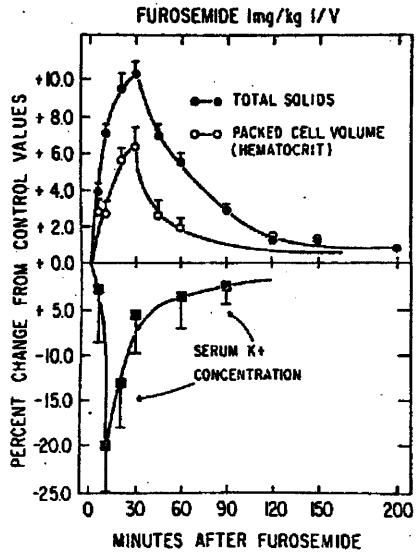


Figure 7-5. Effects of furosemide on some blood parameters. The solid (●) and open (○) circles show the effects of 1 mg/kg of furosemide IV on blood hematocrit (red cell fraction) and total plasma solids. The solid squares (■) show the depression of serum potassium levels produced by this same dose. The effects all peak within thirty minutes after dosing and by two hours postdosing are back to control levels. Reproduced with permission from Tobin et al., *J Equine Med Surg, 2*:216, 1978.

There is a widespread belief that Lasix has an effect on the detection of drugs in horses and that this effect might possibly account for its widespread use in racing horses. One of these is the thought that Lasix might, by increasing the volume of urine, cause a "flushing" of drugs out of the body of the horse. Another is that Lasix might mask or interfere with the detection of other drugs. A third and more likely effect is that the increased volume of urine produced by Lasix simply dilutes some of the drugs normally found in urine. Typical of these views are the following comments cited in a book entitled *The Misuse of Drugs in Horse Racing,* which read as follows:

> Lasix® binds itself to other drugs that may be circulating freely in the horse's system and washes the other drugs out. There is no doubt that some trainers are using Lasix to mask other (illicit) drugs that may be administered before a race. Whether Lasix actually prevents bleeders is iffy. It lowers the blood pressure through dehydration and this may help. But the real interest is in flushing and masking. There is no question that it gives horses a competitive edge.

These comments are particularly surprising. There is no evidence whatsoever that Lasix binds itself to other drugs. There is not a single shred of evidence in the scientific literature that Lasix "washes" or "flushes" drugs out of a horse's body. In a number of studies carried out in Kentucky, Lasix did not act to reduce plasma half-lives ("clearance time") of either procaine (Fig. 7-6) or phenylbutazone (Fig. 7-7) or methylphenidate, and these studies have been confirmed and extended by other laboratories. All one can say, in block letters, is that **LASIX DOES NOT FLUSH DRUGS OUT OF THE BLOODSTREAM OF HORSES, PERIOD.**

The reason that Lasix does not flush drugs out of horses (i.e. reduce their blood levels) is quite simply that Lasix does not move very much fluid out of horses.

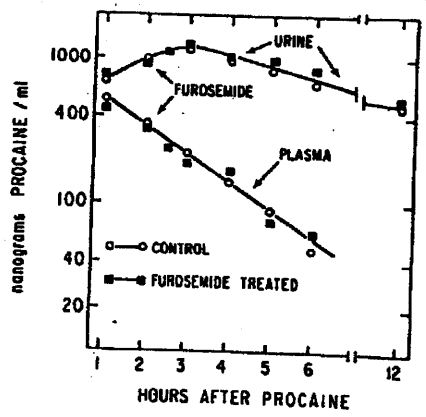


Figure 7-6. Lack of effect of furosemide on plasma and urinary levels of procaine. The open circles (○) show plasma and urinary concentrations of procaine after 10 mg/kg of procaine was given by IM injection. The solid squares (■) show plasma and urinary levels when the same dose of procaine was followed by 1 mg/kg of furosemide IV. The data show that the administration of procaine had no significant effect on plasma or urinary levels procaine. Reproduced with permission from Roberts et al., *J Equine Med Surg, 2*:185, 1978.

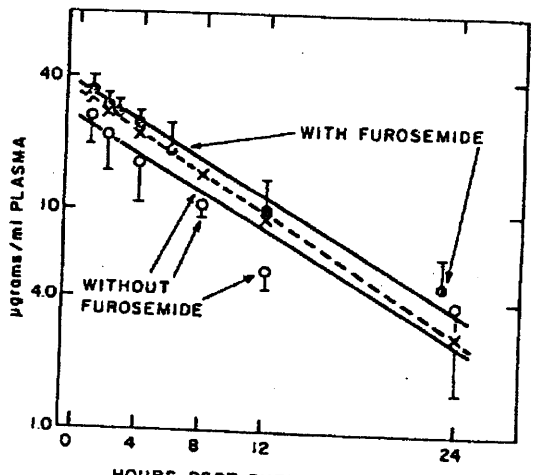


Figure 7-7. Lack of effect of furosemide on plasma levels of phenylbutazone. The open circles (○—○) and crosses (×—×) show plasma levels of phenylbutazone after 6.6 mg/kg (about 3 gms/1000 lb.) of the drug was administered IV. The solid circles (●—●) show the plasma levels of phenylbutazone when the phenylbutazone was followed by 1 mg/kg of furosemide IV at 2.5 hours. The data show that furosemide did not significantly reduce the plasma levels of phenylbutazone. Furosemide, therefore, did not "flush" phenylbutazone out of these horses. Reproduced with permission from Roberts et al., *J Equine Med Surg, 2*:185, 1978.

Although the diuretic response looks very dramatic to the casual observer, the actual volume of urine is only about 2 to 3 percent of the body fluid of the horse. Thus, in its brief period of action, Lasix would be unlikely to lead to the loss of more than 3 percent of the drug in the horse, and for most drugs the effect will be a lot less than this. This inability of Lasix to lead to the elimination of more than a very small percentage of a drug from a horse accounts for its lack of effect on the blood levels of drugs in horses.

Lasix does not reduce the concentrations of certain classes of drugs in equine urine. In studies at the University of Kentucky we administered procaine, a local anesthetic, and methylphenidate, a brain stimulant, to horses and studied the effects of Lasix on urinary concentrations of these drugs. Lasix had no significant effect on the urinary concentrations of these drugs, indicating that their detection in urine is not likely to be significantly affected by Lasix. Experimental work on amphetamine using a closely related diuretic shows that this effect likely also holds for the amphetamines. Thus, it turns out that for these basic, lipid-soluble drugs, Lasix is not able or likely to interfere with urinary detection.

Treatment with Lasix can have marked effects on the urinary detection of other drugs. For example, if a horse is treated with phenylbutazone, the urinary concentration of the drug measured, and then Lasix administered, one finds that Lasix can reduce the urinary concentration of phenylbutazone up to fiftyfold. This is a very substantial reduction in the urinary concentration of phenylbutazone, and it is sufficient to interfere with routine screening for phenylbutazone (Fig. 7-8). This can create problems when both Lasix and phenylbutazone are permitted medications. The answer to this problem, however, is relatively straightforward. Because Lasix *does not change the plasma levels of any drug,* one can readily (and more accurately) determine the presence of phenylbutazone from a blood sample, and this is now routinely done in Kentucky.

Although the specific experimental work has not been done, it appears very

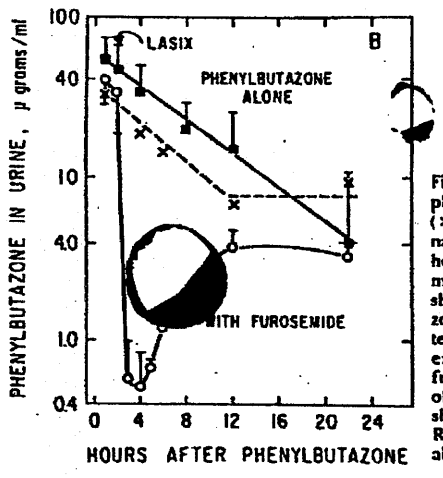


Figure 7-8. Effect of furosemide on urinary phenylbutazone concentrations. The crosses (×—×) and solid squares (■—■) show the urinary concentrations of phenylbutazone after horses were administered 3 gms/1000 lb. (6.6 mg/kg) at zero time. The open circles (o—o) show urinary concentrations of phenylbutazone when 1 mg/kg furosemide was administered IV at 2.5 hours after the Lasix®. The experiment shows that treatment with furosemide can reduce urinary concentrations of phenylbutazone up to fiftyfold or more for a short period after administration of the drug. Reproduced with permission from Roberts et al., *J Equine Med Surg,* 2:185, 1978.

likely that Lasix will similarly dilute urinary levels of other non-steroidal anti-inflammatory drugs such as Equiproxen, Banamine®, Tolectin®, et cetera. Another group of drugs whose detection in urine is highly likely to be influenced by Lasix is water-soluble glucuronide drug metabolites. These drug metabolites enter urine either by glomerular filtration or are pumped into it by the organic acid transport system. Once in the renal tubule, these highly water-soluble drug metabolites are not about to pass back into the blood. Their final concentration in urine, therefore, depends on the degree to which the urine is concentrated. Since Lasix can dilute equine urine up to fiftyfold, it is likely to dilute some water-soluble drug metabolites such as glucuronides by up to fiftyfold.

We have experimentally demonstrated this effect on drug metabolites with Talwin® (pentazocine), a narcotic analgesic often used in the horse. After a dose of Talwin in the horse, relatively large amounts of pentazocine (Talwin) glucuronide are found in the urine. Glucuronides are one of the most important types of water-soluble drug metabolites formed in the horse, and they are not likely to be reabsorbed in the renal tubules. The experiment of Figure 7-9 shows that the glucuronide metabolite of pentazocine is diluted up to fiftyfold after Lasix and that as the effect of Lasix wears off, the urinary concentrations of the drug return to control values.

Because fentanyl is a very potent narcotic that has been used in racing horses, we also tested the effects of furosemide on its urinary detection. We elected to carry out this experiment with the dose of furosemide usually used in the treatment of epistaxis, as the diluting effect of this dose should be both smaller than and of shorter duration than the effect of a 1 mg/kg dose. As shown in Figure 7-10, furosemide produced about a fifteenfold dilution of urinary fentanyl at peak effect, and the effect was over within about 2.5 hours after dosing. The experiment suggests that when a diluting effect of furosemide does occur, the extent of the dilution is closely related to the diuretic response and therefore is dose related.

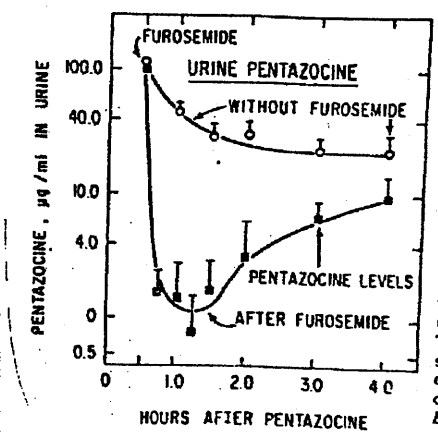


Figure 7-9. Dilution of the glucuronide metabolite of pentazocine by furosemide. The open circles (o—o) show urinary concentrations of pentazocine as pentazocine glucuronide after administration of 0.33 mg/kg of pentazocine (Talwin®). The solid squares (■—■) show urinary concentrations of this metabolite in horses treated with 1 mg/kg of furosemide thirty minutes after the pentazocine was administered. The experiment shows that furosemide transiently dilutes urinary levels of the major glucuronide metabolite of pentazocine. Reproduced with permission from Roberts et al., *J Equine Med Surg,* 2:185, 1978.

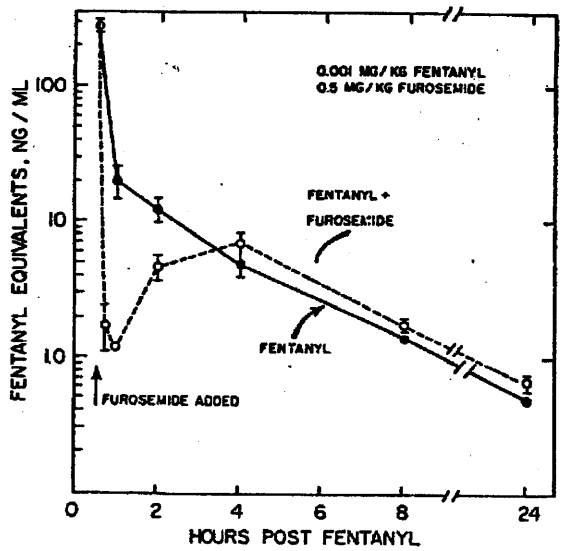


Figure 7-10. Urinary levels of fentanyl after 0.5 mg/kg furosemide. The solid circles (●—●) show urinary concentrations of fentanyl as fentanyl equivalents after administration of 0.5 mg of fentanyl to three horses. The open circles (○—○) show urinary levels of fentanyl equivalents when 0.5 mg/kg of furosemide was administered at thirty-one minutes after the fentanyl. The data show that this dose of furosemide reduced the urinary concentrations of fentanyl about fifteenfold and that the effect lasted for about two and one-half hours.

Because of this shorter period for which the smaller, antiepistaxis dose of Lasix acted to dilute fentanyl in urine, we elected to repeat this dilution experiment with phenylbutazone as the test drug. In the experiment of Figure 7-11 we dosed three horses with 2 gm/1000 lb of phenylbutazone IV and then treated them with 3.5 ml of furosemide (0.33 mg/kg) at two hours after the dose of phenylbutazone. As before, treatment with Lasix produced an immediate and rapid dilution of the phenylbutazone in the horse's urine, but the dilution was very short-lived. By four hours after dosing with furosemide the urinary levels of phenylbutazone were not significantly different from the controls. Comparison of the data of Figure 7-11 with the data of Figure 7-8 therefore shows that the diluting effect of Lasix is much shorter-lived when the dose is reduced to the antiepistaxis dose, and it appears that the duration of the dilution effect is therefore dose related.

This dilution of the major water-soluble metabolite of pentazocine and fentanyl may also occur with other drugs. Thus, apomorphine, morphine, and related narcotics and the phenothiazine tranquilizers are all detected in equine urine as water-soluble glucuronides. Since these are potent drugs that produce their pharmacological effects at quite low doses, the concentration of these glucuronide metabolites in urine can be quite important for their detection. Their dilution by Lasix may be a problem for the analyst and appears to be the only potential problem associated with approval of furosemide for use in racing horses.

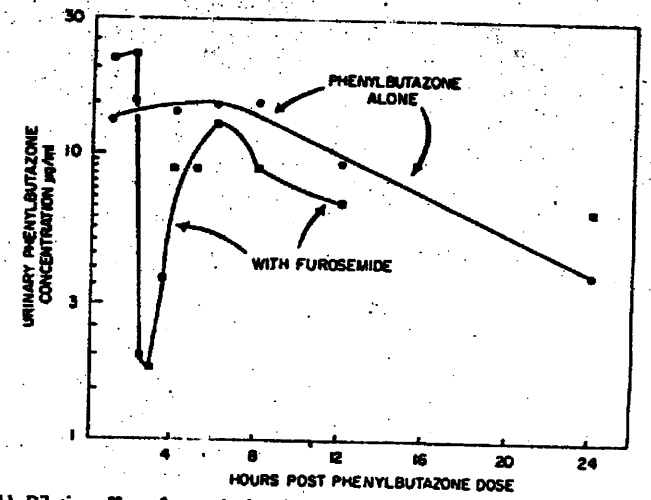


Figure 7-11. Diluting effect of an antiepistaxis dose of furosemide on uinary phenylbutazone. Four horses were dosed with 2 gms/1000 lb. of phenylbutazone IV at indicated zero time and then with either saline or 0.33 mg/kg (3.5 ml) of furosemide at two hours. The solid circles (●—●) show the urinary concentrations of phenylbutazone in the horses receiving saline, while the solid squares (■—■) show urinary levels of phenylbutazone in the horses treated with furosemide.

On the other hand, from what we know about the glucuronide metabolites of both pentazocine, apomorphine, and the phenothiazine tranquilizers, they are excreted over particularly long periods — apparently up to five days in some cases. If this prolonged excretion holds for other glucuronide metabolites, then simply holding horses for three to four hours after the injection of furosemide should yield a reasonably concentrated urine sample and detectable quantities of glucuronides. In this way it may be possible to get around the only potential problem with the approval of Lasix for use in racing horses, which is it diluting effects for some drugs in equine urine.

Another major area of contention about Lasix concerns its effect on equine performance. As suggested earlier, some uncomplicated minds have put forward the simple theory that Lasix treatment just makes a horse eight pounds lighter and therefore eight pounds faster. This proposal ignores the many other known effects of Lasix or perhaps assumes that they conveniently cancel each other out. Theoretical concepts of this caliber do not belong in serious scientific discussions.

A commonsense approach to problems such as this is first to determine whether or not the problem actually exists. Does Lasix actually improve the performance of horses? Performance trials in which horses were raced with and without Lasix have been carried out at both the Ohio State University and the University of Kentucky. These trials were on healthy horses and were conducted double-blind, that is, the people handling and clocking the horse did not know which horses were getting the drugs and which ones were getting the nondrug injections. Both

of these trials showed no statistically significant effects of Lasix on the performance of Standardbred horses.

While these results were encouraging, the number of horses used in the tests was small, and one could reasonably suggest that a bigger study might show a significant improvement of performance in horses on furosemide. At the prompting of Mr. Carl Larsen of the Standardbred Commission in Kentucky, we did a retrospective study on the effect of Lasix on the track times of horses racing at Louisville Downs in the summer of 1977. At this meet, Lasix was the only permitted medication, and its use was monitored by drug testing. Horses could elect to go on furosemide at any time throughout the meet, but once on Lasix they had to stay on Lasix. From this group of horses whose trainers elected to put them on Lasix during the meet, we found 58 horses for whom we had 160 times before they went on furosemide and 232 after they were on furosemide. We thus had a large number of times for 58 horses before and after they went on furosemide. These horses were presumably "put on" furosemide because their owners, trainers, or veterinarians thought that it would improve their performance. When the figures were analyzed, it turned out that the horses were actually 0.14 second slower on Lasix than without Lasix (Table 7-11). This very small difference was not significantly different statistically from the control or pre-Lasix time. From these figures it appears that Lasix does not improve the performance of a horse and, if anything, is likely to add a tenth of a second to his time.

The only conclusion from this study seems to be that the horsemen at Louisville Downs in the summer of 1977 were not, on the whole, able to improve the performance of their horses with furosemide, and it would seem reasonable to extend this experimental result to other horses and horsemen. Lengthy speculations such as one hears, and occasionally sees published, about possible mechanisms by which furosemide improves the performance of horses are therefore not valid.

As well as not improving performance in the horse, Lasix is relatively nontoxic and appears to be a very safe drug in the human as well as in the horse. The usual

TABLE 7-11
LACK OF EFFECT OF FUROSEMIDE ON THE PERFORMANCE OF HORSES RACING AT LOUISVILLE DOWNS, SUMMER, 1977*

	# 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 checked 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. Of 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). Furosemide therefore had no significant effect on the performance of this group of horses.

dose administered in the treatment of "bleeders" is a small dose, and when administered IV, its action is brief. If the horse is given access to water and salt, any drug-induced fluid or salt losses should be rapidly replenished.

In another series of experiments we dosed horses daily with 10 ml (1 mg/kg) doses of furosemide for four consecutive days, and a series of blood values over the four-day test period were observed. No cumulative changes in the blood picture of these horses during this four-day study were observed. However, if these same four doses were given at hourly intervals, a 40 percent drop in serum K^+ was observed. Thus, the only acute problem with large and repeated doses of furosemide would appear to be the possibility of inducing hypokalemia (lowered serum potassium levels) with repeated doses, a situation that is not likely to occur in racing horses.

How furosemide might act to prevent "bleeders" is currently unclear, due at least in part to the fact that no one knows what causes bleeders in the first place. While "true bleeders" are usually assumed to bleed from the lungs, the lung has two blood supplies, and no one knows which blood supply is involved in incidents of bleeding. The lung has a low pressure blood supply to the tissue where the gas exchange takes place and a higher pressure (normal body blood pressure) supply to the supporting structures of the lung such as the tracheobronchial tree and connective tissue. Which of these two circulations is involved in "bleeders" is unknown.

One often suggested cause of bleeding is pulmonary hypertension (i.e. high blood pressure) in the pulmonary circulation, leading to rupture of a blood vessel. The problem with this hypothesis is that high blood pressure in the pulmonary circulation is much more likely to cause massive pulmonary edema than hemorrhage. In other words, the increased blood pressure would cause the horse to drown in his own pulmonary fluids before breakdown of a blood vessel and bleeding from the lungs might be expected. Similarly, upper airway obstruction can be ruled out as a cause of bleeders, since pulmonary edema rather than hemorrhage is the most likely end result of upper airway obstruction.

Giving further consideration to this problem, Robinson* suggests that local lesions in the lung tissue, such as airway obstruction, scar tissue, or pleural adhesions, are critical factors in the incidence of bleeders. Under these circumstances these locally lesioned portions of lung would not expand, while the rest of the normal lung would expand considerably. The horse is among animals that have very poor collateral ventilation in their lungs, and their alveoli thus very easily become blocked off. Under these circumstances a strong inspiratory effort expands all but the lesioned portion of the lung, and capillary rupture occurs at the interface of the normal and affected tissue.

It is also clear that if furosemide does anything in the lungs, it acts to reduce pulmonary edema. As pointed out earlier, this action is rapid, is independent of the diuretic effect of this drug, and appears to be due to a furosemide-induced release of prostaglandins from the kidneys. If Robinson's model is correct and a local lesion in the lungs is the fundamental cause of epistaxis, then furosemide

* N. E. Robinson, Professor of Large Animal Surgery and Medicine, Michigan State University, East Lansing, MI

may act by relieving pulmonary edema, eliminating local blockade of airways in damaged lung tissue, and thus preventing hemorrhage. Similarly, Robinson points out that E-prostaglandins are bronchodilators, and their release by furosemide may act by dilating bronchi to reduce localized airway obstruction and thus the incidence of epistaxis (Fig. 7-12).

Another question that is often asked about Lasix is why, if there are not more than 200 racehorses in California that bleed in any one year, are much higher percentages, reportedly sometimes up to 80 percent of horses at some meets, running on Lasix? The answer to this question that veterinarians give is that Lasix helps a large number of horses with their breathing and therefore helps these horses to perform up to their best form.

Until very recently, there was no experimental evidence to support this clinical impression of practitioners and horsemen. Recently, however, Doctor John Pascoe of the University of California has been studying hemorrhage in the upper respiratory tract of horses post-race and has come up with some results that throw a completely new light on the incidence of bleeding in racing horses.

Doctor Pascoe and his colleagues have been examining the upper respiratory tracts of horses in the test barn immediately post-race with a fiberoptic endoscope. These studies have been carried out in California, Illinois, and Florida, in both Thoroughbred and Standardbred horses, and the basic observations have been essentially similar in each case. What Doctor Pascoe found was that the incidence of overt bleeding from the nostrils post-race was about that which has been reported over the years, i.e. 2 to 3 percent or so. However, fiberoptic endoscope

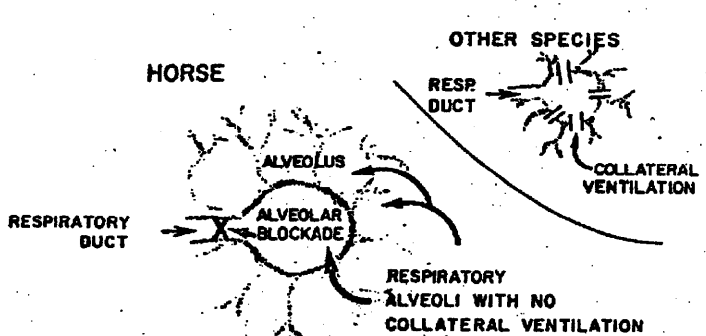
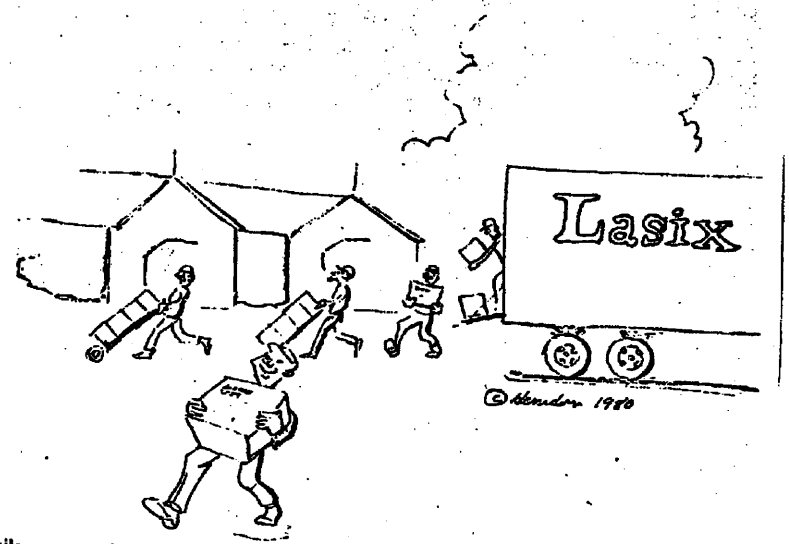
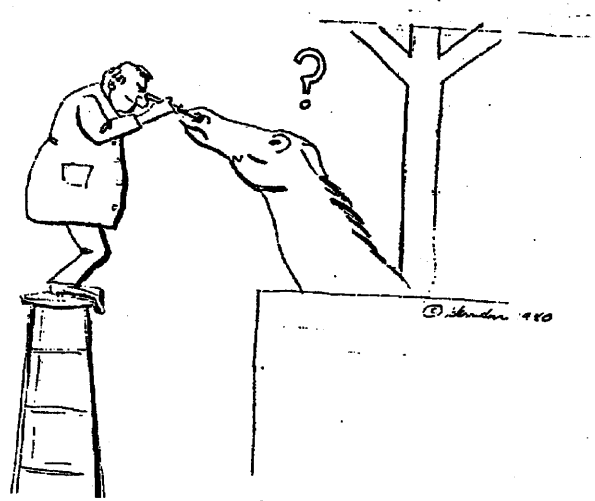


Figure 7-12. Schematic representation of Robinson's equine epistaxis hypothesis. Robinson has suggested that the reason the horse suffers from epistaxis more than other species is anatomical. In the lungs of most species, the small delicate organs called the alveoli, where gas exchange takes place, communicate readily with one another. These communications (inset) are technically called collateral ventilation and make it very difficult to prevent air from entering the alveolus. In the horse, however, there is little collateral ventilation, as indicated by the heavy stippling around the alveolus. Under these conditions, if blockade of the respiratory duct occurs, the alveolus will not expand with the rest of the lung when the animal breathes in. It is thought that the pressure differences between the blocked and unexpanded alveolus (indicated by the dark lines) cause rupture of the alveolar wall and bleeding into the lung tissue, which may appear at the nostrils as epistaxis. Furosemide, by dilating respiratory ducts and reducing pulmonary edema, is thought to reduce alveolar blockade and thus the incidence of epistaxis. Species other than the equine tend to have good collateral ventilation between alveoli (inset), so alveolar blockade and epistaxis in the equine pattern is much less likely to occur.



While some people have the idea that huge amounts of Lasix are used in horses, in actual fact the do used in the treatment of bleeders is quite small.



Use of the endoscope to examine the windpipe of horses post-race has thrown a great deal of light on the incidence of epistaxis.

examination of the upper respiratory tract has shown about 10 percent of horses post-race have very substantial amounts of blood in their windpipes (trachea, Fig. 7-13), that about 10 percent show flecks of blood, and that another 20 percent show intermediate amounts of hemorrhage. Thus, in all, between about 30 and 40 percent of horses show signs of hemorrhage in the upper respiratory tract post-race, although only 1 to 3 percent ever show overt bleeding at the nostrils. It appears likely, therefore, that the small percentage of bleeders seen historically only represents the "tip of the iceberg" with regard to pulmonary hemorrhage, and that pulmonary hemorrhage is far more widespread in racing horses than previously realized. These observations are, of course, in good agreement with the clinical impression that Lasix helps a much greater proportion of horses than just the classic bleeders.

Finally, one might ask, does Lasix actually prevent bleeding in horses, and if so, how? This is a very difficult question to answer, because since such a small percentage of horses bleed from the nose, and then only sporadically, it is not a problem that it is possible to easily approach experimentally. By and large, therefore, the principal evidence that Lasix actually does help prevent bleeding is the experience of veterinarians, which is a clinical impression. In this regard,

Figure 7-13. Post-race tracheal hemorrhage. In this horse, fiberopticscope examination of the trachea post-race revealed a "river" of blood about one-half inch wide on the floor of the trachea. No hemorrhage was visible at the nostrils in this horse. The black dots are due to damaged fibers in the endoscope. Courtesy of Doctor John Pascoe, University of California, Davis.

recent studies* by Doctor John Pascoe of the University of California have shown that the incidence of E.I.P.H. or pulmonary bleeding in furosemide-treated horses varies between about 40 and 60 percent. What is not known, however, is what the proportion of hemorrhage would have been in these horses in the absence of furosemide treatment. Nevertheless, these results do suggest that, at best, furosemide treatment cannot be more than about 50 percent effective in the treatment of E.I.P.H. or pulmonary bleeding.

To summarize the research findings with this drug, it is clear that Lasix is a very safe and effective diuretic in the horse. It is approved for use in the treatment of epistaxis by many racing authorities and is widely used in horses for this effect. However, the evidence that suggests that it is an effective treatment for this problem comes primarily from clinical observations. Lasix has been very widely and also very incorrectly reported to "flush" drugs out of horses. *Lasix does not flush drugs out of horses.* "Masking" of drugs by Lasix is a problem that has not been studied or reported on and does not appear to be significant. Lasix will act to dilute some drugs and drug metabolites in horse urine. This appears to be the only potential problem with the approval of Lasix for use in racing. It can be overcome by waiting about four hours for the diluting effects of Lasix to pass and then taking the urine sample.

Lasix is widely rumored to improve the performance of racing horses. As with most rumors, it is not clear whether or not the horses helped are healthy horses, whom Lasix enables to perform better than their normal ability, or horses with respiratory problems, whom Lasix brings up to normal performance. Many veterinarians have speculated about these rumored performance effects and have proposed mechanisms by which Lasix might improve performance. Unfortunately, all studies on the effects of Lasix on performance in horses have shown no effect, both in healthy horses in University-sponsored studies or in retrospective studies on track times. *The conclusion that Lasix does not improve the performance of horses under racing conditions seems at this time to be inescapable.*

Finally, concluding a remarkably complete compendium of opinions on the effects of Lasix in horses, Robert Baker levelled the accusation against the racing community that it is "now using Lasix to keep pulmonary cripples racing." If Lasix were indeed a "wonder drug" that could keep "pulmonary cripples" racing, one might reasonably expect to see at least some small improvement in performance in a study such as the Louisville Downs study. No trace of such an effect was observed, which strongly suggests that current patterns of Lasix use in horse racing do not represent an abuse of this drug.

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P.O. Box 1571
Spencer, West Virginia 25276
August 28, 1979

James M. Kramon, Esquire
Kramon & Graham, P.A.
Sun Life Building
Charles Center
Baltimore, Maryland 21201

Re: Maxine Pineda, et al v.
Maryland Jockey Club, et al

Dear Mr. Kramon:

Here is my report on the above-captioned case. Prior to preparing this report, I examined the following documents:

1. The Racing Form report of the May 3, 1978 race in which Easy Edith was entered.

2. The portions of Dr. Shelton's treatment diaries which pertain to Easy Edith.

3. The treatment diaries of Dr. Murphy for April 24, 1978 and April 26, 1978, and Dr. Murphy's April 26, 1978 Report of Horses Treated submitted to the Maryland Racing Commission.

4. The following depositions:

Dr. James M. Shelton
Dr. John C. Murphy
Dr. David Gary Zipf
Dr. David L. Paice
Thomas S. Caviness
Thomas Pappagallo
Rudolphe Turcotte
Alfred Leroy Moyers
Gregg McCarron
Charles J. Lang
James A. Callahan

premature closure of these terminal airways.[10] Since equidae have poor collateral ventilation, restricted movement of a portion of the lung resulting from airway obstruction may result in decreasing intra-alveolar pressure as discussed by Robinson.[11] Strenuous exercise with elevated capillary pressure will further increase the transmural pressure and the possibility of rupture of pulmonary capillaries.

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Equine Communications

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Pharmacology Review: A Review of Recent Research on Furosemide in the Horse

—**Thomas Tobin, Kentucky Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, KY**

Furosemide* is a member of the "high ceiling" group of diuretics and is a safe and very effective diuretic in the horse.[1] Outside of the usual medical indications for diuretics, furosemide is also useful in racehorses because it is believed to be effective in the control of epistaxis (nosebleed). Epistaxis, however, only occurs in about 2% of horses, while up to 80% of the horses running in some meets may be on furosemide.[2] This article will review recently acquired knowledge concerning furosemide in the horse and may suggest some reasons for the widespread use of furosemide in racing horses.

Furosemide was introduced into the equine medical literature by Dr. Marvin Beeman and his colleagues in Colorado.[3] It is useful in edema from all causes. It is particularly useful in cases of pulmonary edema because it acts to rapidly remove this fluid, even before it begins to move fluid out through the kidneys.[4] Its ability to increase the volume of urine may also be used to dilute out substances in urine and prevent renal damage. Thus, when a horse "ties up," the released myoglobin may deposit in and damage the kidneys. In situations such as this, the increased urine volume due to furosemide greatly reduces the possibility of renal damage.[4]

The diuretic action of furosemide is due to its inhibition of active reabsorption of chloride in the renal tubules. Sodium and water reabsorption normally follow chloride reabsorption, so administration of furosemide leads to greatly increased losses of sodium, chloride, and water.[2]

In a 1,000-pound horse, 4 ml of furosemide (50 mg/ml) produces about 4 liters of urine in about 40 minutes. This is the dose recommended for use in epistaxis (nosebleed) in the horse. A 10-ml dose, which is a common clinical dose, produces about 9 liters of urine, mostly within 1 hour. Forty milliliters of furosemide will produce about 21 liters of urine within about 2 hours, and this is close to the maximum response in a normal horse.[5]

The diuretic action of furosemide is therefore relatively short lived, especially after small intravenous (IV) doses. This short lived action of furosemide is not due to any inability of the horse to respond, as a second dose of furosemide will usually produce a very good response. Rather, the action of furosemide in the horse is brief because furosemide is rapidly pumped out of the blood into the renal tubules.[6] This pumping accounts for the short plasma half-life ($T_{\frac{1}{2}}$ = about 35 minutes) of the drug, its very rapid onset of action in the kidneys, and the fact that up to 60% of the drug is found unchanged in the urine. This is a very high proportion of a drug to be excreted unchanged in the horse.[6]

Because furosemide is secreted directly into the urine, it is found there in very high concentrations. A good analyst can easily detect furosemide in equine urine for 12 hours after a 10-ml dose, and the drug can be detected for up to 52 hours. Because the pharmacological actions of furosemide are largely over within 2 hours after an intravenous dose, there appears to be no need for detection of the drug for more than 12 hours after its administration. Racing chemists should bear this in mind when setting the level of sensitivity of their test systems.[6]

If furosemide is given intramuscularly (IM), its plasma half-life is greatly increased ($T_{\frac{1}{2}}$ = 86 minutes), and the diuretic response also lasts longer. After IM injection of 1 mg/kg of furosemide, about a 50% greater urinary response is observed than after its in-

Continued on p. 320.

* All the work reported here refers to injectable Lasix®, 50 mg/ml from National Laboratories Corp subsidiary of American Hoechst Corp., Somerville, NJ.

Equine Commun., continued from p. 314.

travenous injection. Onset of the diuretic effect is, however, almost as fast as after IV injection, so IM injection would appear to be the route of choice if a prolonged or greater diuretic effect is required.[6]

Like the diuretic effects of furosemide, the cardiovascular effects of furosemide are also transient. After intravenous injection, furosemide reduces pressure in the pulmonary circulation within minutes. This effect occurs independently of the diuretic effect and may be the basis of the action of furosemide against epistaxis. In the systemic circulation, furosemide produces a decrease in cardiac output with a small increase in heart rate. Furosemide does not alter blood pressure in the systemic circulation. After 1 mg/kg (about 10 ml) intravenously, these effects are usually over within 2 hours.[5]

After intravenous injection, 10 ml furosemide increases total plasma solids about 10%, hematocrit by about 5%, and rapidly reduces plasma K^+ levels. These effects peak between 10 and 30 minutes after administration of the drug and decline back to control levels by 2 hours postdosing.[5,6]

In studies in a number of laboratories, furosemide was found not to reduce the plasma levels of any drugs tested to date.[4] Furosemide does not, therefore, act to "flush" drugs out of the blood or plasma of horses and should not interfere with blood testing for drugs.[7] Similarly, it did not significantly affect the urinary concentrations of a number of drugs tested, such as procaine or methylphenidate, and a closely related drug did not affect amphetamine.[4] These drugs are all basic, relatively lipid soluble drugs, and it appears likely that furosemide has little or no effect on urinary concentrations of this group of drugs.[7]

On the other hand, furosemide treatment caused up to 50-fold reductions in the urinary concentrations of both phenylbutazone and the major metabolite of pentazocine, which is a water soluble pentazocine glucuronide conjugate. In the case of phenylbutazone, this reduction in concentration was sufficient to render the drug difficult to detect in our routine screening tests on racetrack samples at the University of Kentucky. Since both furosemide and phenylbutazone are permitted medications in Kentucky, we now routinely take both blood and urine samples for testing. In our opinion, blood samples are necessary to accurately test for medication with phenylbutazone if the use of furosemide is also permitted.[7]

Some racing jurisdictions, such as Illinois or California, have rules which set upper limits on the concentration of phenylbutazone permitted in horse urine on race day. These rules make little sense if furosemide is also a permitted medication. This is because simple administration of furosemide either IV or IM as close to race time as possible will easily reduce the concentration of phenylbutazone in the postrace urine to below the critical level.[7]

By and large, however, the actions of furosemide on phenylbutazone concentrations in urine are not a serious problem for the racing chemist, in contrast with the likely actions of furosemide on some other groups of drugs. The 50-fold dilution of the major water soluble metabolite of pentazocine also probably occurs with all other drugs detected as water soluble metabolites, such as apomorphine, morphine, and related narcotics and the phenothiazine tranquilizers. Since these drugs are effective at very low plasma concentrations and are difficult or impossible to find in equine plasma, their dilution by furosemide in equine urine is a serious problem for the analyst. The potential for furosemide to interfere with detection of these drugs in urine is probably the most serious problem associated with the approval of furosmide for use in racing horses.[7]

The performance effects of furosemide have been studied in some detail at both the University of Kentucky and the Ohio State University.[8] As pointed out earlier, furosemide has a very rapid action against pulmonary edema and is thought by track veterinarians to greatly assist the breathing of horses with respiratory problems, such as follicular pharyngitis. However, double blind trials on healthy Standardbred horses at both the University of Kentucky and the Ohio State University showed no statistically significant effects of furosemide on the performance of Standardbred horses.[1,8]

Because the numbers of horses involved in these trials were small and the horses were all clinically normal, we elected to perform a further study on the effects of furosemide on racing performance under track conditions. To this end, the Kentucky Harness Commission made available to us the "Lasix list" for the Louisville Downs Summer 1977 Harness Racing Meet. 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 furosemide. From the "Lasix list," 58 horses were selected, and for these horses 160 times prefurosemide and 232 times postfurosemide were obtained. It turned out that the horses were actually 0.14 seconds slower on furosemide than before they went on furosemide, though this difference was not statistically significant. The only conclusion from this experiment seems to be that the horsemen at Louisville Downs or their veterinary advisors were, on the whole, not able to improve the performance of their horses with furosemide.[8]

Furosemide appears to be a very safe drug in the horse. The usual dose administered in the treatment of epistaxis is a relatively small dose, and when administered IV, its action is brief. Given access to water and salt, any drug-induced losses of fluid or ions should be rapidly replenished. In another series of experiments 10-ml (1 mg/kg) doses of furosemide were given daily to horses for 4 consecutive days, and a series of hematological parameters followed. No cumulative changes in any of these parameters over the 4-day test period were observed. However, if these same 4 doses were given at hourly intervals, a 40% drop in serum K^+ was observed. Thus, the only likely acute toxicity with furosemide would appear to be the possibility of inducing hypokalemia with rapidy (hourly) repeated doses.[5]

In conclusion, furosemide is a very effective, safe, and rapidly acting diuretic in the horse. It clearly acts to dilute out and interfere with testing for certain drugs in equine urine. It does not appear to affect the performance of Standardbred horses. Clinical experience suggests that furosemide is effective in the treatment of epistaxis, and it may be useful in horses with respiratory problems, but no hard evidence to support these clinical impressions is currently available.

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Piperazine Toxicity in Horses

P. H. McNeil, B.V.Sc. and G. B. Smyth, B.V.Sc., M.A.C.V.Sc., Dept. of Veterinary Clinical Sciences, University of Melbourne, Veterinary Clinical Centre, Werribee, 3030, Victoria, Australia

Clinical signs of horses involved in an epidemic of iatrogenic piperazine citrate poisoning are described. The disease was reproduced by similarly overdosing an experimental horse. No treatment was necessary for a complete recovery.

Introduction

Toxic reactions subsequent to oral administration of piperazine compounds for the treatment of helminth infestations of man and domestic animals are uncommon. Vomiting, urticaria, blurred vision, weakness, nystagmus, and convulsions may occur with normal or excessive dosing in humans.[1,2,10,12] Occasional emesis, diarrhea, and transient neurotoxic symptoms are seen with excessive dosing in small animals.[4,5,16,18,20] Mild gastrointestinal signs are reported after administering high doses to calves.[13] Therapeutic activity is attributed to the neuromuscular blocking activity of these compounds in the parasite, which allows normal intestinal peristalsis of the host to expel the intact parasite from the gut. The effects of the drug on neuromuscular activity within the host are minimal.[10,16]

Reports of toxicity in horses are rare. Toxic signs were not produced in foals treated with 6 times the normal dosage levels of piperazine adipate.[17] However, in a more recent study, involving trials with both levamisole and piperazine, neurotoxic signs were produced in a horse dosed with piperazine monohydrochloride at 6 times the normal dosage levels.[7] This paper reports the clinical signs associated with an outbreak of iatrogenic piperazine citrate poisoning on a Standardbred stud and the experimental reproduction of the disease.

Case History

A group of 10 weanling Standardbred colts were running in a 50-acre paddock grassed with improved pasture. All had suffered a mild upper respiratory tract infection during the 3 weeks prior to anthelmintic treatment. There was no other history of illness in any of the horses. During a 2-day period, every horse on the property had been treated via nasogastric intubation with piperazine citrate and thiabendazole pow-

Furosemide in Horses: A Review

Albert A. Gabel, D.V.M., M.S.
Thomas Tobin, D.V.M., Ph.D.
Richard S. Ray, D.V.M., M.S., Ph.D.
George A. Maylin, D.V.M., M.S., Ph.D.

From the Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by: Gary P. Carlson, University of California; Harold E. Garner, University of Missouri; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; William W. Muir, Ohio State University, and David Snow, University of Glasgow, and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Finley, A.V. Villattico, Robert E. Vessing, Dr. Francis Ozog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Bierhaus, and General Wayne Kester.

1. Injectable furosemide[a] is a potent, effective, safe diuretic which at the usual dose has its peak diuretic effect in about 20 minutes in horses, at which time it increases urine output up to 40-fold for a short time. Most of its diuretic effect is within the first 2 hours after injection.

2. During the first 2 hours, it decreases the pressure in the right side of the heart, decreases left atrial pressure, decreases cardiac output, and concurrently it increases peripheral (systemic) blood vessel resistance and heart rate.

3. It reduces edema in the lungs and airway of horses with certain respiratory diseases by 1 or more mechanisms, perhaps by lowering the pressure of the left atrium and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.

4. The mechanism by which furosemide helps prevent epistaxis may result from the beneficial effect outlined in (3) above since most horses bleed from the lungs. Lasix[a] also appears to affect the platelets of horses during exercise.

5. The blood (plasma) level of furosemide declines rapidly. It can be detected in the urine by screening methods for the first 24 hours and by more sophisticated methods for up to 48 hours after injection.

6. Furosemide does not affect the blood levels of other drugs.

7. Furosemide has little effect on the concentration of certain drugs, such as procaine, in the urine. However, during its peak diuretic effect, within 2 hours after injection, furosemide causes up to 40-fold dilution of certain other drugs such as phenylbutazone. However, furosemide administered more than 4 hours before race time does not significantly reduce the ability to detect those drugs studied to date.

[a] Lasix, National Laboratories, Inc., Somerville, NJ 08876

8. There is no evidence that furosemide directly or indirectly affects the behavior of the horse.

9. Furosemide cannot make horses race faster or perform better than their innate ability, but in many cases it restores normal performance of horses which bleed.

Introduction

There is no scientific evidence that furosemide will increase the speed or improve performance of a horse beyond its innate capability. In a recently completed double-blind study there was no significant difference in the times of time-trial miles of clinically normal Standardbreds after intravenous furosemide injection compared to saline injection, 2 hours and 10 minutes before the trials.[13] A mechanical prompter was used for uniform psychological stimulation. However, clinical observations indicate that the drug may help a horse to more nearly reach his racing capacity if he is a bleeder, or has certain other conditions, including perhaps early obstructive lung disease "heaves" or diseases in which pulmonary edema occurs, even transiently, during exercise.[12,14]

Mechanism of Action (Diuretic Effect)

The primary effect and use of injectable furosemide has been as a potent diuretic. It has a high degree of efficacy, low-inherent toxicity, and a high therapeutic index in horses.[11] Furosemide produces the increase in urine by inhibiting active electrolyte (inorganic ions such as chloride) transport in that part of the kidney known as the diluting segment of the ascending limb of the loop of Henle.[4,9,11,25] Furosemide produces increased losses of chloride, sodium, other electrolytes, and water.[11] There is little potassium loss in horses.[11] In normal horses, no dehydration or electrolyte imbalance occurs if they have access to water and salt before and after the injection.[12] Its onset of action is rapid.[11,14,20,21,25] The diuretic effect of furosemide peaks within 15 to 30 minutes, when there is up to a 40-fold increase in urine production for a short time.[21,24] Dosed at 1 mg/kg body weight, most of its diuretic effect occurs within 2 hours.[24] Doses between 0.01 and 1.0 mg/kg (the dose recommended by the manufacturer) produce a graded increase in urine volume and decrease in its specific gravity.[21,24] The average urine production during a 2-hour period after injection of the usual dose of furosemide is 3 to 12 liters and is dependent on the horse's state of hydration when the drug is given.[20,21,24] Only 1 liter of urine is produced during an average 2-hour control period.[24] Due to the loss of fluid during the period of increased urine production, horses which are dehydrated before the injection make less urine during several hours which follow; therefore, it is sometimes difficult to get a urine sample during this period.[11] Race Commission veterinarians do not find this to be a significant problem.[11]

Furosemide is used by many veterinarians in the treatment of acute tieing-up and azoturia to increase urinary output, since high concentrations of the pigment from damaged muscles can cause kidney shutdown.

Cardiovascular Effects

In resting horses, in addition to increased urine production, the following changes were found to be statistically significant ($P<0.05$)[20] from 15 minutes to 110 minutes after intravenous injection of the usual dose (1 mg/kg) of furosemide:

1. Decreased blood pressure in the right side of the heart, pulmonary artery, and left atrium. This may be due to hypovolemia and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.
2. Decreased venous return and therefore decreased cardiac output.
3. No significant change in systemic arterial blood pressure, although there was a significant increase in peripheral (systemic) vascular resistance and reflex increase in heart rate.
4. Hemoconcentration. Packed cell volume and total protein percentage in the blood increased.

In a recent double-blind study intravenous furosemide injection was compared to saline injection under simulated race conditions using Standardbreds.[14] Hemodynamic and biochemical data were recorded before and during the first "warm-up miles" 15 minutes after injection and before, during, and after the third (time-trial) mile 2 hours and 10 minutes after injection. After furosemide, compared to saline, pulmonary artery pressure was lower during the first warm-up mile, 15 minutes after injection, but not during the time-trial mile 2 hours and 10 minutes after injection. There was no difference in cardiac output immediately after and 10 minutes after each of the miles, nor in the pulse rate during or after each of the miles.

These experiments suggest that, if given during the 2-hour period prior to a race, furosemide might have a negative effect on performance because of a decreased cardiac output.

Respiratory Effects

A clinically important action of furosemide in both man and small domestic animals is its rapid action against pulmonary edema. Since this effect also occurs in patients in which this drug does not produce diuresis, and in patients which are kept hydrated, the volume change is not totally due to diuresis.[1,6]

Recent observations suggest that the pulmonary effects of furosemide may be mediated by prostaglandins released from the kidneys.[1,6] Another theory is that there may be a tendency for lung edema to develop when mean pulmonary artery pressure exceeds 40 mm Hg by very much.[1,6,22] Aver-

age mean pulmonary artery pressures of approximately 70 mm Hg have been measured in Standardbreds during time trials.[17] Edema (which may occur transiently) has a negative effect on breathing reflexly through the J-fibers of the vagus nerve.[12] The more severe the exercise, the greater the tendency for pulmonary edema to occur. This may explain why furosemide is used more in Thoroughbreds, which work harder, than in Standardbreds.[21]

The experience of race track veterinarians indicates that the drug appears to have an effect of producing a more nearly optimal performance in Thoroughbreds which have heavy or labored breathing while training or racing, in those which have nasal exudate after racing, and in some which become excited in the paddock.[19]

It is thought that horses with edema and narrowing of the upper airway, especially the pharynx by such conditions as follicular pharyngitis, may race more normally following the use of furosemide, because tissue fluid is removed, enlarging the airway toward normal size.[14,21]

Epistaxis

It is thought that in most cases the blood comes from rupture of small vessels in the lungs, and sometimes the upper airway, when horses have epistaxis following work.[6,19] The experience of many veterinarians shows that furosemide injection approximately 4 hours before exercise is effective in preventing epistaxis in a high percentage of Thoroughbred and Standardbred horses which bleed during or after racing or hard workouts.[21]

In a double-blind study under simulated racing conditions, Standardbred horses were injected with 1 mg/kg of furosemide or saline 15 minutes before the first warm-up mile.[15] Hemostatic function including the blood clotting mechanism and platelets were evaluated prior to injection, immediately following the first warm-up mile, and after the time-trial mile 2 hours and 10 minutes after the injection. There was a slight increase in the retention of platelets in glass bead columns, but this platelet function change could not be substantiated in a follow-up study using furosemide injections in nonexercised horses. No significant changes were found in screening tests of the blood clotting mechanism.

Furosemide may be effective in horses which bleed by reducing the blood pressure of the left atrium and increasing capacitance of the lung vessels.[19]

Blood (Plasma) and Urine Levels—Detection

Furosemide is usually administered to horses by intravenous (IV) or intramuscular (IM) injection at doses of 1 mg/kg or somewhat less. After IV administration of 1 mg/kg, its plasma levels decline rapidly, from about 10 μg/ml (parts per million) initially to 500 ng/ml (parts per billion) at 20 minutes.[16] Thereafter, plasma levels fall more slowly with a half-life of about 30 minutes, to less than 20 ng/ml at 4 hours.[6] Urinary levels of the drug start at about 30 μg/ml and fall rapidly over the first 12 hours to about 500 ng/ml.[16] This is about the detection limit of the thin layer techniques used in routine drug testing screens.[16] However, using gas chromatographic (G.C.) methods, urinary levels of the drug can still be detected up to 48 hours postdosing.[16]

Effects of Furosemide on the Concentration of Other Drugs in Equine Blood and Urine

In no instance is furosemide known to significantly reduce the blood or plasma level of any drug.[24,26]

Treatment with furosemide does not appear to significantly affect the concentration of basic lipid soluble drugs in equine urine.[16] Thus, furosemide has no effect on either plasma or urinary concentrations of procaine in horses treated with 10 mg/kg of procaine HCl intramuscularly.[16] Similar results were observed with methylphenidate[8,16] In both cases, urinary output of the drug was increased by the greatly increased urine volume, and the concentration did not decrease enough to cause trouble in detection of the drugs.[16] The same effect probably occurs with amphetamine.[16] Glucuronide and sulfate conjugates of narcotics can be diluted up to several fold.[17]

In contrast, however, urinary concentrations of phenylbutazone are reduced up to 40-fold at peak diuresis.[16] This concentration change can greatly reduce the reliability of routine UV screening for phenylbutazone.[25] Concentrations of phenobarbital also are probably affected, though not quite so severely.[8]

In doses usually used, furosemide can greatly reduce the urinary concentrations of some drugs, but only during the first 2 hours and for a much lesser extent up to 4 hours after it is administered; in other drugs it has little effect. In particular, use of furosemide can interfere with routine screening for phenylbutazone during this period of increased urine production (2 to 4 hours). For this reason, blood testing for phenylbutazone should be recommended where medication rules permit the use of furosemide within 4 hours of racing.

Ritalin - Ciba Pharmaceutical Co., 556 Morris Ave., Summit, NJ 07901.

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

Thomas Tobin, D.V.M., Ph.D.
Brian L. Roberts, B.Sc.
T.W. Swerczek, D.V.M., Ph.D.
Mark Crisman

From the Kentucky Equine Drug Research Program, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506

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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®[1]) is a member of the "high ceiling" group of diuretics and is the member of this group most widely used in equine medicine.[2] 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.[3] 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.[4] 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.[5]

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,[6] in particular, with respect to dose and time response relationships. Beyond recent work from this laboratory,[7,8] 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.[9,10,11] 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.[5]

In this study, we report on dose and time response relationships for the natriuretic, 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,[7,8] and preliminary reports of this work have been presented.[12,13]

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® 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.[a] 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

[1] Lasix®, Original Hoechst Russel Pharmaceuticals, Somerville, NJ

[a] Becton-Dickenson Co., Rutherford, NJ
Model 143, Instrumentation Laboratories, Inc., Lexington, MA

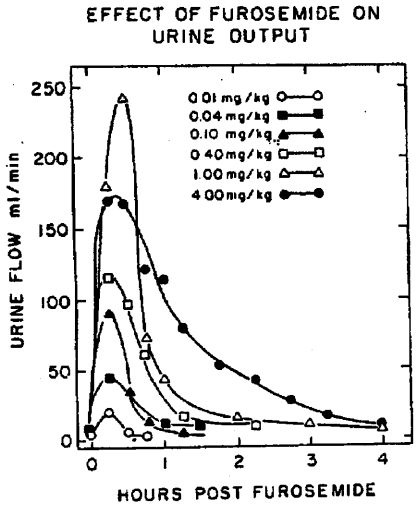

Fig 1—Time course of urinary flow rate response to intravenous furosemide. The symbols show flow rate in ml/minute after the indicated doses of furosemide intravenously. Open circles (○-○), 0.01 mg/kg; solid squares (■-■), 0.04 mg/kg; solid triangles (▲-▲), 0.1 mg/kg; open squares (□-□), 0.4 mg/kg; open triangles (△-△), 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.[7] Hematocrits were determined by centrifugation,[8] red blood cell counts on a Coulter counter,[9] and hemoglobin on a hemophotometer.[10] Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and creatine phosphokinase (CP) were determined by the Spin Chem method.[11] Other methods, drugs, and chemicals were as previously outlined.[6,12] 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 between 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.

[6] Optical Refractometer, Bausch & Lomb, Rochester, NY
[7] "Autocrit" Centrifuge, Clay Adams, Inc., NY
[8] ZBI Coulter Counter, Coulter Electronics, Inc., Columbus, OH
[9] Hemophotometer, Fisher, Inc., Cincinnati, OH
[10] Spin Chem, Smith-Kline Instruments, Ltd., Palo Alto, CA

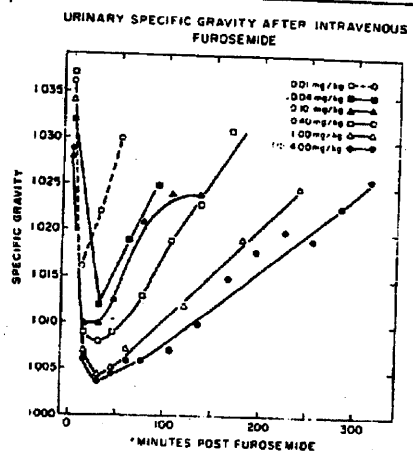

Fig 2—Effect of intravenous furosemide on urinary specific gravity. Furosemide at the indicated dose levels was administered to Thoroughbred horses by rapid IV injection. The symbols show urinary specific gravity after 0.01 mg/kg (open circles, ○-○); 0.04 mg/kg (solid squares, ■-■); 0.1 mg/kg (solid triangles, ▲-▲); 0.4 mg/kg (open squares, □-□); 1.0 mg/kg (open triangles, △-△); 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.

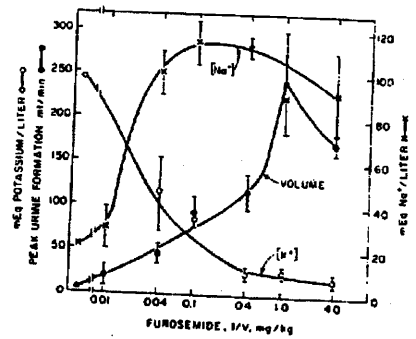

Fig 3—Dose response relationships of urinary output and cation concentrations at peak diuresis due to intravenous furosemide. Furosemide at the indicated concentrations was given by rapid intravenous injection to horses. The solid circles (●-●) show peak urinary output at each of the indicated doses. The crosses (X-X) and open circles (○-○), 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.

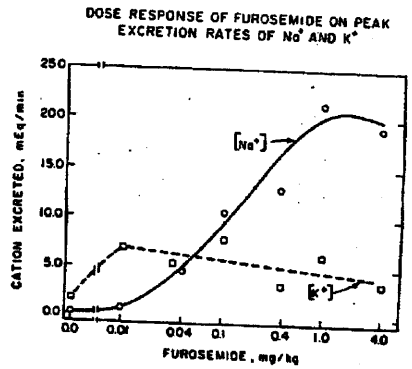

Fig 4—Dose response relationship for intravenous furosemide on cation excretion rates. All data points represent cation excretion rates at peak drug effect. The open circles (○-○) show peak urinary output of Na^+ after the indicated concentrations of furosemide, while the open squares (□-□) show peak 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.

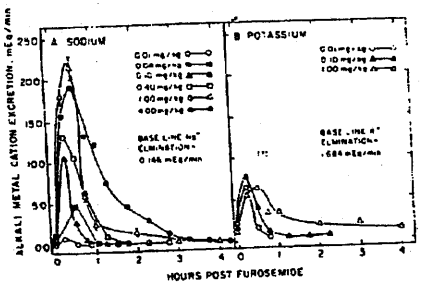

Fig 5—Effect of intravenous furosemide on rates of urinary loss of Na^+ and K^+. The left-hand panel shows the effect of the indicated doses of furosemide on Na^+ output/minute; the right-hand panel the effect of the indicated doses on K^+ loss/minute. All points are means ± standard deviation of determination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

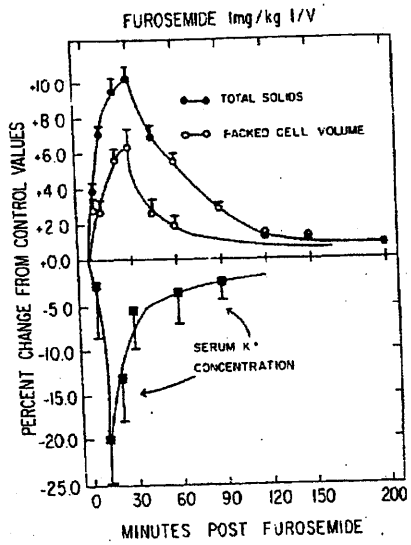

Fig 6—Effect of 1 mg/kg furosemide intravenously on blood values. Horses were administered 1 mg/kg furosemide intravenously and blood samples drawn at the indicated times. The solid circles (●-●) show the percent increase in total plasma solids, while the open circles (○-○) show the percent increase 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.

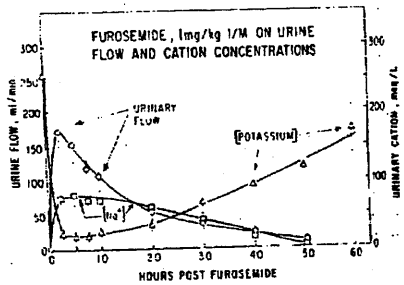

Fig 7—Effect of 1 mg/kg furosemide intramuscularly on urine volume and cation concentration. The open circles (○-○) show urinary output after the indicated dose of furosemide, while the open squares (□-□) and triangles (△-△) show urinary concentrations of Na^+ and K^+, respectively. All experimental 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*	732	8.4*	621
2 hours	9.0	744	12.6	887
4 hours	10.5	748	16.2	994

*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.[11]

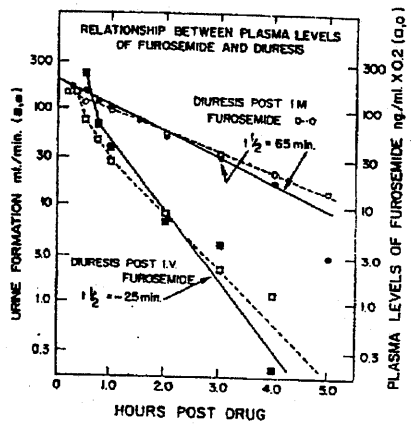

Fig 8—Relationship between plasma levels of furosemide and diuresis. The solid symbols and lines show rates of urine formation in ml/minute after IV injection (solid squares, ■-■, replotted from Fig 1) and after IM injection (solid circles, ●-●, replotted from Fig 6) of 1 mg/kg furosemide. Control rates 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]).

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

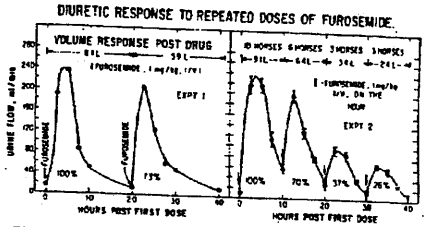

Fig 9—Diuretic response to repeated doses of furosemide. The left panel (Experiment 1) shows the diuretic response to 1 mg/kg of furosemide IV at indicated zero time and 2 hours, respectively. All data points are the means of experimental determinations on 4 different horses. The right-hand panel (Experiment 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.

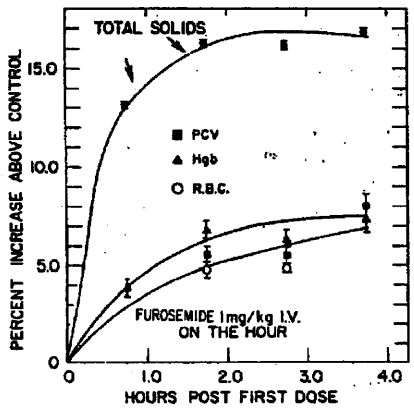

Fig 10—Effect of repeated doses of furosemide on total solids, hematocrit, hemoglobin, and RBC content. Blood samples were drawn from the horses of Experiment 2 (Fig 9) at 45 minutes after each dose of furosemide. The solid circles (●-●) show total plasma solids, the solid squares (■-■) hematocrit, the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

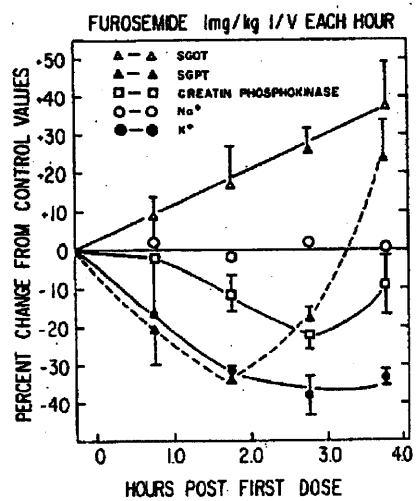

Fig 11—Effect of repeated doses of furosemide on plasma enzymes and monovalent cations. Blood samples drawn from the horses of Fig 9 (Experiment 2) at 15 minutes prior to furosemide and 45 minutes after each dose were analyzed for Na^+ (open circles, ○-○), K^+ (solid circles, ●-●), SGOT (open triangles, △-△), 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 Love Bug Wilson	143	142	− 1
9		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 hetwen 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.*[9] 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 dioretic 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.*[9] reported no significant effects on K^+ excretion. The discrepancy between the results reported here and those of Garner and coworkers[9] 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.*[9] 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.*[13] 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[15] and more recently furosemide,[1] 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[2] 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

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

of Gabel and co-workers[2] 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.*[2] 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[2] 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 withfurosemide 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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The Pharmacology of Furosemide in the Horse
I. Effects on the Disposition of Procaine, Methylphenidate, Phenylbutazone, and Pentazocine

Thomas Tobin, D.V.M., Ph.D.
Brian L. Roberts, B.Sc.
J. Richard Miller, B.Sc.

From the Kentucky Equine Drug Research Program and The Graduate Program in Toxicology, Department of Veterinary Science, University of Kentucky, Lexington, KY 40506.

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The effects of furosemide (1 mg/kg IV) on the dispositions of procaine, methylphenidate, phenylbutazone, and pentazocine in Thoroughbred horses were investigated. Furosemide did not reduce plasma levels of procaine, methylphenidate, or phenylbutazone and had little effect on urinary concentrations of procaine and methylphenidate. Furosemide reduced urinary concentrations of phenylbutazone and the major glucuronide metabolite of pentazocine about 40 to 50-fold.

These results and experience in our laboratory show that furosemide may substantially reduce the probability of detection of phenylbutazone and pentazocine in urine. For phenylbutazone, this problem may be approached either by determining plasma levels of the drug or by using more sensitive detection methods in urine. For pentazocine and other drugs excreted as glucuronides, the solution is not clear-cut. Because of limitations in current analytical technology, it appears likely that furosemide treatment may substantially reduce the probability of detection of many drugs excreted as water soluble metabolites.

Introduction

The extensive use of furosemide[1] in horses racing in some jurisdictions raises questions as to its effect on other drugs with which it may be administered. Thus furosemide might affect the pharmacological response to, disposition of, or urinary elimination of other drugs.[2] Further, and of particular interest to horsemen and racing authorities, furosemide treatment may affect routine forensic screening for other drugs.[3] This study was designed to provide answers to some of these questions.

The most likely mechanism for an effect of furosemide on drug disposition is via its renal effects. In the kidney, furosemide enters urine primarily via the tubular organic acid transport system.[1] In the diluting segment of the loop of Henle, it blocks active reabsorption of chloride and thus indirectly that of sodium and water.[2] Furosemide might therefore theoretically affect urinary concentrations of drugs by (a) competing with the tubular secretion of acidic drugs or (b) blocking the renal concentrating mechanism and preventing any concentration of drugs in urine.[3]

Other possible sites for drug interaction also exist. Furosemide might displace other acidic drugs from their plasma protein binding sites and thereby affect their disposition and pharmacokinetics.[4] Furosemide might also affect the rate of metabolism of other drugs and thus their disposition.[5] Conversely, since at least some of the effects of furosemide are mediated via the prostaglandins, drugs such as phenylbutazone might directly antagonize some of the actions of furosemide.[1] To determine the practical significance of these various theoretical possibilities, we studied the effects of furosemide on the disposition of phenylbutazone,[6] procaine,[7] methylphenidate,[8] and pentazocine[9] in horses. Preliminary reports have been communicated.[10,11]

Materials and Methods

(a) Animals.—Care and maintenance of horses and collection of urine samples was as previously described.[11] Unless otherwise noted, all experimental points are the means of experiments on at least 4 horses.

(b) Phenylbutazone and Procaine.—Plasma and urinary concentrations of phenylbutazone and procaine were determined as previously described.[6,12]

(c) Methylphenidate.—Methylphenidate levels in biological fluids were determined by the method of Tobin.[7] One milliliter of saturated sodium tetraborate and 2 ml of cyclohexane were added to 4 ml of the biological fluid or appropriate aqueous standards. The mixture was then "rotoracked" for 5 minutes and centrifuged. Occasionally the "rotoracking" produced a partial emulsion which was resolved by centrifuging at 2,000 x g for 20 minutes. After centrifugation the cyclohexane layer was transferred to another tube and 50 µl of HFBA added. This mixture was allowed to react at room temperature for 3 minutes. At the end of this period, 5 ml of 0.5 M NaOH was added to the mixture and it was again "rotoracked" for 10 minutes. After centrifugation the cyclohexane layer was transferred to a clean tube, and an aliquot taken for gas chromatography. Chromatography was on a 3-ft OV-101 column at an injection temperature of about 300° C. Under these conditions, the retention time for derivatized methylphenidate on the column averaged about 3 minutes.

(d) Pentazocine Glucuronides.—Hydrolysis of pentazocine glucuronides was performed by adding to 0.5 ml of urine 1.0 ml of saturated KH_2PO_4, to bring the pH to about 5.0, and 0.8 ml of bovine liver glucuronidase.[9] These were then incubated overnight at 37° C (about 16 hours). Hydrolysis with this relatively large amount of enzyme for this time was required for complete release of all glucuronidase releasable pentazocine.

At the end of the incubation period, 3-4 drops of concentrated NH_4OH were added to bring the pH of the system to about 9.0. Then, 4 ml of dichloromethane was added, the whole rotoracked for 5 minutes, and centrifuged at 1,000 x g for 5 minutes. The dichloromethane phase was then separated and evaporated at 55° C in a water bath to dryness. 0.5 ml of benzene added, and an aliquot of this sample taken for gas chromatography.

Chromatography was on a Perkin-Elmer 3920A equipped with a 3-foot OV-101 column and a nitrogen phosphorus detector. Injection temperature was 210° C, column temperature 220° C, and manifold temperature 250° C. Gas flows were 1 ml/minute H_2 at 8 psi, 100 ml/minute air at 30 psi. The pentazocine peak recovered from equine urine had the same retention time as authentic pentazocine over 3 different column temperatures. Derivatization of the material recovered from equine urine with pentafluoropropionic acid (PFPA) also produced material which chromatographed with similar retention times as PFPA-treated pentazocine.

(e) Drugs, Chemicals and Reagents.—Pentazocine was the commercially available injectable form. All other drugs and reagents were as previously described.[1,6]

Results

Although phenylbutazone is known to be highly protein bound in many species, there are no reports on its plasma protein binding in the horse. Figure 1 shows that over the range of drug concentrations found in equine plasma,[6] phenylbutazone is about 95% protein bound. Since furosemide is also acidic and highly protein bound, phenylbutazone and furosemide may share binding sites and thus are candidates for mutual displacement interactions.

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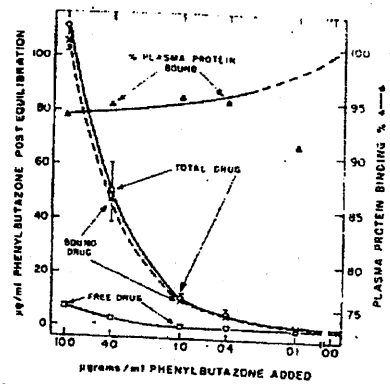

Fig 1—Plasma protein binding of phenylbutazone. The indicated concentrations of phenylbutazone were added to 5 ml of freshly drawn equine plasma and 280 ml of 50 mM phosphate buffer, pH 7.4. The plasma was then placed in a spectraphane membrane dialysis bag, suspended in the phosphate buffer, and the system incubated at 37° C for 18 hours. At the end of this period, the concentrations of phenylbutazone in both phases were determined as described in "Methods." The open squares (□-□) show the concentration of phenylbutazone remaining in the buffer phase, while the open circles (○···○) show concentrations in plasma. The crosses (X-X) represent the difference between these values, which is the concentration of drug plasma protein bound at each point. The solid triangles (▲-▲) represent the percent of drug in the plasma protein bound. All experimental points are the means, ± standard error of the mean, of experiments on 3 different equine plasmas.

Figures 2, 3, and 4 show the diuretic effect of 1 mg/kg furosemide IV in these horses and also its effect on the disposition of phenylbutazone in the horse. Phenylbutazone, 6.6 mg/kg, was administered IV at indicated zero times to all animals. The test animals were challenged with 1 mg/kg furosemide IV 2.5 hours after administration of phenylbutazone. Figure 2 shows plasma concentrations of phenylbutazone in these horses. The solid lines show matched experiments in which the same horses were used in both experiments. The crosses represent additional comparable control data from other experiments.[6] Plasma levels of phenylbutazone declined with approximately the same 7-hour half-life in the presence or absence of furosemide, suggesting no clinically significant effect of furosemide on the plasma half-life of phenylbutazone in the horse.

In contrast to the lack of effect on plasma concentrations, urinary concentrations of phenylbutazone were markedly decreased after administration of furosemide (Fig 3). Urinary levels dropped 40-fold within 15 minutes of administration of furosemide and remained depressed for up to 12 hours. Again, the dotted line in Fig 3 is a second control for normal urinary excretion of phenylbutazone taken from other data;[6] the solid line represents a matched control experiment.

Figure 4 shows the effect of furosemide on the rate of urinary excretion of phenylbutazone. As well as reducing the concentration of phenylbutazone in urine, furosemide also tended to reduce the rate of elimination of phenylbutazone since its rate of elimination declined to a low point about 2.5 hours after administration of furosemide. Thereafter, urinary output of phenylbutazone increased, in contrast with the consistent decline observed in controls.

Figure 5 shows the effect of furosemide on plasma and urinary concentrations of procaine. Horses were administered procaine HCl intramuscularly (IM) (10 mg/kg) at indicated zero time, and the test animals were challenged with 1 mg/kg of furosemide IV at 2 hours post-procaine. The open symbols show plasma and urinary levels in the absence of furosemide. The solid symbols show the values in furosemide-treated horses. No statistically significant differences between either group were observed.

Figure 6 shows the rate of excretion of procaine with and without furosemide. Procaine excretion rates after furosemide treatment transiently rose to 20-fold or more than control values, then returned to control values by 4 hours after furosemide treatment. Procaine is lipid soluble at physiological pH and readily distributes throughout the body.[7] As urine volume increased after administration of furosemide, procaine apparently readily entered this expanding compartment and was excreted at a rate proportional to urine flow. Thus furosemide considerably enhanced the urinary excretion rate of procaine.

The effect of furosemide on the disposition of methylphenidate is shown in Fig 7. Plasma levels of methylphenidate in control horses declined with a half-life of about 1 hour after subcutaneous injection of 0.33 mg/kg methylphenidate. Administration of 1.0 mg/kg of furosemide IV 2 hours after the methylphenidate reduced the plasma half-life of methylphenidate while also decreasing the concentration of the drug in urine (Fig 8). The change in urinary concentration observed after furosemide, however, was relatively small, and urinary output of methylphenidate was therefore increased more than tenfold after administration of furosemide (Fig 9).

Figure 10 shows the effects of furosemide on urinary concentrations of a glucuronide metabolite of pentazocine. At indicated zero time, 0.33 mg/kg pentazocine was administered IV to all horses, and the test horses were challenged with 1 mg/kg furosemide IV 30 minutes later. In the absence of furosemide, urinary concentrations of pentazocine fell slowly, from about 100 µg/ml at 30 minutes postdosing to 25 µg/ml at 4 hours. In the presence of furosemide, however, urinary concentrations of the glucuronide metabolite fell sharply, from 100 µg/ml at 30 minutes

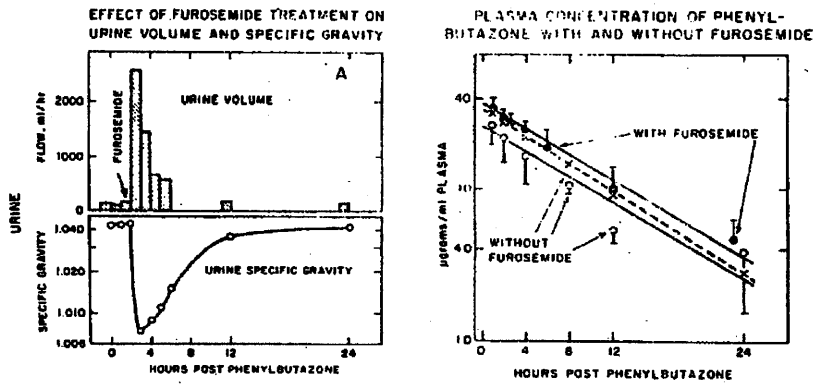

Fig 2—Diuretic effect of furosemide in Thoroughbred horses and its effect on plasma levels of phenylbutazone. The left-hand panel shows urinary output (upper panel) and specific gravity (lower panel) when 6 horses, treated with 6.6 mg/kg phenylbutazone IV at zero time, were challenged with 1 mg/kg furosemide IV at 2.5 hours postphenylbutazone. In the right-hand panel the open circles (○ - ○) and crosses (X - X) show plasma levels after phenylbutazone alone. The solid circles (● - ●) show plasma levels when the phenylbutazone was followed at 2.5 hours by 1 mg/kg furosemide IV. All points are the means of experiments in at least 4 horses.

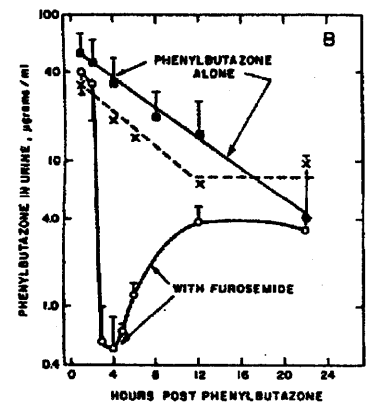

Fig 3—Effect of furosemide on urinary concentrations of phenylbutazone. The crosses (X - X) and solid squares (■ - ■) show urinary concentrations of phenylbutazone in 4 horses administered 6.6 mg/kg IV at zero time. The open circles (○ - ○) show urinary concentrations of phenylbutazone in 4 horses in which the phenylbutazone was followed 2.5 hours later by 1 mg/kg of furosemide IV.

to 1.2 μg/ml at 1 hour postdosing. Thereafter, urinary concentrations of the metabolite recovered and were about 50% of control levels by 4 hours after dosing.

Discussion

Treatment with furosemide did not act to reduce plasma levels of any drugs whose plasma levels were tested and appeared to prolong the plasma half-life of methylphenidate. Though statistically significant (Fig 7), the pharmacological basis of this action on methylphenidate is not clear. It does not appear to be of sufficient magnitude to be clinically or forensically important.

In racing circles, it is occasionally suggested that diuretics act to "flush" drugs out of the body and that administration of a diuretic can reduce plasma levels of a drug and hinder its forensic detection in plasma. These results clearly show that this is not so for the drugs tested. Even in the case of phenylbutazone, where furosemide might be expected to displace the drug from plasma protein binding sites (Fig 1), no differences in plasma levels that cannot be accounted for by the normal half-life of the drug were observed. Furosemide, therefore, is not likely to interfere in any pharmacokinetic way with plasma levels or detection of drugs in plasma in pre- or postrace testing.

For some drugs furosemide treatment may also have little effect on their urinary concentrations. Furosemide had no effect on urinary concentrations of procaine and a relatively small effect (<40% reduction) on urinary concentration of methylphenidate. These observations are in good agreement with those of Frey *et al.*[1] who found little effect of bumetanide treatment on urinary levels of amphetamine and a marked increase in urinary output of this drug during diuresis.

Procaine, amphetamine, and methylphenidate are all basic, relatively lipid soluble drugs, and it seems likely that this accounts for the lack of effect of furosemide on their urinary concentrations. Presumably these drugs can readily diffuse across the renal tubule and equilibrate with urine at concentrations dependent on their pH and the pH of urine. In agreement with this hypothesis, Evans and Lambert[2] have shown that urinary concentrations of procaine are closely related to urinary pH changes consistent with a rapid equilibration of procaine across renal tubules. Thus, it may be reasonable at this point to assume that furosemide will tend to have little effect on the concentration of basic, highly lipid soluble drugs in equine urine.

In contrast to our observations with basic drugs, urinary concentrations of phenylbutazone were markedly reduced by treatment with furosemide. This effect can be of considerable forensic importance. Our attention was first

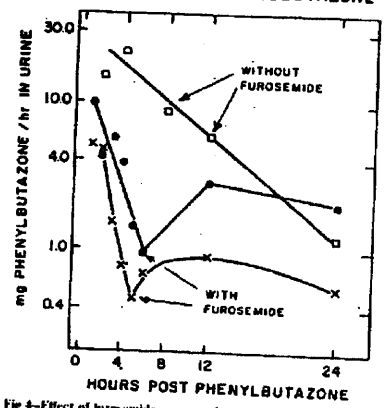

Fig 4—Effect of furosemide on rate of urinary excretion of phenylbutazone. The open squares (□ - □) show the rate of urinary excretion of phenylbutazone after 6.6 mg/kg phenylbutazone IV at zero time. The solid circles (● - ●) and crosses (X -X) show the excretion rates when this dose of phenylbutazone was followed by 1 mg/kg furosemide IV at 2.5 hours. All experimental points are the means of experiments on 4 different horses with standard error of the means omitted for clarity.

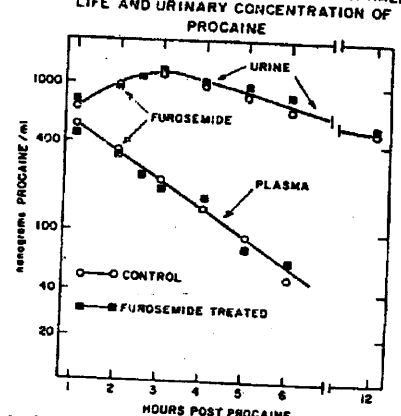

Fig 5—Effect of furosemide on the disposition of procaine in the horse. The open symbols show plasma and urinary concentrations of procaine after intramuscular administration of 10 mg/kg procaine HCl at zero time. The solid symbols show plasma and urinary levels when the administration of procaine was followed by 1 mg/kg of furosemide intravenously. All experimental points are the means of experiments on 4 different horses with standard error of the means omitted for clarity.

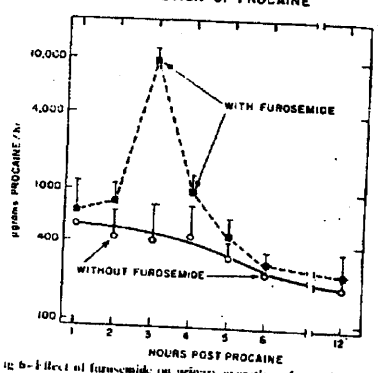

Fig 6—Effect of furosemide on urinary excretion of procaine. The open circles (○ - ○) show urinary elimination of procaine after 10 mg/kg of procaine HCl IM, the solid squares (■ - ■) show elimination of procaine after 1 mg/kg furosemide IV at 2 hours. All experimental points are the means of determinations of 4 different horses ± standard error of the mean.

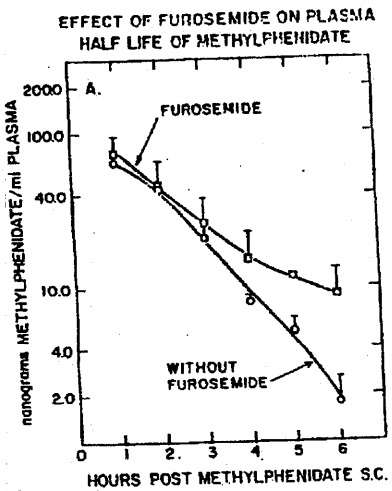

Fig 7–Effect of furosemide on plasma levels of methylphenidate. Methylphenidate, 0.33 mg/kg, was administered subcutaneously to 4 Thoroughbred mares at indicated zero time. The open circles (○ - ○) show plasma levels after methylphenidate alone, the open squares (□ - □) plasma levels after administration of 1 mg/kg of furosemide at 2 hours. The slopes of a least squares fit to each set of points are significantly different (t test, $P < 0.01$). All points are the means ± standard error of the mean of experiments of 4 different horses.

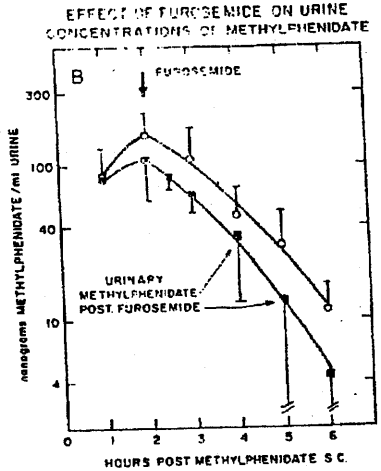

Fig 8–Effect of furosemide on urinary concentrations of methylphenidate. The open circles (○ - ○) show urinary concentrations of methylphenidate after 0.33 mg/kg of methylphenidate subcutaneously. The solid squares (■ - ■) show urinary concentrations when 1 mg/kg furosemide was given IV 2 hours after the methylphenidate. All experimental points are the means of determinations on at least 4 horses ± the standard error of the mean.

drawn to this phenomenon when routine screening of racetrack samples from furosemide-treated horses showed phenylbutazone consistently present in plasma samples but only occasionally in urine.[1] As shown in the experiments of Figs 2, 3, and 4, phenylbutazone concentrations are reduced to close to the limit of routine UV screening and flame ionization detection, while plasma levels are unaffected. Furosemide treatment, therefore, can cause a potentially important reduction in urinary concentrations of phenylbutazone for a period of hours after its administration.

The pharmacological basis for this effect on urinary concentrations of phenylbutazone is unclear. It appears to be a combination of (a) a dilution effect and (b) a reduction in tubular transport of phenylbutazone. These conclusions are based on the following observations.

While plasma and urinary concentrations of phenylbutazone are usually quite similar (i.e., about 40 μg/ml in plasma and urine after 6.6 mg/kg to a horse),[1] this equivalence of blood and urine levels is deceptive. Because phenylbutazone in plasma is 95% plasma protein bound (Fig 1), only about 2 μg/ml or less of phenylbutazone is free to be excreted by glomerular filtration. This filtered 2 μg/ml must then be increased to 40 μg/ml by (a) the renal concentrating mechanism, or (b) active tubular secretion.

Simple interference with the renal concentrating mechanism would be sufficient to account for most of the observed reduction in phenylbutazone concentration in urine. However, simply changing urine volume would not affect the total rate of excretion of phenylbutazone, while the data of Fig 4 show a reduction in total urinary output of phenylbutazone. This means less net entry of phenylbutazone into the increased volume of urine in the presence of furosemide. Possible mechanisms for this effect include reduced glomerular filtration, less diffusion of phenylbutazone into urine, and/or less tubular transport of phenylbutazone. Because of the acidic nature of both phenylbutazone and furosemide, an action at the level of the tubular transport appears likely to account for the data of Fig 4.[1]

Many drugs used in the illegal medication of racing horses are detected in urine primarily as their glucuronide metabolites.[2] Glucuronide metabolites enter urine either

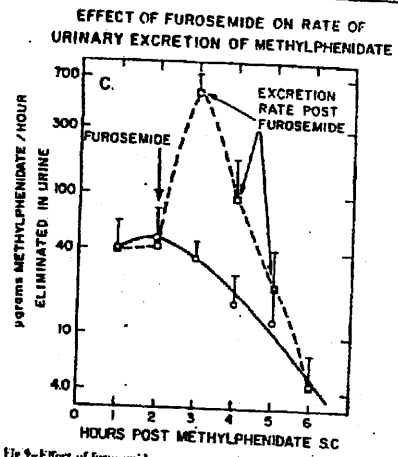

Fig 9–Effect of furosemide on rate of urinary excretion of methylphenidate. The open circles (○ - ○) show the rate of urinary excretion of methylphenidate (0.33 mg/kg) alone, or after 1 mg/kg furosemide IV (open squares, □ - □) 2 hours after administration of furosemide.

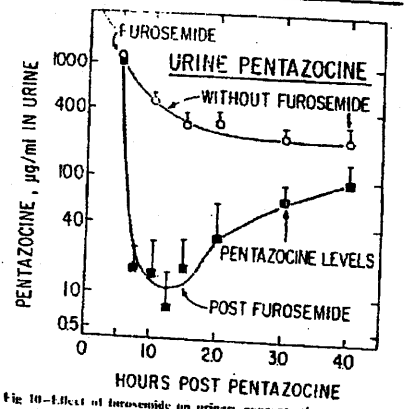

Fig 10–Effect of furosemide on urinary concentrations of a glucuronide metabolite of pentazocine. Horses were injected IV with 0.33 mg/kg pentazocine at indicated zero time. The open circles (○ - ○) show urinary concentrations of a glucuronide metabolite of pentazocine in control horses. The solid squares (■ - ■) show urinary concentrations of this metabolite in horses treated with 1 mg/kg of furosemide IV 30 minutes postpentazocine. All data points are means ± standard error of mean of experiments on at least 4 different horses.

by glomerular filtration or by the organic acid transport system. Since these metabolites are poorly lipid soluble, they are concentrated in urine by the renal concentrating mechanism. Urinary concentrations of these drugs may thus be many times greater than plasma levels of these drugs. Further, because sensitive analytical methods are not available for many drugs excreted as glucuronides, this concentrating effect is important in that it enables analysts to identify many of these drugs during routine forensic screening.[2]

Pentazocine is rapidly and apparently completely metabolized to a glucuronide metabolite in the horse. Figure 10 shows the effect of furosemide treatment on urinary levels of this metabolite. Urinary concentrations are markedly reduced, by about 50-fold, at peak effect in drug treated animals.[3] As with phenylbutazone, drug levels recovered slowly and were about 50% of control levels at 4 hours postdosing. The experiment shows marked effects of furosemide treatment on urinary levels of a glucuronide metabolite in the horse.

It appears likely that this diluting effect observed for the glucuronide metabolite of pentazocine will apply to other drugs excreted as glucuronides, such as apomorphine, the phenothiazines, fentanyl, and other narcotic drugs. In good agreement with this probability are some recent experiments by Ozog[4] who reports substantial reductions in concentrations of urinary apomorphine in furosemide-treated horses supporting the hypothesis that glucuronide metabolites are particularly susceptible to diuretic dilution.[1,2] Further, because many of these drugs are active at extremely low dose levels (phenothiazines) and because analytical methodology for their detection is either marginal or nonexistent (fentanyl), this effect of furosemide on glucuronide metabolites is likely to be highly significant for routine analytical work. Abolition of the renal concentrating effect by furosemide renders detection of these drugs in urine as technically challenging as is their detection at the low levels observed in plasma.

In some recent experiments Frey *et al.*[5] concluded that the diuretic bumetanide "did not interfere with the detection of doping drugs." The results reported here and experience in our Equine Drug Testing Laboratory suggests that this conclusion is in error.[6] Because sample size is limited in routine dope testing, changes in urinary concentrations of the magnitude reported in this report may be critical for drug testing and can mean the difference between success and failure.

As a practical matter, these results show that administration of furosemide can reduce the concentrations of some drugs or their metabolites in urine from 40 to 50-fold. The practical significance of this effect depends entirely on the techniques and skill of the analyst. In our test-

ing laboratory, more sophisticated gas chromatographic methods had to be substituted for our TV and thin layer screening for phenylbutazone. Given adequate equipment it appears unlikely that detection of phenylbutazone and other acidic drugs should be seriously compromised by furosemide. Further, plasma levels of these drugs are not likely to be affected, and detection of plasma levels of acidic drugs is readily possible with current technology.[10]

The situation for drugs such as morphine, acepromazine, and apomorphine, which are detected as glucuronide metabolites, is far less clear-cut. Since these drugs are effective at very low concentrations, the analyst is operating close to the detection limit of available methodology, and the urinary excretion mechanism assists in their detection. It seems highly likely that use of furosemide in combination with these drugs substantially reduces the probability of their detection given the current state of the art of drug screening. Blood testing, recently recommended where the use of furosemide within 4 hours of racing is permitted, is not likely to be helpful with this group of drugs, since satisfactory blood testing methods are not available for many members of this group.

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The Pharmacology of Furosemide in the Horse. II. Its Detection, Pharmacokinetics, and Clearance From Urine

Brian L. Roberts, B.Sc.
J.W. Blake, Ph. D.
Thomas Tobin, D.V.M., Ph. D.

From the Kentucky Equine Drug Research and Testing Programs and The Graduate Program in Toxicology, Department of Veterinary Science, University of Kentucky, Lexington, KY 40506.

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After intravenous (IV) injection of furosemide, plasma levels of this drug declined rapidly (t½ = 5 minutes), then somewhat less rapidly to give a terminal or metabolic phase half-life of between 32 and 38 minutes. After intramuscular (IM) injection, complete and rapid absorption of the drug was observed, but plasma levels of the drug declined more slowly (t½ = 86 minutes). Furosemide was about 95% bound to equine plasma proteins.

Up to 60% of the amount of furosemide injected was rapidly excreted unchanged in urine, apparently due to secretion by the organic acid transport system of renal tubules. Urinary concentrations of furosemide therefore ranged up to 1,000-fold greater than plasma levels of the drug, and furosemide was detectable in equine urine for up to 3 days after its administration. Less sensitive analytical methods, such as thin layer chromatographic (T.L.C.) screening, detected furosemide for about 12 hours after its IV or IM routine injection. Because the cardiovascular and diuretic effects of furosemide are over within 2 hours after its IV injection, thin layer chromatographic screening seems adequate for routine testing.

Introduction

Furosemide[a] is a member of the high ceiling group of diuretics which is widely used in equine medicine.[5-17] 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.[12] It is also suspected in racing circles of being used to "dilute out" prohibited drugs in the urine of racing horses.[20] Its approval in recent years by some racing authorities for the prophylaxis of epistaxis in horses has resulted in its greatly increased use in horses racing in these jurisdictions. However, this increased use of Lasix[®][a] is, in many instances, much greater (up to 80% of all horses) than the known incidence of epistaxis in horses (<5%).[3]

Because of this widespread use of furosemide in racing horses, many questions concerning its actions and use in the racing horse have arisen. The diuretic effect of this drug is not well characterized in the horse, particularly with respect to dose and time response relationships.[7] The pharmacokinetics and disposition of this drug in the horse are essentially unknown, and in particular the "clearance" time for this drug in the urine of horses has not been reported. Recent

[a] Lasix®, Hoechst-Roussel Pharmaceuticals, Somerville, NJ.

reports from this laboratory have presented information on the effects of furosemide on the disposition of other drugs in the horse.[20]

In this study, we report on a specific and sensitive detection method for furosemide in equine plasma and urine, its pharmacokinetics in the horse, and how these pharmacokinetics relate to dose response and time response data for this drug[16] and to routine tests for the clearance of this drug from equine urine. Preliminary reports have been communicated.[4,18]

Materials and Methods

Animals—Mature Thoroughbred and half-Thoroughbred mares (375–500 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® tubes[b] from the jugular veins. Urine samples were collected by bladder catheterization. Urine samples for analysis were collected "midstream" after catheterization. Bladders were drained at time periods and volumes were recorded to calculate the quantity of the drug or electrolytes excreted over a time period. All samples were held in ice until they could be taken to the laboratory for analysis or for further storage at -40°C until the samples were analyzed.

Chemical Method — A modification of the gas chromatographic method of Lindstrom and Molander[12] was used to quantitate furosemide for all *in vivo* experiments.

Furosemide was extracted by pipetting 1.0 ml plasma or 0.5 ml urine plus an equal aliquot of 1 N HCl and 6.0 ml of dichloromethane (DCM) into a 16 x 125 mm screw cap culture tube. The samples were "rotoracked" for 3 minutes and centrifuged. The aqueous phase was aspirated and the DCM phase decanted into a 16 x 150 mm screw cap culture tube. The tubes were placed into a 60°C water bath and evaporated to dryness.

To derivatize furosemide, 2.0 ml of 0.2 M NaOH, 50 μl of THAHS (tetrahexyl-ammonium-hydrogen-sulfate), and 5.0 ml of 0.5 M methyl iodide (in DCM) were added to each tube. The tubes were capped tightly and secured horizontally in a shaker/water bath at 50°C for 20 minutes. Methylation occurred rapidly under these conditions (Fig 2). The samples were then removed from the water bath, centrifuged, and stored at 4°C. Allowing the tubes to stand for a period of time at this point gave cleaner chromatograms. The aqueous phase was removed and the dichloromethane (DCM) phase decanted into another tube and evaporated to dryness at 60°C in a water bath. Two milliliters of hexane and 1.0 ml of 0.2 M NaOH were added to each tube, "rotoracked," and centrifuged. Samples were then ready for gas chromatographic analysis.

A Perkin-Elmer 3920-B gas chromatograph equipped with a ^{63}Ni electron capture detector was used for all determinations. This machine was equipped with a 6 foot x ¼ inch O.D. glass column packed with 3% OV-101 absorbed on 100/200-mesh GasChrom Q.[c] The injection port temperature was 310°C, column temperature 290°C, interface 300°C, and ECD 330°C. Prepurified N_2 was the carrier gas at a flow rate of 55 ml/minute. A sample volume of 2.0 liters was used throughout.

In the fluorometric assay, the DCM extract of plasma was layered over 2.0 ml of 50 mm phosphate buffer, pH 7.4. After rotoracking and centrifuging the samples again, 1.0 ml of aqueous phase was removed and mixed with 1.0 ml of 0.5 N HCl to give a final pH of 2.0. Within 15 minutes of addition of acid, the samples were scanned at 345 nm_{ex} and 410 nm_{em}. Furosemide concentrations were calculated by comparison with standards.[9]

For thin layer chromatographic detection of furosemide, 14 ml of urine was acidified to a pH of 2.0 by the addition of about 10 drops of concentrated HCl. Four milliliters of dichloromethane (DC) was then added to the system, the whole "rotoracked" for about 5 minutes and then centrifuged. The urine layer was discarded and the DCM layer transferred to another tube and evaporated to dryness. The residue was then taken up in about 100 μl of DCM and streaked on silica gel 60, F254 plates. The plates were then developed in a solution of 6 parts chloroform, 4 parts cyclohexane, and 1.5 parts glacial acetic acid for about 90 minutes. After developing, the plates were air-dried and immediately examined under short-wave UV light for bright spots corresponding with furosemide. This is the usual method used for routine screening in Kentucky. To develop a permanent record these plates were then sprayed in sequence with 50% H_2SO_4, 0.7% $NaNO_2$, 1.0% ammonium sulfamate, and 0.5% N (1-naphthyl) ethylene diamine dihydrochloride (NEDD).[d] A bright pink spot developed immediately corresponding to the position of furosemide on the control plates. This color spot[21] was stable for several weeks.

[b] Vacutainer® tubes, Becton-Dickinson Co., Rutherford, NJ.

[c] Applied Science Laboratories, State College, PA.

[d] P. Baumler, personal communication, Maxx Testing Laboratory, Toronto, Ontario, Canada.

Chemicals and Biochemicals — Authentic furosemide was a kind gift of the Hoechst Pharmaceutical Company, Somerville, NJ. Commercial injectable furosemide (50 mg/ml) for *in vivo* experiments was purchased from the W.A. Butler Company, Columbus, Ohio. The methyl iodide was from Fisher Scientific Company and was more satisfactory than methyl iodide from other sources. The quaternary ammonium compound, tetra-hexyl-ammonium-hydrogen-sulfate (THAHS) was obtained from Labkemi Ab, Stockholm, Sweden. All other reagents and solvents used were of "nanograde" purity.

Kinetic Analysis — Pharmacokinetic analysis of these data was performed by applying the appropriate equations for a 2-compartment open model[8] to the date of Fig 6 or with the assistance of the SAAM 23 program as described by Tobin *et al.*[19] The IV data were fitted to a 2-compartment open model by fixing 1/apparent volume of distribution of the central compartment obtained graphically and allowing all other values to float. Unless otherwise noted, all values are the means ± SEM of experiments on at least 4 experimental Thoroughbred mares. For the IM data, the best fit was obtained with a 1-compartment open model with first order absorption and elimination. Areas under the plasma concentration time curve were determined by the trapezoidal rule.

Results

Figure 1 shows the mass spectrum of the material formed from furosemide by the process outlined in Materials and Methods. This material shows a molecular ion of 372, consistent with the structural formula shown in Fig 1. Figure 2 shows that formation of this material was complete within less than 10 minutes. Figure 3 shows that the peak heights obtained by gas chromatographic analysis of this material are directly proportional to the concentrations of furosemide added to the system. Figure 4 shows that about 80% of the furosemide added to water, plasma, or urine was recoverable by the process outlined in Materials and Methods.

Other experiments (data not presented) showed that furosemide added to plasma or urine was stable for up to 48 hours at 0°C. Similarly, the methylated derivative of furosemide was stable for up to 3 days.[16] Thus, both the drug itself in plasma and urine and the derivative formed by the analytical method used were sufficiently stable to be useful for routine pharmacokinetic analysis.

Figure 5 shows plasma protein binding of furosemide in equine plasma. Over the concentration range of 0.1 μg/ml to 10 μg/ml added to the binding system, furosemide was about 95% plasma protein bound. Other experiments (data not presented) showed that the blood plasma partitioning of furosemide in equine blood was about 92.5% in plasma and 7.5% in the red blood cell fraction at drug concentrations of about 100 ng/ml.[14]

Figure 6 shows the pharmacokinetics of furosemide in the horse after rapid intravenous injection of 1 mg/kg furosemide. Plasma levels of the drug fell rapidly at first, with an initial alpha (α) phase half-life of about 5 minutes. Thereafter, plasma levels of the drug fell more slowly, with a beta (β) or terminal phase half-life of about 38 minutes. Furosemide was no longer detectable in equine plasma after 4 hours, by which time plasma levels had dropped to less than 4 ng/ml. In the first hour after dosing by the IV route, 36% of the drug administered was recovered unchanged in the urine.[14]

This plasma level pattern was well fitted by a 2-compartment open model as shown in Fig 6 and described by the kinetic parameters presented in Table 1, which parameters were calculated using the standard equations describing this model and from the date of Fig. 6.[8] In addition, these data

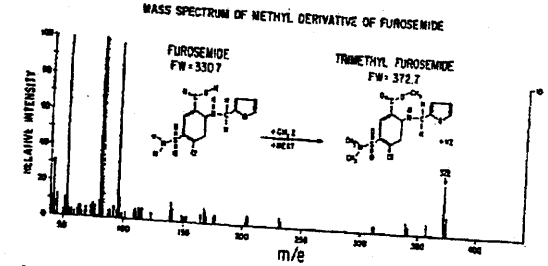

Fig 1—Mass spectrum of the trimethyl derivative of furosemide. Furosemide was reacted with methyliodide, as outlined in Materials and Methods, to yield material with a mass spectrum consistent with that of trimethyl furosemide.

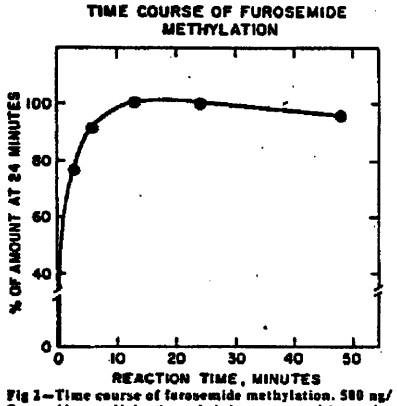

Fig 2—Time course of furosemide methylation. 500 ng/ Furosemide was added to the methylation system, and the reaction stopped when indicated. The solid symbols (● - ●) are the percentage of the amount of derivative at 24 minutes formed at the indicated time.

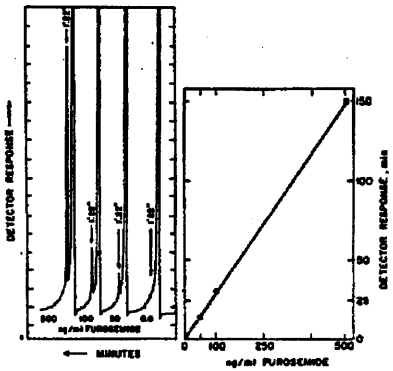

Fig 3—Gas chromatograms of trimethyl furosemide. The left hand panel shows gas chromatograms of derivatized spiked furosemide standards. The right hand panel shows that detector response was linearly related to the amount of furosemide added over the range of 50—500 ng/ml furosemide.

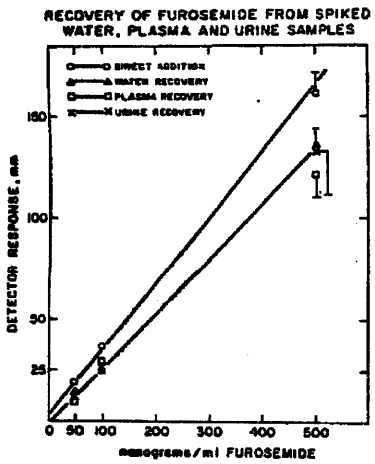

Fig 4—Recovery of furosemide from spiked water, plasma, and urine samples. The indicated concentrations of furosemide were added directly to the methylating system (open circles O - O) or to 1 ml buffered water (Δ–Δ), plasma (□–□), or urine (X - X). The furosemide was then extracted into dichloromethane, derivatized, and the detector response compared with that obtained after direct addition of furosemide to the methylation system.

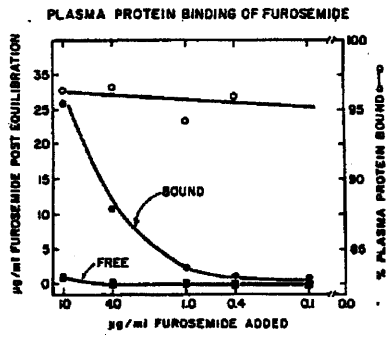

Fig 5—Plasma protein binding of furosemide. The indicated concentrations of furosemide were added to 45 ml of 50 mm phosphate buffer, pH 7.4, and to 5 ml of freshly drawn equine plasma in a spectrapor dialysis bag. The system was then incubated overnight at 37°C with gentle shaking. At the end of this period, 4-ml aliquots of the buffer and plasma were withdrawn and assayed for furosemide fluorometrically. The solid squares (■ - ■) show concentrations of furosemide remaining in the buffer phase, the solid circles (● - ●) show the concentration of furosemide found in the plasma phase. The open circles (O - O) in the upper portion of the figure show the percentage of furosemide plasma protein bound under these conditions.

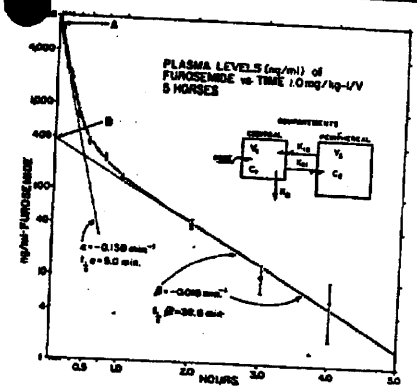

Fig 6—Plasma levels of furosemide after its rapid IV injection. The solid circles (● - ●) show plasma levels of furosemide after rapid IV administration of 1 mg/kg furosemide. The solid line from 1.5 hours on is that of a least squares regression fit to all points after 45 minutes. The rest of the solid line was fitted by eye to the data points. The dotted lines A and B represent the 2 components into which this curve may be resolved, the Alpha or distribution phase with a half-life of about 5 minutes and the Beta or elimination phase with a half-life of about 39 minutes. The inset shows a 2-compartment open model which may be used to describe these data.

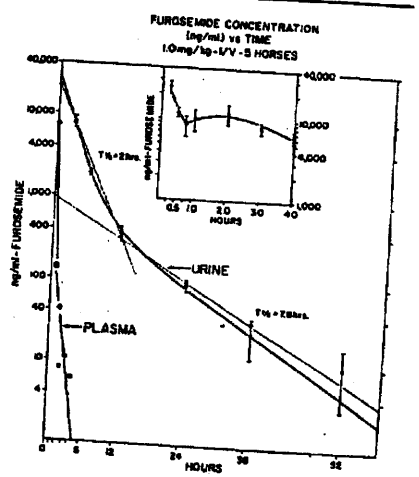

Fig 7—Urinary concentrations of furosemide after 1 mg/kg furosemide by rapid intravenous injection. The solid circles (● - ●) show urinary concentrations of furosemide after IV administration of 1 mg/kg of furosemide. The inset shows a detail of urinary concentrations during the first 4 hours postdosing. The dotted lines represent least squares fit to urinary concentrations of the drug observed over the first 12 hours and for subsequent 48-hour periods together with the respective apparent half-lives. The open circles (O - O) show the corresponding plasma levels (Fig 6) reported for comparative purposes. All data points are means ± SEM of experiments on 5 horses.

were modelled using a SAAM 23 program[18] to fit the data to the differential equations describing a 2-compartment open model. Since the SAAM program used a weight of $\frac{1}{(SD)^2}$ for all points, slightly different parameters were obtained. The pharmacokinetic parameters describing this model obtained by both analyses are presented in Table I.

Figure 7 shows urinary concentrations of furosemide observed in these horses. Urinary concentrations of furosemide fell from an initial value of about 25 μg/ml (25,000 mg/ml) to less than 9 μg/ml at 45 minutes postdosing. Thereafter, urinary concentrations remained at a plateau for a period, then declined with an apparent half-life of about 2 hours. After the 12th hour, by which time urine levels had reached 400 ng/ml, the apparent urinary half-life of the drug had increased to about 7.5 hours. By 52 hours urinary concentrations of the drug had fallen to about 10 ng/ml, at which point the experiment was terminated.

Figure 8 shows plasma levels of furosemide after the IM injection of 1 mg/kg of furosemide. Plasma levels of the drug peaked very rapidly (at about 8 minutes) and then declined exponentially over the next 12 hours, from about 00 ng/ml initially to about 2 ng/ml at 12 hours. Over the rst 4 hours after dosing by this route, about 62% of the ad-

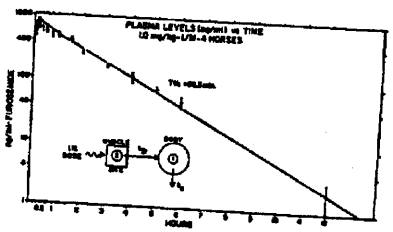

Fig 8—Plasma concentrations after 1 mg/kg furosemide intramuscularly. The solid circles (● - ●) show plasma concentrations of furosemide after 1 mg/kg IM. The solid line is a least squares fit to all data points subsequent to the peak plasma drug concentration. The inset shows a 1-compartment open model which may be used to describe these data. All data points are the mean + SEM experiments on 4 horses.

TABLE 1

Pharmacokinetic Parameters for Furosemide after Intravenous Injection

The pharmacokinetic parameters for furosemide in the horse were calculated from the graphically determined values in Fig 6 and also by means of a SAAM 23 fit to the 2-compartment open model of Fig 6.

A. Pharmacokinetic Parameters Calculated from Fig 6.	B. Pharmacokinetic Parameters from SAAM 23 Program Fit.
A = 8839 ng/ml	k_{12} = 0.035 minute^{-1}
B = 372 ng/ml	k_{21} = 0.030 minute^{-1}
α = 0.138 minute^{-1}	k_1 = 0.125 minute^{-1}
β = 0.018 minute^{-1}	β = 0.022 minute^{-1}
$t_{1/2}$ = 5.0 minutes	$t_{1/2}$ = 31.5 minutes
$t_{1/2}$ = 38.6 minutes	V_β = 272.6 liters
K_a = 0.108 minute^{-1}	$\int_0^\infty$ Cdt = 1513 ng/ml x hour
k_{21} = 0.022 minute^{-1}	Clearance 304.7 liters/hour
k_{12} = 0.024 minute^{-1}	
V_1 = 50.1-liters	
= 0.11 liters/kg	
Vd_β = 2.7 liters/kg	
Vd_{area} = 54 liters/kg (corrected for plasma protein binding)	

ministered dose was recovered as unchanged drug in the urine (Table 2).

These data could not be fitted to a 2-compartment open model by assuming rapid first order absorption of the drug because of the relatively slow decline in the plasma half-life of this drug. These data were well fitted by a 1-compartment open model (Inset, Fig 8), assuming very rapid absorption of the drug and slower elimination of the drug than was observed after its IV administration. Table 3 shows the pharmacokinetic parameters generated by a SAAM 23 fit of the differential equations describing this model fitted to the data of Fig 8.

Figure 9 shows urinary concentrations of furosemide in the experiment of Fig 7. Urinary levels of the drug started at

TABLE 2

Excretion of Unchanged Furosemide in Urine after Intramuscular Injection

Hours post-injection	Cumulative percentage of dose recovered from urine
1.0	27.2 ± 5.3
2.0	45.0 ± 8.6
4.0	61.8 ± 10.9

TABLE 3

Pharmacokinetic Parameters for Furosemide after Intramuscular Injection

k_{21} = 0.448 minute^{-1}

k_e = 0.008 minute^{-1}

V/F = 649 liters

$\int_0^\infty$ Cdt = 1642 $\frac{\text{ng hour}}{\text{ml}}$

Clearance = 290.5 liters/hour

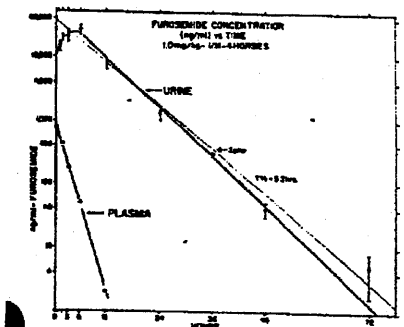

Fig 9—Urinary concentrations after 1 mg/kg of furosemide intramuscularly. The solid circles (● - ●) show urinary concentrations of furosemide after IM injection of 1 mg/kg furosemide. The dotted line (- - - -) represents a least squares regression fit to the data points from 12 hours on, which yielded an apparent urinary half-life for the drug of about 5.3 hours. The open circles (O - O) show some plasma levels from Fig 8 replotted for comparative purposes. All data points are the means of determinations on 4 horses.

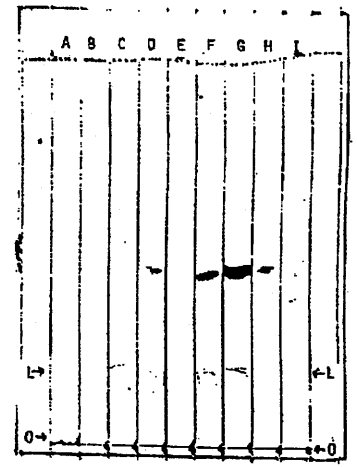

Fig 10—Thin layer chromatogram of furosemide recovered from equine urine. A Thoroughbred mare was dosed with 1 mg/kg furosemide IM and urine samples taken over the next 24 hours. Urine samples were extracted, chromatographed, and sprayed as outlined in Methods. The chromatograms are as follows: A, control urine prior to furosemide; B, urine spiked with 15 μg furosemide; C,D,E,F,G,H, urine samples, 2,4,8,12, and 24 hours post-dosing; I, urine spiked with 5 μg/ml urine. The arrows marked L point to the furosemide spots, which are bright red in color. The arrows marked O point to the origin. The circles on samples B and H encompass a faint pink spot in B, and the position of a trace of fluorescence in H. Sample I yielded no detectable fluorescence or color. This thin layer plate typical of about 12 experiments.

about 11.0 μg/ml, slowly increased to about 30 μg/ml at 6 hours, then declined exponentially with a half-life of 5.3 hours to about 4 ng/ml at 72 hours, when the experiment was terminated.

Figure 10 shows the detection of furosemide in urine by the routine method used for racetrack urine samples in Kentucky as described in Materials and Methods. By this method furosemide was detected in equine urine for about 12 hours after its injection into the horse. The limit of detection of this method was about 7.5 μg of furosemide, or about 0.5 μg/ml in a 15-ml urine sample.

Discussion

Modification of the method of Lindstrom and Molander[12] yielded a stable methylated derivative of furosemide with good chromatographic and electron capturing properties (Figs 1—4). Data obtained with this and other methods showed that furosemide was highly protein bound to equine plasma over the concentration range within which this drug is found *in vivo* (Fig 5). After its rapid IV injection, the pharmacokinetics of this drug in the horse appeared to be adequately described by a 2-compartment open model with a β phase half-life of between 32 and 38 minutes (Fig 6 and Table 1). After its IM injection, plasma levels of the

drug peaked very rapidly (within 10 minutes) and then declined relatively slowly, with an apparent half-life of 86 minutes (Fig 8). Urinary concentrations of this drug were invariably much higher than plasma concentrations, and the drug was detected in urine long after it was no longer detectable in plasma (Fig 9). Routine forensic screening methods for furosemide appeared to be able to detect the drug in urine for up to about 12 hours postdosing (Fig 10).

In our hands, the gas chromatographic method reported here was the only satisfactory method for detection of furosemide in equine plasma and urine. Preliminary experiments with the fluorometric method used by other workers[9] did not yield a satisfactory β phase half-life after IV injection of this drug and were totally incapacitated by the fluors present in equine urine.[14] A colorimetric reaction based on the Bratton Marshall reaction such as was used for the thin layer plates (Materials and Methods) encountered problems with high and variable blanks. Furosemide could not be derivatized with pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA), or thionylchloride. Because of these problems the current somewhat laborious technique was the only method available to us to detect furosemide in equine plasma and urine.

The plasma levels of furosemide observed after its rapid IV injection were well fitted by a 2-compartment open model described by the parameters in Fig 6 and Table 1. The initial α distribution phase for furosemide was relatively fast, with a $t_{1/2}$ of about 5 minutes. This suggests that despite the presence of the ionized carboxyl group the drug is relatively lipid soluble and leaves the central compartment rapidly. The β or metabolic phase was also relatively rapid, with an apparent half-life of between 32 and 38 minutes, depending on the statistical weighing of individual values. This is a relatively short half-life for any drug in the horse, particularly one as highly protein bound as furosemide. The apparent volume of distribution of furosemide in the horse was 2.7 liters/kg. When this value is corrected for 95% protein binding, it becomes about 54 liters/kg, a very large volume of distribution indeed. Only relatively small concentrations of free furosemide were thus available for metabolism and excretion. These considerations might lead one to expect a relatively long plasma half-life for this drug as is observed in the horse with phenylbutazone, another acidic, highly protein bound drug.[20]

Despite these considerations, the plasma half-life for furosemide observed in these experiments was only about 1/10 of the 5—6 hour plasma half-life of phenylbutazone.[19] Further, while urinary concentrations of phenylbutazone are approximately the same as plasma levels of the drug, urinary concentrations of furosemide may be 1,000 times greater than total plasma levels of the drug, of which only about 1/20 is free to be excreted. Finally, phenylbutazone is almost completely metabolized in the horse,[6] while up to 60% or more of a dose of furosemide can be excreted unchanged in the horse. These observations suggest fundamental differences in the handling of these 2 drugs by the horse, and it appears likely that excretion of furosemide by the organic acid transport system in renal tubules can account for the majority of these differences.

The renal tubules have long been known to possess an active transport system for the excretion of organic acids. Studying this system, Hirsch *et al.*[10] produced clear-cut evidence for accumulation of furosemide by renal tissue *in vitro*. Similarly, Cohen *et al.*[2] have shown that in the dog renal tissue accumulates furosemide *in vivo* to concentrations about 10 times those observed in plasma. Again, Hook and Williamson[11] have shown that probenecid, a specific inhibitor of the renal tubular organic transport system, reduces the diuretic response to furosemide in the dog. These observations show that furosemide is actively transported into the renal tubule and suggest that this is necessary for its diuretic action.

Based on the assumption that furosemide is actively secreted into the renal tubule, the rather unique pharmacokinetics of furosemide become explainable. Thus, the relatively short plasma half-life of this highly protein bound drug suggests very efficient secretion by the kidney. Efficient secretion of the parent drug accounts for the very high concentrations of the drug observed in urine and the very high proportion of the drug excreted unmetabolized. It would thus appear that the pharmacokinetics and disposition of furosemide in the horse are largely dependent on its secretion by the renal organic acid transport system.

The β phase half-life of 32—38 minutes reported for furosemide in the horse is in good agreement with plasma half-life values reported for other species. Thus, Wallin *et al.*[22] report a 25-minute β phase half-life for furosemide in the rat. Similarly, Cutler *et al.*[4] report a β phase half-life for furosemide in man of about 29.5 minutes, in close agreement with the values reported here for the horse. However, earlier studies by Calesnick and other workers have reported longer plasma half-lives for furosemide in man.[1] The short plasma half-life for furosemide after IV injection reported in this work is also in good agreement with the very brief diuretic effect elicited by 1 mg/kg furosemide.[14,16] In contrast, after IM injection of this same dose both plasma levels of the drug and its diuretic effect are relatively prolonged.[14]

Secretion of furosemide into the renal tubule by the active transport system presumably also accounts for the high urinary concentrations of furosemide and their slow decline relative to plasma levels of the drug. As shown in Fig 7, after

IV injection, urinary concentrations of procaine started at about 25 μg/ml and fell relatively rapidly for the first 30 minutes. This initial rapid decline in urinary levels then ceased abruptly, and urinary concentration of furosemide then appeared to increase slowly over the next hour. This clear-cut change in urinary concentration of the drug appears related to its peak diuretic effect. It is likely that the sudden cessation in the decline in urinary concentrations of the drug is due to reestablishment of the urinary concentrating mechanism, increasing the concentration of furosemide in the urine.

Thereafter, urinary concentrations of furosemide fell rapidly with a half-life in the order of about 2 hours. However, after about 12 hours the rate of decrease of urinary concentrations of the drug slowed with the final apparent half-life of the drug in urine being about 7.6 hours. The tendency for furosemide to concentrate in the kidney presumably explains the much higher concentrations of the drug observed in urine and the prolonged clearance time. In interpreting these experiments it should be kept in mind that since plasma furosemide is about 95% protein bound, the concentrations of furosemide which are free in plasma and available for excretion in the urine are very low indeed, and presumably rarely greater than 50 ng/ml.

After IM injection of furosemide, plasma levels peaked within 8 minutes and then declined. This decline had an apparent half-life of about 86 minutes and continued unchanged through almost 3 orders of magnitude, until furosemide levels became undetectable at about 12 hours. Urinary excretion data showed that about 62% of the dose administered was excreted unchanged in the first 4 hours (Table 2). The relatively prolonged plasma half-life of furosemide after injection by the IM route also corresponds well with the relatively prolonged diuretic response seen after administration of the drug by this route.[14]

Pharmacokinetic analysis of these data showed that the initial peak of drug levels and the slow subsequent decline could not be fitted to the 2-compartment open model of Fig 6 and Table 1 assuming first order absorption of the drug from a single intramuscular injection site. The best fit to these data were obtained by assuming a 1-compartment open model. Table 3 presents the kinetic parameters describing this model. Because the areas under the IV and IM curves are comparable, the absorption fraction or bioavailability of this drug after IM injection appears to be close to 1, and plasma clearance values at between 290 and 385 liters/hour by both routes were also comparable.

After IM injection of furosemide, urinary concentrations rose for the first 6 hours and then declined exponentially over the next 66 hours, at which point they became undetectable. Again, urinary concentrations of furosemide are very much greater than plasma, either bound or free plasma concentrations, and the drug remains detectable in equine urine long after it is no longer detectable in plasma.

As a practical matter, these results show that furosemide is rapidly eliminated from the bloodstream of the horse. Since the drug is eliminated largely by secretion unchanged into urine, very high concentrations of the drug are found in urine long after it can no longer be detected in plasma and its pharmacological effects have ceased. Because this might create a problem in routine testing, we studied the efficacy of routine screening tests for furosemide, such as are used in the Kentucky Equine Drug Testing Program. The data of Fig 10 shows that, using a simple acidic extract and thin layer chromatography, extraction of a 15-ml sample of equine urine allowed detection of the drug for up to about 12 hours after its administration. In view of the fact that the diuretic, cardiovascular, and significant drug diluting effects of furosemide appear to be complete within 2 to 4 hours of the normal IV dose, there is no rational need to detect furosemide beyond this time. Thus, simple acidic extraction and either U.V. or colorimetric detection should suffice for fair and effective (rather than irrational) drug screening for furosemide, and urinary concentrations of this drug of less than 500 ng/ml should not be considered pharmacologically significant.

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Furosemide, Patella vulgata β-Glucuronidase and Drug Analysis: Conditions for Enhancement of the TLC Detection of Apomorphine, Butorphanol, Hydromorphone, Nalbuphine, Oxymorphone and Pentazocine in Equine Urine.

Joan Combie, J. W. Blake, Thomas E. Nugent, and Thomas Tobin*

Department of Veterinary Science
College of Agriculture
University of Kentucky
Lexington, KY 40546-0076

ABSTRACT

We have investigated the action of five sources of β-glucuronidase enzymes on the hydrolysis of glucuronides of apomorphine, butorphanol, hydromorphone, nalbuphine, oxymorphone and pentazocine in equine urine.

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*To whom correspondence should be addressed.

For all glucuronides tested, Patella vulgata β-glucuronidase yielded the largest thin layer chromatographic (TLC) spots. For oxymorphone, P. vulgata was the only treatment to yield detectable TLC spots under test parameters.

For these six drugs, TLC spot size and chromatographic quality were compared between control horses and horses pretreated with furosemide four hours earlier. Furosemide pretreatment produced a statistically significant increase in spot size and was found to enhance chromatogram quality.

These findings support previous suggestions that P. vulgata is a superior drug-glucuronide hydrolyzing enzyme. They also support earlier reports that administration of furosemide at four hours prerace is unlikely to result in significant interference with routine drug testing procedures.

INTRODUCTION

Furosemide (Lasix®) is the treatment of choice for exercise-induced pulmonary hemorrhage (EIPH) or epistaxis (Tobin, 1981). The only problem with this approach is that furosemide may interfere with the detection of certain drugs in urine for a period after its administration.

In 1977, Gabel, Tobin, Ray and Maylin (1977) suggested that this interference or dilution effect would be minimal four hours after furosemide administration. Recently, Combie and co-workers (1981) confirmed

this estimate, showing that after 0.4 to 0.5 mg/kg furosemide IV the dilution effects were over in three hours or less. The present study was undertaken to extend the scope of this earlier work. Thin layer chromatography was chosen as the testing mode because this is the most widely used drug screening method. The drugs examined were apomorphine, butorphanol, hydromorphone, nalbuphine, oxymorphone and pentazocine.

Current screening methodology used by the Kentucky Equine Drug Detection Laboratory includes hydrolysis of urine samples by β-glucuronidase from P. vulgata before analysis by thin layer chromatography. Although previous work has shown that the P. vulgata is the preferred source of β-glucuronidase for cleaving morphine glucuronide (Combie, Blake, Nugent, Tobin, 1981), no studies have yet reported the applicability of this procedure to cleave other drug-glucuronide complexes. Therefore, samples were hydrolyzed by β-glucuronidase from five different sources, including P. vulgata, to determine whether the Patella preparation remained superior in the presence of other aglycones.

MATERIALS AND METHODS

Apomorphine was obtained from Eli Lilly & Co, Indianapolis, IN; butorphanol was from Bristol Laboratories, Syracuse, NY; hydromorphone was from Wyeth Laboratories, Inc, Philadelphia, PA; nalbuphine and oxymorphone were from Endo Laboratories, New York, NY; pentazocine was from Winthrop Laboratories, New York, NY; and furosemide was from National Laboratories Corp, Somerville, NJ.

Experiment 1:

Six mares were dosed intravenously (IV) with one of the following preparations: 0.012 mg apomorphine per kg, 0.05 mg butorphanol

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(Stadol®) per kg, 0.01 mg hydromorphone per kg, 0.14 mg nalbuphine (Nubain®) per kg, 0.005 mg oxymorphone (Numorphan®) per kg, and 0.25 mg pentazocine (Talwin-V®) per kg. A urine sample was obtained 75 min later by bladder catheterization from the horse dosed with oxymorphone. Urine samples were obtained from the other five horses 50 min after dosing.

Five sources of β-glucuronidase from Sigma Chemical Co, St. Louis, MO, were utilized: P. vulgata (Type L-II), Helix aspersa (Type HA-4), Helix pomatia (Type H-1), bovine liver (Type B-3) and Glucurase®. The first four were lyophilized powders stored at -20 °C which were weighed out and dissolved in distilled, deionized water to make solutions equivalent to 5000 U of β-glucuronidase per mL immediately before an assay was run. Glucurase® was a bovine liver β-glucuronidase solution, acetate buffered to pH 5 and stored at 4 °C. It was used as obtained from the manufacturer. Urine samples to be hydrolyzed by either H. aspersa or H. pomatia were adjusted to pH 4.5; those to be hydrolyzed by P. vulgata or bovine liver were brought to pH 5; and those to be incubated with the Glucurase® were adjusted to pH 5.5 with acetic acid.

Hydrolysis was performed by mixing 200 µL of urine from all horses, except the one given oxymorphone, with 200 µL of each enzyme solution and incubating for 1 h in 1 mL sealed, glass ampules in a water bath. For oxymorphone, 400 µL of urine and an equal volume of enzyme were incubated. An incubation temperature of 65 °C was used for samples containing P. vulgata as the β-glucuronidase source, while all other samples were incubated at 55 °C.

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Following incubation, 200 µL of the urine-β-glucuronidase mixture (600 µL for samples containing oxymorphone) was mixed with 500 µL of a 1.5 M carbonate buffer, bringing the pH to 8.9 with the exception of urine from the horse dosed with apomorphine. Urine containing apomorphine was adjusted to pH 7. The buffered urine samples were extracted with 4 mL dichloromethane:isopropanol (9:1). Following separation by centrifugation, the solvent was transferred to clean tubes and 1 mL of 0.1 N H_2SO_4 was added to each. The tubes were mixed gently for 15 min. The layers were separated by centrifugation and the solvent was discarded. Two mL of the carbonate buffer was added to each tube, bringing the pH to 8.9 except for apomorphine-containing samples. For samples from horses dosed with apomorphine, only 0.5 mL of the buffer was added to adjust the pH to 7. Four mL of fresh solvent was added to each tube. Following extraction and separation by centrifugation, the dichloromethane:isopropanol was transferred to a clean set of tubes and evaporated to dryness under a stream of nitrogen.

The residue was redissolved in 25 µL of dichloromethane and spotted on high performance thin layer chromatography plates (Brinkmann Instruments, Inc, Westbury, NY; E. Merck, manufacturer) pre-coated with silica gel 60, using long capillaries drawn from 22.9 cm Pasteur pipettes. The plates were developed in ethyl acetate:methanol:glacial acetic acid (80:10:10). The solvent front was allowed to traverse a distance of 5 cm. Following air drying, the plates were sprayed with modified Folin-Denis reagent.[1]

[1] A mixture of 10 g sodium tungstate, 2 g 12-molybdosilicic acid, 5 mL concentrated phosphoric acid and 50 mL water was refluxed for 2 h. The mixture was diluted to 100 mL with additional water and stored at 0-5 °C (can be stored at room temperature).

The plates were then exposed to fumes of ammonium hydroxide to maximize the blue-green color reaction.

The plates were labeled with a code unknown to the person reading them. A score of 10 was assigned to the largest, most distinct spot for each drug. Failure to detect the presence of a drug was recorded as a zero. The other spots were then ranked on this 0 to 10 scale by the independent reader.

Experiment 2:

TABLE I

Experimental Design

-4 h	administered saline (control) or 0.4 mg furosemide/kg; water withheld.
-45 min	administered nalbuphine, oxymorphone, pentazocine.
-30 min	administered butorphanol, hydromorphone.
-20 min	administered apomorphine.
0 min	POST TIME (hypothetical).
+20 min	allowed water ad libitum.
+30 min	urine sample collected.
+ 1 h	urine sample collected.
+ 2 h	urine sample collected.

Six mares were dosed with saline for the control part of this experiment and on a separate occasion were given 0.4 mg furosemide (Lasix®) per kg for the test section of this experiment. This was done in random order. The time of administration of the saline or the diuretic was set at -4 h, corresponding to 4 h before hypothetical race time. Water was withheld

at this time. Each horse was given one of six drugs in the same dosages as listed in Experiment 1. The timing of this drug administration ranged from 20 to 45 min before post-time as listed in Table I. Twenty min after post time, waterbuckets were returned to the stalls housing these horses and they were allowed water ad libitum for the duration of the experiment. Urine samples were collected by bladder catheterization at 30 min, 1 h and 2 h after the time of the hypothetical race. Samples were stored at -20 °C until they could be assayed. The specific gravity of all samples was determined.

Urine samples were adjusted to pH 5. Either 80 µL or 500 µL of urine was incubated with an equal volume of a 5000 U/mL solution of β-glucuronidase from P. vulgata for 3 h at 65 °C. The hydrolyzed urine samples were assayed as outlined under Experiment 1. The samples were assigned a number with the aid of a random number generator. The plates were read and scored by one of us (JWB) with no knowledge of either the code or the experimental design. A score of 10 was given for the largest, most distinct spots for the entire experiment. Failure to detect the presence of a drug was assigned a zero and other spots were ranked on this 0 to 10 scale.

RESULTS

The ability of β-glucuronidase from 5 sources to hydrolyze six different drug-glucuronide complexes was checked (Fig 1). For all six drugs, hydrolysis by the P. vulgata preparation, gave the maximum size spot on the TLC plate and was assigned a score of 10. With the exception of apomorphine, use of β-glucuronidase from the powdered bovine liver resulted in the lowest score, indicating that for 5 of the drugs in this study, the

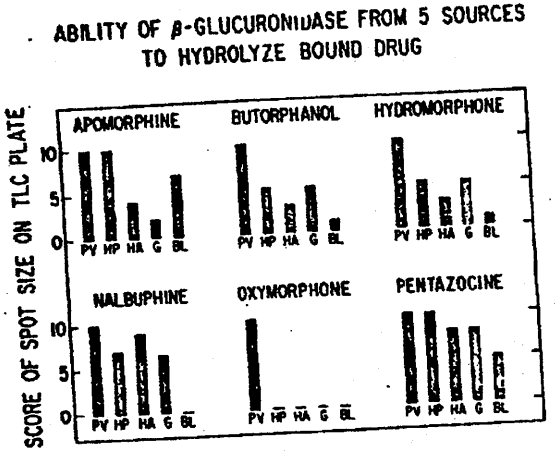

Fig 1 The ability of β-glucuronidase from 5 sources (PV - Patella vulgata; HP - Helix pomatia; HA - Helix aspersa; G - Glucurase®; BL - powdered bovine liver) to hydrolyze six different drug-glucuronide complexes is shown as the score of spot sizes on TLC plates following a 1 h hydrolysis.

bovine liver preparation had the poorest ability to hydrolyze the drug-glucuronide complex. β-glucuronidase from H. pomatia, H. aspersa and the Glucurase® solution, gave intermediate results with the exact order of the scores of TLC spot sizes varying somewhat among the six different drugs. The choice of the source of β-glucuronidase appeared to be most critical for oxymorphone, where the drug was detected only in the sample submitted to hydrolysis by the P. vulgata enzyme under parameters of experiment 1. All other enzyme preparations resulted in insufficient hydrolysis for the presence of oxymorphone to be detected. The amount of pentazocine administered to the horse was sufficient to give acceptable results regardless of the source of β-glucuronidase.

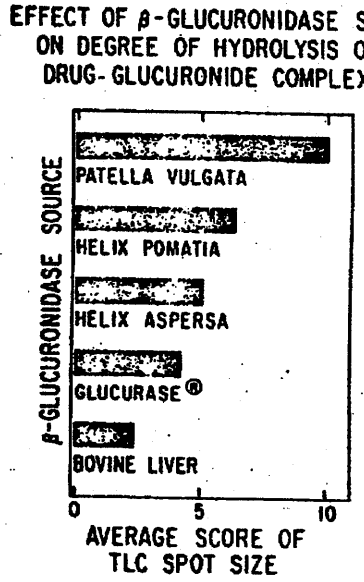

Fig 2 Six mares were dosed IV with one of the following drugs: apomorphine, butorphanol, hydromorphone, nalbuphine, oxymorphone, and pentazocine. Urine samples were hydrolyzed with 5 β-glucuronidase preparations, extracted and spotted on TLC plates. The plates, labelled with a code unknown to the person reading them, were scored on a 0 to 10 scale (10 - maximum size spot, 0 - no drug detected). The average score of TLC spot size is normalized to the scores obtained from the use of Patella vulgata β-glucuronidase.

The mean scores obtained with each individual drug and enzyme preparation are illustrated in Fig. 2. While hydrolysis by the P. vulgata preparation freed the maximum amount of drug in each case, the bovine liver β-glucuronidase hydrolyzed only 23% as much drug-glucuronide complex on the average, resulting in a mean score of 2.3. Of the five β-glucuronidase preparations tested, enzyme from P. vulgata in each case gave the best results, regardless of the aglycone.

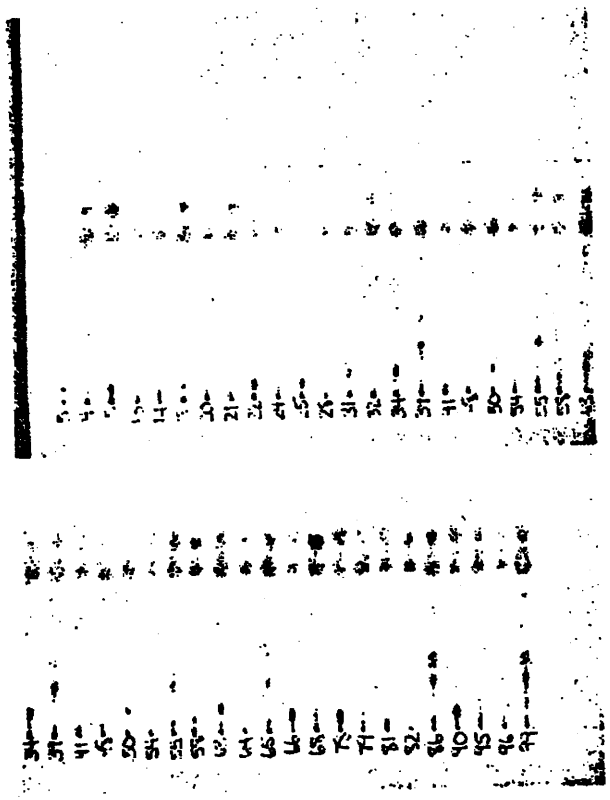

Fig 3 Six horses were pre-treated with saline for the controls and, on a separate occasion, with 0.4 mg furosemide per kg. They were dosed with one of 6 drugs, apomorphine, butorphanol, hydromorphone, nalbuphine, oxymorphone or pentazocine and urine samples were collected according to the schedule in Table I. Samples were hydrolyzed by ß-glucuronidase from Patella vulgata for 3 h at 65 °C, extracted and spotted on TLC plates such as the one shown here. Samples were assigned numbers by random number generator to eliminate reader bias.

The effect of furosemide on drug detection is shown in Figures 3 and 4. Figure 3 is a photograph of the TLC plate obtained following analysis of the 500 µL of urine from horses treated and not treated with furosemide before being dosed with one of the six drugs used in this study (Experiment 2). The photo shows the random numbers assigned to the samples. This was one of the plates scored to determine the effect of Lasix® pre-treatment on the size of the drug spot on a TLC plate. Before the code was broken, the test reader noted that some samples appeared to be much "cleaner" than others, and these observations were recorded. Upon analysis of the data, it was found that the samples from horses pre-treated with the diuretic gave cleaner, more distinct and easier to read thin layer chromatograms. For example, although #18 and #58 samples were from the same horse, at the same time following drug administration, and assigned comparable scores, #18 gave a more distinct spot and exhibited the presence of less extraneous material, thus less smearing. Sample #18 was obtained after the horse had been given furosemide, while sample #58 was from the same horse not pre-treated with the diuretic.

The effect of furosemide on the TLC spot size for six different drugs is shown in Fig 4. The values plotted were an average of those obtained following analysis of both 80 and 500 µL of urine. A 1-tailed, paired data t-test indicated that, in this experimental design, administration of furosemide resulted in a statistically significantly higher TLC score ($t = 1.902$, $\alpha = .05$) than control samples. Statistical analysis of the specific gravity of all urine samples in this experiment revealed that the overall slight increase in urine specific gravity following pre-treatment with furosemide was not significant (1-tailed, paired data t-test, $t = .670$, $\alpha = .05$).

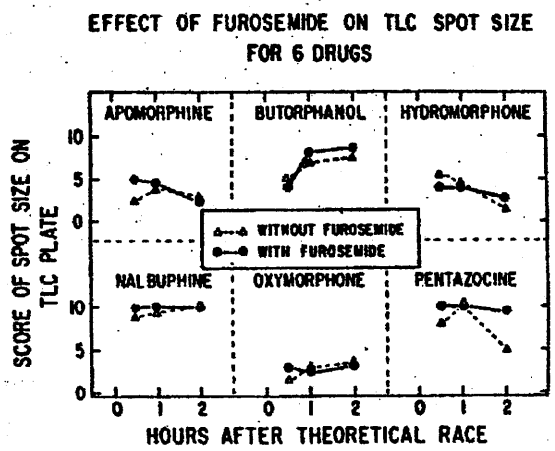

Fig 4 Six mares were pre-treated with saline (shown by the open triangles, Δ-Δ) and, on a separate occasion, with 0.4 mg furosemide per kg (shown by the closed circles, ●-●). They were dosed with one of the six drugs indicated according to the schedule in Table I. Urine samples were hydrolyzed by β-glucuronidase from Patella vulgata. They were analyzed by thin layer chromatography and the spots were scored according to size.

DISCUSSION

P. vulgata was the most useful of the five β-glucuronidase preparations evaluated for activity against the glucuronide metabolites of six different drugs. When 5,000 U of P. vulgata β-glucuronidase was incubated with the drug-glucuronide complexes of apomorphine, butorphanol, hydromorphone, nalbuphine, oxymorphone and pentazocine in 500 µL or less of urine, we found larger TLC spots after use of the P. vulgata enzyme. This held for all drugs tested; one drug, oxymorphone, was detected only after hydrolysis of the urine sample with the Patella vulgata β-glucuronidase under test parameters.

Based on this experience, we tested the efficacy of this glucuronide hydrolysis system in a situation where the efficient hydrolysis of drug-glucuronide metabolites may be forensically important. The circumstance chosen was pre-treatment of horses with furosemide. This is because the diuresis due to furosemide can dilute out certain drugs and drug-glucuronide metabolites in urine, rendering their efficient recovery important (Tobin, 1981).

In the test model, we elected to treat our horses with furosemide at 0.4 mg/kg four hours "pre-race". Four hours was selected because this is the current Kentucky rule and has been suggested by the Veterinary Chemists' Advisory Committee to the National Association of State Racing Commissioners (Gabel et al, 1977). The test drugs were administered between 45 and 20 min prior to the hypothetical post time. The times of administration of the drugs were selected so that the drugs would have maximal behavioral effects at post time, and the doses selected were below threshold doses for measurable behavioral effects.

One objective of this experiment was to determine whether or not furosemide treatment would interfere with routine post-race TLC screening for drugs in horse urine. For this reason, the extraction and TLC methodology used was similar to that currently used in the Kentucky Equine Drug Testing Program (Blake et al, 1979). All TLC plates were coded and read "blind" by one of us (JWB) to obviate observer bias in the interpretation of these tests.

In these experiments, pre-treatment of horses with furosemide did not appear to significantly reduce the detectability of any of the

drugs tested. In fact, when the spot size scores were compiled and compared with the control values, the score from the furosemide-treated horses was actually statistically significantly better than in the untreated samples. Further, the reader of the plates commented that the quality of the plates from the furosemide-treated horses was superior to those from the untreated horses. It appears that Lasix® tends to reduce the concentration of materials which would tend to co-extract with the drug, thus leaving a "cleaner" sample. This results in less smearing and better spot definition. The experiment is in good agreement with earlier work which showed that the drug diluting effects of these small doses of furosemide is over within three hours or less of dosing (Combie, Nugent, Tobin, 1981). The experiment also suggests that the earlier recommendation of Gabel, Tobin, Ray and Maylin (1977) on lack of significant drug dilution effect of furosemide at four hours is essentially correct.

Since the diuretic effects of furosemide are characteristically brief, the lack of interference by these doses of furosemide with drug testing was not unexpected. However, the apparently clear-cut enhancement of the quality of the thin layer chromatograms in the presence of furosemide was surprising. On the other hand, previous work from this laboratory has shown that urinary concentrations of fentanyl and its metabolites and free morphine in horse urine were found to be higher four hours after furosemide treatment than in controls (Combie, Nugent, Tobin, 1981), which supports these thin layer chromatographic findings. In association with the fact that some drugs are not affected by the

diluting effects of furosemide, these results show that under some circumstances furosemide may actually enhance the detection of drugs in horse urine.

In summary, therefore, these results support suggestions that P. vulgata β-glucuronidase is a superior drug-glucuronide hydrolyzing enzyme for the structures tested. They further support earlier studies and estimates of the duration of the drug "diluting" effects of furosemide and support suggestions that four hours pre-race is a relatively conservative time to administer this drug.

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THE PHARMACOLOGY OF FUROSEMIDE IN THE HORSE
V. The Duration of Reduction of Urinary Concentration of Drugs

Joan Combie MSc; Thomas Nugent, and Thomas Tobin MVB, PhD, MRCVS

SUMMARY

The administration of furosemide to horses in IVdoses of 0.5 mg/kg or less reduced drug concentrations in urine for less than 4 hours. The most prolonged reduction observed was that of the glucuronide metabolite of morphine, which required three hours post-dosing to return to control. Urinary concentrations of phenylbutazone were not significantly different from control by two hours post-dosing, while urinary concentrations of fentanyl appeared to return to normal within about two and one-half hours of dosing. Other experiments showed that blood levels of morphine were not significantly reduced by furosemide treatments.

INTRODUCTION

Furosemide (Lasix®)[a] is a potent, high ceiling diuretic which is the recommended treatment for epistaxis or exercise induced pulmonary hemorrhage (EIPH) in the horse. Since at least 43% of racing horses have some degree of EIPH, a number of racing jurisdictions have allowed the pre-race use of furosemide in horses.[105] However, the principal problem with the approval of furosemide for use in racing horses is that it acts to dilute out certain drugs and drug metabolites in equine urine. This dilution is important because it might reduce the efficacy of testing for some drugs.

Because furosemide is a very short acting drug in the horse, these diluting effects are quite transient. Therefore, one approach to the problem of the diluting effect of furosemide in the horse is to avoid taking urine samples during the period of the diluting effect.

Reviewing the problem in 1977, Drs. Gabel, Tobin, Ray, Maylin and the other members of the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners concluded that "furosemide administered more than four hours before race time does not significantly reduce the ability to detect those drugs studied to date."[4] These are the only published recommendations on the duration of diluting effects of furosemide. However, since these workers based this 4-hour estimate on research done using 1.0 mg/kg doses of furosemide, we elected to investigate the duration of the diluting effect of furosemide after administration of smaller (0.5 mg kg or less) doses of this drug.

It is important that the duration of the diluting effect of prophylactic doses of furosemide be determined. Although the diuretic effects of furosemide are relatively short-lived, the duration of other pharmacological actions of this drug may be more prolonged. Therefore, to enable the use of this drug in horses without undue interference with drug testing, the minimal period for which small, prophylactic doses of furosemide produce their diluting effects should be accurately known. Preliminary reports of this work have been communicated.[5]

Senior author's address: The Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40546.

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[a] A. Butler Co., Columbus, Ohio.

MATERIALS and METHODS

Animals: Mature thoroughbred and standardbred horses between 400 and 550 kg were used. The animals were kept at pasture until the day of an experiment when they were housed in box stalls and allowed hay and water *ad libitum*.

All drugs were injected intravenously into the left jugular vein. Blood samples were drawn from the right jugular vein and urine was obtained by bladder catheterization.

Experiment 1:

Phenylbutazone and Furosemide: Three mares were dosed with 4.4 mg phenylbutazone[b] per kg body weight. Urine samples were collected at intervals for 24 hours. The following week, the procedure was repeated on the same animals except that 0.385 mg furosemide per kg body weight was injected intravenously immediately after collection of the 2-hour sample. All samples were stored at 4° C until the analysis was performed.

Two hundred µl of each urine sample was mixed with 2 ml hexane:dichloromethane (2:1) and 2 ml sodium acetate buffer (pH 4.5). The tubes were rotoracked for 5 minutes and centrifuged at 1150 g, 2° C for 15 minutes. A 1 ml aliquot of the solvent phase was evaporated to dryness under nitrogen. The residue was redissolved in 50 µl hexane, 2 µl of which was injected into a Varian 2700 gas chromatograph equipped with a tritium on scandium electron capture detector. Separation was done on a 6-foot glass column packed with 3% OV-101 maintained at 249° C.

Experiment 2:

Fentanyl and Furosemide: Four horses were given 0.001 mg fentanyl[c] per kg body weight and urine samples were collected at intervals (Figure 2) during the next 24 hours. The following week, the same horses were again dosed with fentanyl. Immediately after the 30-minute urine sample was obtained, the animals were given 0.5 mg furosemide per kg body weight. All samples were stored at -20° C for later analysis.

Levels of fentanyl in the urine were determined by a radioimmunoassay method utilizing a commercially available kit.[d] The procedure has been described elsewhere.[1] Briefly, 0.05-0.5 ml urine was incubated with radioactive tracer and antiserum for 2 hours at pH 7.4. Fentanyl in the sample competed with the ^{3}H-fentanyl for binding sites on the fentanyl antibody. After formation of

[b] Sodonal Laboratories Corp., Somerville, N.J.
[c] McNeil Laboratories, Fort Washington, PA.
[d] Institut National des Radioelements, Fleurus, Belgium.

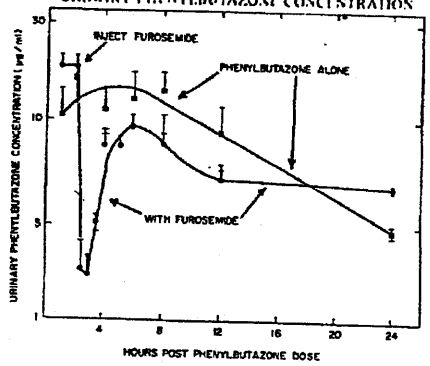

Figure 1. Effect of furosemide treatment on urinary phenylbutazone concentration. Urinary phenylbutazone concentrations following IV dosing with phenylbutazone alone are shown by the solid squares (■-■) and following IV dosing with phenylbutazone and furosemide (0.385 mg/kg) are shown by the solid circles (●-●). Vertical bars represent ± SEM. (Reproduced with permission from "Drugs and the Performance Horse," Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981).

the immunocomplex was completed, bound and free fentanyl were separated by selective adsorption onto dextran-coated charcoal. The charcoal complex was removed by centrifugation and the radioactivity in the supernatant, due to bound ^{3}H-fentanyl, was measured in a liquid scintillation counter.

Experiment 3:

Morphine and Furosemide: Mares were injected IV with 0.1 mg morphine[a] per kg body weight. Blood and urine samples were collected (Figure 3-5) during the next 6 hours. Six months later, the same horses were again dosed with the same amount of morphine. Following collection of the 1-hour samples, 0.4 mg furosemide/kg body weight was injected. Blood samples were allowed to clot and serum was removed. Serum and urine samples were stored at -20°C until the analysis was performed.

The methodology for the determination of morphine levels in equine biological samples has been previously described.[2] Urine samples were split, 1 portion analyzed for free morphine, while the other portion was subjected to hydrolysis with β-glucuronidase to allow measurement of total morphine. Samples were cleaned by a combination of liquid-liquid extraction and column chromatography. A strong electron capturing compound was formed by the derivatization of the extracted morphine with pentafluoropropionic anhydride (PFPA). Separation was performed on a SP 2250-DB on 100/120 Supelcoport[c] column at 235° C in a Varian 3700 equipped with a ^{63}Ni electron capture detector.

RESULTS

Urinary levels of phenylbutazone were reduced by an average of 10-fold during peak diuresis.

Experiment 1 — Urinary concentrations of phenylbutazone quickly recovered, there being no significant difference (paired data t-test, $t = 2.370$, $t_{\alpha} = 0.05$) between the treated and untreated horses from 2 hours after the horses had been given the diuretic.

Experiment 2 — Horses were dosed with 0.001 mg/kg of fentanyl and this dose was followed 30 minutes later by either furosemide, 0.5 mg/kg, or normal saline. Urine samples were collected at the indicated periods and analyzed for fentanyl equivalents by RIA. Administration of furosemide caused urinary levels of "fentanyl" to fall about 18-fold within 30 minutes of dosing but urinary "fentanyl" levels had returned to control within about 2½ hours of dosing.

Experiment 3 — Morphine (0.1 mg/kg) was administered to 6 horses and followed 1 hour later with 0.4 mg furosemide/kg body weight. Free morphine plus β-glucuronidase releasable morphine (referred to here as total morphine) fell 13-fold within 15 minutes after the diuretic had been given (Figure 3). Total morphine found in the urine rose rapidly from this low point until the levels were not significantly different (paired data t-test, t

[a]Eli Lilly and Co., Indianapolis, Ind.
[c]Supelco, Inc., Bellefonte, PA.

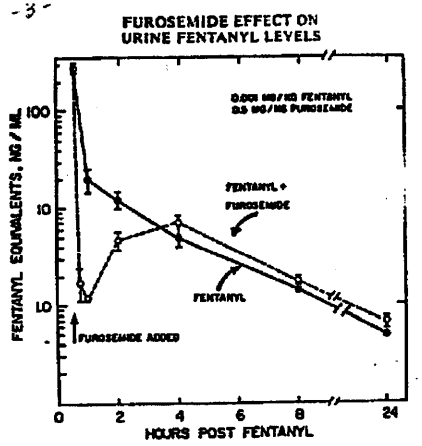

Figure 2. Furosemide effect on urine fentanyl levels. Urinary fentanyl levels were determined by RIA in horses given fentanyl alone (shown by the solid circles, o-o) and given fentanyl and furosemide (shown by the open circles, o-o). Vertical bars represent ± SEM. (Reproduced with permission from "Drugs and the Performance Horse," Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981).

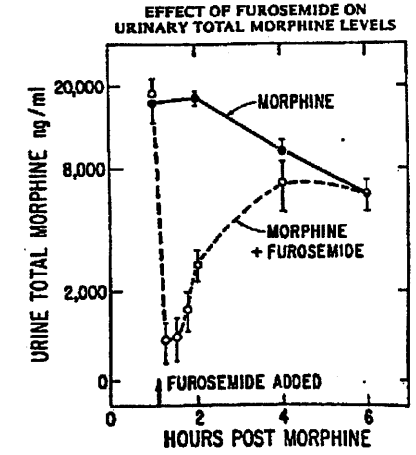

Figure 3. Effect of furosemide on urinary total morphine levels. Horses were dosed with 0.1 mg morphine per kg body weight and urine levels of morphine were analyzed following hydrolysis with β-glucuronidase (shown by the solid circles (o—o). Later, the experiment was repeated but 0.4 mg furosemide/kg body weight was injected 1 hour after the horses were given morphine (shown by the open circles, o-o). Vertical bars represent ± SEM.

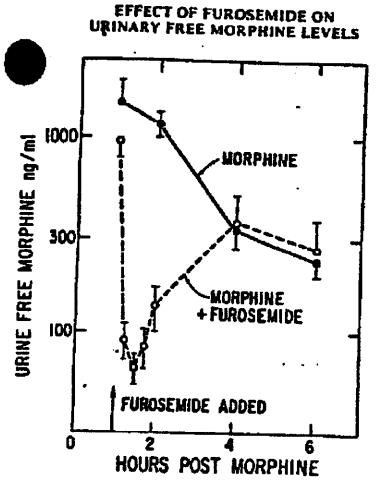

Figure 4. Effect of furosemide on urinary free morphine levels. Urine concentrations of free morphine in horses dosed with morphine alone are shown by the closed circles (o-o) and in the same horses later dosed with morphine and furosemide are shown by the open circles (o-o). Vertical bars indicate ± SEM.

= 1.026, t_{α} =0.05) from control values of furosemide by 3 hours after furosemide administration. Furosemide appeared to have broadly similar effects on total and free morphine levels as seen by comparing Figures 3 and 4.

Serum samples from these same horses were analyzed for morphine and the results are presented in Figure 5. Throughout the entire 5-hour period immediately following furosemide administration, there was no significant difference (2 sample t-test, $t = 1.354$, $t_{\alpha} = 0.05$) in serum levels of morphine whether or not the horses had received furosemide.

DISCUSSION

Epistaxis, or bleeding from the nose, following exercise has occurred since the earliest days of thoroughbred racing. Historically, the incidence of epistaxis has been low (less than 2%),[3] and a variety of treatments for this condition have been recommended. In the early 1970's, equine practitioners and horsemen began recommending furosemide for the treatment of epistaxis. Since then, it has become the treatment of choice for this condition.

In the late 1970's, work by Pascoe and his associates showed that the classical 2% incidence of bleeding from the nose was a manifestation of a much more extensive problem.[4] Studying horses post-race with a fiberoptic

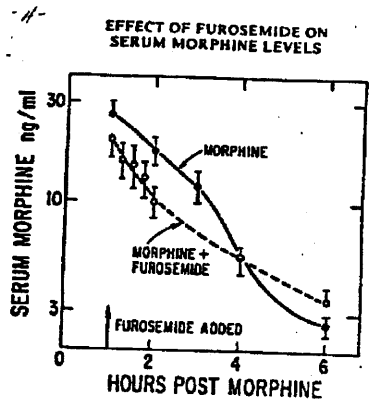

Figure 5. Effect of furosemide on serum morphine levels. Serum morphine levels of 6 horses given 0.1 mg morphine/kg body weight are shown by the closed circles (o-o). Later, the same animals were given the same amount of morphine but were also treated with furosemide (0.4 mg/kg). These serum levels are shown by the open circles (o-o). Vertical bars represent ± SEM.

endoscope, Pascoe showed that up to 40% of horses showed evidence of blood in the larynx or trachea post-race. Pascoe therefore renamed the syndrome exercise-induced pulmonary hemorrhage (EIPH)[5] and other work since then has supported Pascoe's observations and conclusions.[6]

The horse appears to be unusually prone to epistaxis, and this predisposition has been attributed to the architecture of the horse's lungs. As a species with poor collateral ventilation at the alveolar level, blockade of the direct entry of air to an alveolus has been theorized to cause blocked alveoli to rupture when the lung expands.[7] Furosemide may act to alleviate EIPH by improving the entry of air into a partially blocked alveolus, either by reducing pulmonary edema or by dilating bronchioles.[8] Alternatively, it may act to reduce epistaxis by reducing congestion.[9] While scientific studies on the efficacy of furosemide for EIPH are not available, there is a clear medical rationale and anecdotal clinical experience to justify its use in EIPH.

The only technical problem with the approval of furosemide for use in racing horses is its ability to dilute out certain drugs in equine urine.[10] The maximum duration of this dilution effect must, therefore, be determined to allow evaluation of its potential for use in racing. In this investigation the duration of the diluting effect after small doses of furosemide was determined, and the amount of apparent dilution of post-race urine samples associated with pre-treatment with furosemide was determined.

These experiments were based on the hypothesis that

both the duration and extent of the diluting effects of furosemide would be reduced if the dose of furosemide was reduced. Thus, one might expect that the duration of the dilution effect would be considerably less after a dose of furosemide in the order of 0.4 mg/kg, i.e. the dose commonly used in the treatment of epistaxis, than after 1 mg/kg, the manufacturer's recommended dose for pulmonary edema as was reported earlier.[7,8] In general, this expectation has been borne out by the results obtained.

Both the duration and extent of the dilution after the lower doses of furosemide (less than 0.5 mg/kg) were less than those reported in earlier experiments. Thus, 1 mg/kg of furosemide IV produced about a 40 to 50-fold dilution of the urinary concentrations of both phenylbutazone and the major glucuronide metabolite of pentazocine in a previously reported work.[8] Reducing the dose, however, produced a correspondingly smaller maximal dilution effect, about 10-fold for phenylbutazone, about 18-fold for fentanyl, and about 13-fold for morphine.

Similarly, the duration of the dilution effect was markedly reduced. While the effect lasted for longer than 4 hours when the dose of furosemide was 1.0 mg/kg,[7,8] reducing the dose of furosemide reduced the duration of the effect to about 3 hours or less. For example, the dilution effect when phenylbutazone was used about two hours, and three hours or less when morphine or fentanyl were the test drugs. Four hours is therefore a conservative time pre-race to administer a prophylactic dose of furosemide. In fact, these data suggest that the drug could be administered in less than 0.5 mg doses as close as three hours prior to sampling with little effect on drug detection in post-race urine samples.

These data further suggest that the diluting effect of furosemide on urinary drug concentrations in actual practice may be relatively small. In other experiments, we measured and compared the concentration of phenylbutazone and its metabolites in the post-race urines of horses with and without furosemide pre-treatment.[9] These data show that the apparent dilution of phenylbutazone and its metabolites in equine urine amounts to less than a 50% reduction in drug concentration in equine urine. Since all practical analytical methods have a margin of safety of much greater than 50%, this reduction in concentration is unlikely to be forensically significant.[9]

It is highly unlikely that less than 0.5 mg furosemide per kg body weight will significantly affect the detection of drugs in blood. This is because small doses of furosemide lead to the elimination of only about 4 liters of fluid from the horse, which is between 1 and 1.5% of the body water of a horse. In general, therefore, furosemide at less than 0.5 mg/kg is unlikely to lead to the elimination of more than 1.5% of the drug in a horse, which is unlikely to be a forensically significant amount.

Further, the fraction of the dose of drug eliminated in the urine in response to furosemide will generally be a lot less than 1.5%. This is because most drugs, either acidic or basic, have an apparent volume of distribution in the horse of much greater than total body water. For example, the tranquilizer acepromazine distributes in the horse in a manner equivalent to its distribution in a volume of over 800,000 liters. The proportion of the total amount of drug eliminated after a dose of furosemide will, therefore, in many cases be only a fraction of 1% of the dose present in the horse at the time of treatment with furosemide.

As a practical matter, therefore, the results reported here show that the diluting effects of doses of furosemide less than 0.5% mg/kg administered IV are essentially over within about three hours of dosing. These data, therefore, support the original conclusions of Gabel, Tobin, Ray and Maylin that dilution is not likely to be significant if the drug is administered four hours before post-time.[4] These results further suggest that four hours prior to post-time may be a relatively conservative time restraint to put on administration of this drug if the dose administered is equal to or less than 0.5 mg/kg.

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AnaCor Laboratories
7300 Jefferson Street, N.E.
Albuquerque, NM 87109
(505) 345-8964

Joint Venture of AnaChem, Inc. and Assaigai Analytical Laboratory

May 12, 1983

David Vienna and Associates
510 C Street, N.E., Suite 100
Washington, D.C. 20002

Dear Mr. Vienna:

Thank you for the opportunity to add some perspective to the testimony by Ted Cochran. As requested by Mr. Vienna, I will address only the comments by Mr. Cochran that Mr. Vienna has underlined and I will discuss them sequentially.

At the present time there is a radioimmunoassay kit available to the racing chemist for detecting fentanyl. This has enabled the analytical racing chemist to easily screen for fentanyl. To go back to Mr. Cochran's analogy, if one is looking for a needle in almost 100 tons of hay, this task is easy when you use a strong magnet. i.e., the fentanyl radioimmunoassay kit.

How does Mr. Cochran know how easy it is for a horseman to get M-99, "elephant juice"? Is Mr. Cochran a horseman? According to one veterinarian, the current black market price for "elephant juice" is between $8,000 - $16,000 for a vial or approximately $200/dose. In addition, my connections have indicated that most horsemen and veterinarians have not found "elephant juice" to be as effective a stimulant as was once hoped. Opiate derived narcotics appear to be more effective and much less dangerous.

Progress in racing chemistry as in other scientific fields has been largely made by individuals associated with university or government facilities supported by grants. These researchers do not necessarily screen urine for drugs as the official racing chemist for a particular racing jurisdiction. In order to become an associate member of the A.O.R.C. one needs to be the designated official racing chemist of a racing jurisdiction and to have passed a test in which you have correctly identified the ten drugs in ten urine samples. In the past, many chemists would not qualify nor have a need to be members of the A.O.R.C. At the last N.A.S.R.C.- A.O.R.C. meeting in Portland, Oregon, Dr. Richard Sams and Dr. George Mylin joined the A.O.R.C.

I am a relatively recent associate member of the A.O.R.C., however, my experience in communication with other racing chemists has been very positive. I can call any racing chemist and have called them to discuss problems and questions. In fact, I have called Dr. Mylin concerning a

REFERENCES

1. See attached paper, "Determination of Trace Quantities of Dimethylsulfoxide in Aqueous Solutions."
2. See attached paper, "Natural Occurring Levels of Dimethyl Sulfoxide in Selected Fruits, Vegetables, Grains, and Beverages."
3. See attached paper, "Dimethyl/Sulfone: Isolation from Human Urine."
4. Unpublished data, John McDonald, Illinois Racing Board Laboratory
5. See attached paper, "The Metabolism of Dimethyl Sulfoxide and its Metabolic Effects in Man and Animals."
6. See attached paper, Excerpt from book "Dimethyl Sulphoxide"
7. See attached paper, "Oxidation of Dimethyl Sulfoxide to Dimethyl Sulphoxide to Dimethyl Sulphone in the Rat and Man."

An Evaluation of Pre- and Postrace Testing and Blood and Urine Drug Testing in Horses

T. Tobin, D.V.M., Ph.D.
G.A. Maylin, D.V.M., Ph.D.
J. Henion, Ph.D.
C. Woodward, Ph.D.
R. Ray, D.V.M., Ph.D.
J. Johnston, B.S.
J.W. Blake, Ph.D.

From the Department of Veterinary Science, University of Kentucky (Tobin and Blake); the New York State Racing and Wagering Board, Drug Testing and Research Program, Cornell University, Ithaca, NY (Maylin and Henion); the Pennsylvania State Harness Racing Commission, Drug Testing Program (Woodward); the Department of Veterinary Clinical Sciences, Ohio State University (Ray); and the Association of Official Racing Chemists (Johnston); with additional input by other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee, Tom Finley, B.S.; A.V. Villatico, B.S.; Robert E. Vessiney, B.S.; Francis Ozog, Ph.D.; Robert Schoenfeld, Ph.D.; Albert Gabel, D.V.M., M.Sc.; Joe Solomon, D.V.M.; Marvin Beeman, D.V.M.; Joe O'Dea, D.V.M.; Gene Bishaus, D.V.M.; General Wayne Kester, D.V.M., and W.D. Roberts, D.V.M.

1. Blood and urine are the major biological fluids used in drug testing in North America. Blood is used in both prerace and postrace testing, urine in postrace testing only.
2. Most drugs and drug metabolites are detectable in blood and urine, although certain drugs may be more readily detected in one than the other.
3. Although no testing program can analyze for all possible drugs, best coverage is obtained by testing both blood and urine. The addition of blood to all testing programs is recommended.
4. In prerace testing, blood samples are taken from all horses in every race within two hours of racing. The samples are analyzed at trackside laboratories. Horses with illegal medication are scratched.
5. In postrace testing, urine and/or blood are taken from selected horses after the race.
6. The combination of prerace and postrace testing of blood and urine offers the best drug coverage and prevents the racing of many illegally medicated horses. An affordable alternative is postrace blood and urine testing.
7. In the last analysis, each racing jurisdiction must evaluate the operational and scientific merits of prerace and postrace testing and blood and urine testing. The procedures which best support their rules of racing and are operable within their financial structure should be selected.

Definitions

Positive Test

For the purposes of this report, a positive is a drug finding which violates a medication rule. An analyst reports a positive when there is sufficient data to substantiate the presence of a specific drug or foreign chemical substance. Partial data, which suggests, but does not satisfy the analyst that a specific drug is present constitutes a "suspicious." A "suspicious" may be upgraded to a positive by the acquisition of more data, depending on the medication rules of the particular jurisdiction.

Prerace Testing

Prerace testing involves the testing of all horses in every race for illegal[a] drugs before racing. In the United States, horses are detained in a secure paddock, and an initial 20-ml blood sample is drawn within two hours of post time.[16,27,32,40,41] These samples are analyzed[b] immediately for the presence of drugs at a trackside laboratory. All drug findings are confirmed by resampling the horse(s) involved, and results are reported before opening of the pari-mutuel windows. The judges or stewards may withdraw any horse(s) from the race.[c] A subsequent hearing may be held to investigate a drug positive in accordance with the rules of racing. Prerace testing is currently in operation at harness tracks in New York, New Jersey, Pennsylvania, and Ohio, and is always performed in conjunction with postrace blood and urine testing at trackside laboratories. In New Jersey, New York, and Ohio, all drug findings are reconfirmed at a central research facility.

Postrace Testing

Postrace testing involves the testing of selected horses for the presence of illegal drugs after racing.[23] Urine and/or blood from these horses are analyzed for drugs in a central laboratory, usually remote from the track. Drug positives are reported to the designated official who may take disciplinary action.

Selection of Biological Fluids

The biological fluids available for drug testing are sweat, saliva, blood, and urine. The selection of a fluid depends on the disposition of drugs, the analytical techniques available, the medication rules involved, and the degree of regulation required by the racing jurisdiction. In general, drugs are distributed to body tissues by the blood and are then eliminated or excreted from the body in urine, feces, and sweat. In certain instances drugs are detectable in saliva. Drugs and, in particular, drug metabolites, can be present in higher concentrations in urine which is essentially an ultra-filtrate of blood. If biological fluids which have lower concentrations of drugs are selected, more expensive and sensitive analytical techniques are required. Depending on the available resources, the rules of racing, and the amount of regulation required, a single fluid or combination of fluids may be analyzed.

[a] For the purposes of this report, an illegal medication is a medication whose use is prohibited in the jurisdiction in question.

[b] Throughout this report analytical work is reported as the state-of-the-art in March, 1978.

[c] Withdrawing a horse from a race is commonly referred to in racing circles as "scratching."

Sweat

Limited data are available concerning the detection and excretion of drugs in sweat.[13,30] Another objection to the use of sweat is that it is difficult to refute a defense that drugs reported in sweat were surface contaminants and were never actually present in the horse. Sweat is usually not available prerace, which precludes its use in prerace testing.

Saliva

Collection of the sample. Saliva is collected by swabbing the horse's mouth with a gauze pad held in a forceps. The pad may have been moistened with a dilute solution of acetic acid. This procedure yields between 3 and 15 ml of undiluted saliva. Saliva is rapidly and easily obtained in this way, and collection of a single sample takes about four minutes.[14] Unfortunately, however, the concentrations of many drugs in saliva are low.

Properties of the sample. Acidic, highly protein-bound drugs do not appear to enter saliva in sufficient concentrations to be detectable.[8,10,14]

Because saliva is alkaline (basic) relative to plasma, the entry of basic (alkaline) drugs into saliva is restricted.[1,15,42] Thus, concentrations of certain drugs in equine saliva are only about 25% of the concentrations found in plasma.[11] Many basic drugs are pharmacologically active at very low plasma concentrations, so this reduction in concentration in saliva is important. Neutral drugs are found in about the same concentrations in plasma and saliva.

The volume of saliva which can be obtained is small, being not more than 20 ml and often as low as 2 to 3 ml.[14] Because of the reduced concentration of many drugs in equine saliva, this small volume can be a serious problem. Saliva is not, to our knowledge, routinely used as a biological fluid in most drug testing programs in the United States.

Blood

Collection of the sample. Blood sampling using prepackaged, sterile vials and needles is rapid and inexpensive. For sampling alone, minimal facilities are required. Since a professional veterinarian does the blood sampling, ancillary information about the clinical condition of the horse is obtained. Blood is, *per se*, the simplest, most economical, and rapidly obtainable biological sample to take from the horse. It is the only sample suitable for prerace testing under North American racing conditions. There is no evidence that venipuncture as utilized in pre- and postrace testing harms horses.

Advantages of blood testing. The prinicpal advantage of blood testing is that almost all drugs detectable in blood are present in an unchanged form. Unlike urine, in general, only very small concentrations of metabolites are present, provided large doses of drugs are not administered.[31] For this reason, drugs which are excreted totally in urine as unidentified metabolites are detectable only in blood.[2,10,19,22,23,27,40] Blood samples are readily and easily obtained almost without exception. Normal endogenous biochemical material or "background" is significantly lower in blood than urine, so that very low concentrations of drugs can be detected without extensive sample cleanup and preparation. Because drugs are actually found in blood at or about the time of racing, the significance of the finding is usually relatively clear-cut.[28] Blood levels of drugs are essentially not affected by other drugs such as diuretics,[28] in contrast with the marked dilution effects observed on some urinary drug concentrations.[22] Because the disappearance of most drugs from the blood occurs relatively rapidly in the horse, the possibility of an analyst finding traces of a drug in blood days after administration is in most cases remote.[1]

Disadvantages of blood testing. An apparent problem with blood testing is the small size of the sample, which is usually not greater than 20 ml. Compounding this problem is the fact that some drug or drug metabolite concentrations in blood are a good deal lower than in urine.[4,6,28,29] The upshot of this is that the analytical techniques required in blood testing are somewhat more exacting than those required for urine.[2,4-6,12,18,22,40] Some classical analytical techniques used in urine testing are not readily applicable to blood testing.[4,18] Given sensitive analytical techniques, the small size of blood samples is not a problem. Another disadvantage is that some drugs and drug metabolites detected in urine may not be detected in blood.[2,3,17,21,28,40] Use of a blood sample also limits the time during which drug administration can be detected.

Relationship Between Blood and Urinary Concentrations of Drugs

The principal difference between blood and urinary levels of drugs is that urinary concentrations of drugs or their metabolites tend to be higher than plasma concentrations of drugs. In the kidney, all drugs and drug metabolites pass freely into a freshly formed plasma filtrate which is the first step in urine formation.[39] During urine formation any drug which is predominantly water soluble, and especially drug metabolites, can be concentrated several-fold.[39] For this reason, some drugs which are marginally detectable in plasma, are readily detectable in urine. On the other hand, drugs which are excreted as metabolites in urine may be detectable as the parent drug in plasma.

Urine

Collection of the sample. Urine collection is slow, difficult, and expensive compared to blood and saliva. While up to 90% of horses will urinate within one hour, some horses must be held for up to three hours to produce a urine sample, and occasionally no sample is obtained for testing. Routine use of diuretics to facilitate urine collection is not recommended, as these drugs interfere with urinary concentrating mechanisms and result in reduced urinary concentrations of many drugs.[21,22] If a large number of horses is being tested, this means that a considerable holding facility must be available. Further, urine collection is apparently an acquired skill, so a staff of trustworthy and skilled urine collectors must be maintained. Due to these limitations, usually only several horses from a given race are sampled. The time constraint virtually eliminates the use of urine as a prerace testing medium in North American racing, although urine is used in Europe and Asia.[7,31,41]

Advantages of urine testing. The major advantage of urine testing is that relatively large quantities (200 to 500 ml) are usually available compared to blood or saliva. Many drugs and drug metabolites are found in urine in concentrations greater than those found in plasma.[4,23,35-37] These concentrations render the analyst's job with urine easier if classical analytical techniques are used. A sufficient sample is also available for a number of confirmatory tests.

Disadvantages of urine testing. The principal disadvantage of urine testing is that many drugs are excreted in urine as water-soluble metabolites which are difficult to extract and in some cases exist as unidentified compounds.[2,9,11,17,20,25,26,29,34] Drug metabolites of adequate quality for analytical standards are very difficult to obtain commercially. Another problem with urine testing is that the volume and pH of urine can vary considerably in response to changes in the animal's environment and to drugs which may be administered. Thus, diuretics can greatly increase the volume of urine and dilute much of the renal concentrating effect.[38]

Prerace Testing

Sampling Procedures

The only biological sample used in prerace testing in the United States is blood. An initial blood sample is drawn from all horses within two hours of racing and analyzed at the adjacent laboratory. Once sampled, the animals must be held under close supervision until race time to insure that no drugs are administered between sampling and racing. All drug findings are confirmed by resampling the horse(s) involved. In the event the horse is

scratched, urine samples are also collected for additional confirmatory analysis.

Analytical Procedures

The general analytical approach used in prerace blood testing involves a preliminary screening procedure to test for a wide variety of drugs and use of confirmatory techniques to indentify positive samples. Solvent extraction is used to isolate drugs from blood according to functional groups present. The extracts are analyzed using a combination of ultraviolet spectroscopy, thin layer chromatography, gas chromatography and, in Pennsylvania, high pressure liquid chromatography.[46] Based on these analytical techniques, a drug is identified to the satisfaction of the analyst and a report is made to the designated official prior to post time.

Benefits of Prerace Testing

Prerace testing is the best mechanism by which the actual running of illegally medicated horses can be prevented. Its presence on a track acts as a highly visible and immediate deterrent to illegal medication, and as such, creates an atmosphere of confidence in racing. The integrity of the testing system is increased because the chain of evidence is shortened. Because the results of the test are available while the animal is still in the secure area, the animal may be isolated and resampled for confirmatory or independent testing if necessary. Since all horses entered are tested prerace, testing coverage is more equitable than postrace testing of selected horses. Because prerace testing is completed before the race, it simplifies regulatory problems for both horsemen and track management. Because the horse is disqualified before racing rather than after winning a purse, both the incentive to and incidence of legal challenges are reportedly reduced.

Blood is the only biological fluid presently used in prerace testing in North America. The benefits of blood from a technical point of view are discussed above under advantages of blood testing.

Limitations of Prerace Testing

The major limitations associated with prerace testing are the technical disadvantages associated with the use of blood as a biological fluid. These are discussed above under disadvantages of blood testing. In addition, the time constraints imposed on prerace testing are more demanding than in postrace analysis. It is imperative that strict security be maintained between sampling time and post time.

Costs of Prerace Testing

Prerace testing and postrace urine testing are used in combination in New Jersey, New York, Ohio, and Pennsylvania. Each operating racetrack requires a separate laboratory located at trackside. In some instances, two tracks may share the same facility if racing dates are compatible.

Each laboratory requires approximately 600 to 700 sq ft of fully equipped space. Total cost is $80,000—approximately—$30,000 for the facility and $50,000 for analytical instrumentation. Instrumentation costs are usually amortized over a five-year period. Salaries, benefits and supplies for operation of each laboratory are approximately $350/day, thus, the average cost of testing 80 bloods prerace and 20 urines postrace for a 200-day racing season is $70,000.

Postrace Testing

Sampling Procedures

Postrace testing is performed on either urine and/or blood. Usually a few selected horses such as winners, beaten favorites, or others in accordance with the rules of racing are tested in each race. After the race, the horse is taken to a detention barn and cooled-out. At this point a blood sample is taken, if required, and a urine collector left in the stall to collect the urine sample. Once collected, the samples are cooled, frozen, or otherwise preserved and prepared for shipment to the testing laboratory.

Analytical Procedures

The general analytical approach used in postrace blood and/or urine testing involves a preliminary screening procedure to detect a wide variety of drugs and/or metabolites followed by confirmatory techniques to identify the exact chemical involved. A combination of solvent extractions and resin extractions are used to separate the drugs from endogenous biochemical material. Most analytical protocols involve the use of ultraviolet spectroscopy, thin layer chromatography and gas chromatography. An increasing number of laboratories are now using high-pressure liquid chromatography, infrared spectroscopy and mass spectrometry in addition to the above techniques.

Benefits of Postrace Testing

Postrace testing has been and remains the backbone of drug testing systems in most jurisdictions. The major benefit of postrace testing is the technical advantage of testing urine as a biological fluid. These are outlined above under advantages of urine testing. In addition, the time constraints imposed on prerace testing are more demanding than postrace analysis. Thus, a greater number of tests and analytical techniques could be used for screening and identification purposes.

Oral dosing with slowly absorbed drugs or "time release" capsules immediately before prerace sampling may not give rise to detectable blood levels of drugs. However, if dosing in this way gives rise to blood levels of drugs effective at the time of racing, detectable drugs will show up in postrace blood and/or urine testing.

Limitations of Postrace Testing

For reasons previously indicated, postrace testing strongly deters, but does not totally prevent, the running of illegally medicated horses. It has no effect on betting payoff. Because the principal disciplinary action is confiscation of purses, fines and suspensions, there can be substantial financial inducement for legal challenge of the postrace testing process.

Postrace testing, as presently employed, samples 25% or less of the horses in a race on a selective basis. In the event a sample is accidentally lost in shipment or in the laboratory, no replacement can be taken.

Costs of Postrace Testing

The cost of contract postrace testing is variable, ranging from $10 to $20/urine or combined blood and urine analysis.

Relationships of Blood and Urine and Prerace and Postrace Testing

Combined prerace and postrace testing offers the best and most comprehensive medication control system available today. In the absence of prerace testing, selective postrace testing of blood and urine offers an affordable alternative. What is lost, of course, is the ability to scratch any of the postitive horses before they race.

Postrace urine testing offers good drug coverage. The inclusion of a blood sample helps under some circumstances and must be considered well worth the extra cost. The advantages of postrace blood samples include the ability to detect many drugs in blood which may be diluted in urine by diuretics, whose use is permitted in some jurisdictions. It also helps to detect a parent drug which may be found in urine only as metabolites. Finally, under some circumstances it may not be possible to obtain a urine sample, in which case the postrace blood sample may be the only sample available.

An ideal testing situation would be one where blood or urine levels of drugs would be detected in all horses before post time, with any offender of the rules of racing scratched. Prerace blood testing approaches this ideal and should be encouraged and regarded as the method which could ultimately be the best approach to racing's medication control problems.

Future Developments

Applied research in analytical chemistry and equine pharmacology is needed in order to improve the state of the art of drug testing. The chemist must be capable of detecting a wide variety of potent new drugs and the veterinarian must have knowledge of drug action in the horse, so that the use of illegal medication can be controlled. This is no small task.

New analytical methods are constantly being developed for use in drug analysis. These must be evaluated for use in drug testing and applied whenever possible.

There is a dearth of reliable information concerning the effects that drugs have on racing performance or speed of a horse.[18] Drugs must be administered to horses in doses comparable to racing situations under controlled, experimental conditions. Studies in drug metabolism, pharmacokinetics (clearance times) and exercise physiology must be expanded if we are to improve our knowledge of equine pharmacology.[18] A number of research programs are now conducting these studies using swimming pools, treadmills and simulated race track conditions, but much work remains to be done.

In the last analysis, each racing jurisdiction must evaluate the operational and scientific merits of prerace and postrace testing, and blood and urine testing. The procedures which best support their rules of racing and are operable within their financial structure should be selected.

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(continued from p. 83)

May 11 and 12, *Mastitis in Depth II*. Sponsored by the New York State College of Veterinary Medicine, Cornell University. Further details are available from the Office of Continuing Education, N.Y.S. College of Veterinary Medicine, Ithaca, NY 14853, (607)256-7700.

May 14–19, *Veterinary Mini-Course Series*. Sponsored by the Tuskegee Institute, School of Veterinary Medicine. *Orthopedic Surgery*. Instructor: Dr. C. D. Newton. *Clinical Dermatology*. Instructor: Dr. A. A. Stannard. For further information contact: Mrs. C. T. Lyman, School of Veterinary Medicine, Office of Associate Dean, Tuskegee Institute, AL 36088, (205)727-8176.

May 17, *Mid-Atlantic States Veterinary Clinic*. Sponsored by the Maryland and Pennsylvania Veterinary Medical Associations. For more information contact: Mr. Ray Thompson, 330 N. Charles St., Baltimore, MD 21201, (301)539-1700.

May 17–19, *Sixth Equine Nutrition and Physiology Symposium*. Sponsored by the Equine Nutrition and Physiology Society. Various papers will be given. For further information contact: Gary D. Potter, Secretary-Treasurer, Animal Science Department, Texas A&M University, College Station, TX 77843.

May 19–20, *Diagnostic Dermatology (A Team Concept)*. Sponsored by the Auburn University of Continuing Education School of Veterinary Medicine. A Team presentation. For further information contact: Mrs. Marion Moore, School of Veterinary Medicine, Auburn University, Auburn AL 36803, (205)826-4546.

May 28–31, *Veterinary Acupuncture Workshop*. Sponsored by the Tuskegee Institute, School of Veterinary Medicine. For further information contact: Dr. E. W. Adams, School of Veterinary Medicine, Tuskegee Institute, AL 36088.

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25301

ALFRED K. HAYS
EXECUTIVE SECRETARY

May 10, 1983

Mr. James Wise
c/o David Vienna & Associates
510 C Street, N.E., Suite #100
Washington, D.C. 20002

Dear Mr. Wise:

Appertaining your request of Chairman Roger Ramey to provide a letter refuting a claim voiced by a representative of the American Horse Council before Congressman Conyers sub-committee hearing in Washington, a statement of clarification is made.

"The West Virginia Racing Commission does not have a policy allowing any owner, trainer, jockey, etc. to serve suspended racing days for medication or any other violation of W.Va. Rules when the subject race track is closed. Also, by reciprocal agreement, a suspended permit holder is prohibited from racing at any other track under the jurisdiction of the National Association of State Racing Commissioners."

You are hereby authorized to use this letter for verifying the denial, and we will direct a letter directly to Congressman Conyers, if you so desire.

Respectfully,

Alfred K. Hays

Alfred K. Hays
Executive Secretary

cc: Commission Members
H.B.P.A. - Charles Town
H.B.P.A. - Waterford Park

PHARMACOLOGY, PHARMACOKINETICS, AND BEHAVIORAL EFFECTS OF ACEPROMAZINE IN THE HORSE

S. BALLARD AND T. TOBIN

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PHARMACOLOGY, PHARMACOKINETICS, AND BEHAVIORAL EFFECTS OF ACEPROMAZINE IN THE HORSE
S. BALLARD AND T. TOBIN

ABSTRACT

Acepromazine is a phenothiazine tranquilizer that finds frequent use in equine medicine. When administered intravenously to mature thoroughbred and standardbred horses, acepromazine distributed according to the "two-compartment open model" with an A phase half-life of 4.17 minutes, a B phase half-life of 184.4 minutes, and a resulting volume of distribution of 6.6505 l/Kg. The percent of acepromazine bound to plasma proteins was determined as being greater than 99.0%. The drug distributed almost evenly between the plasma and erythrocyte phases in blood. Acepromazine exerted a profound effect upon hematocrit levels in the horse and was found to significantly depress packed cell volumes at doses as low as 0.002 mg/kg IV. Penile prolapse occurred at doses of 0.01 mg/kg of acepromazine. Higher dosages increased the duration and magnitude of the depression of respiratory rate. Therapeutic doses of acepromazine were shown to reduce the horse's capacity to respond in operant conditioning trials by as much as 50%. The fentanyl-induced locomotor response of the horse was also reduced. Hematocrit effects were by far the most sensitive response of the horse to acepromazine administration. Penile protrusion, operant behavior, and respiratory effects were approximately similar in sensitivity but required doses greater than 0.004 mg/kg.

Acepromazine, a phenothiazine derivative (Fig. 1), is a widely used tranquilizer in equine medicine. Like most phenothiazines, it blocks a range of central effects including locomotor activity, respiratory response, and control of body temperature[7]. Since horses retain much of their co-ordination and alertness while becoming easier to handle with acepromazine, the drug is frequently used in the transport of valuable or unruly animals. It may be used in competition in small doses to calm an otherwise excitable animal and allow it to perform in a more relaxed manner[4]. It is also used in veterinary medicine as a pre-anaesthetic during surgical procedures, sometimes in conjunction with other agents such as potent analgesics. Because acepromazine is widely used and is prohibited in most racing situations, sensitive and specific methods of detection are needed to test for its presence in performance horses[2]. Further, acepromazine metabolites may persist in urine for long periods[3]. We therefore elected to characterize the pharmacological effects of this drug in horses with particular reference to their duration of action.

In our initial recovery experiments, we were surprised to find that the recovery of acepromazine from equine plasma at pH 9.4 was relatively poor. When we repeated the experiments with human and ovine plasma much the same results were observed (Fig. 2). After a number of possible variables were investigated we were surprised to find that the recovery of acepromazine from plasma was atypical[1]. While recovery from buffer was essentially complete at basic pH, recovery from plasma was optimal at a pH of about 6.0. (Fig 3). This is despite the fact that acepromazine is a basic drug and might be expected to be readily available from plasma at basic pH values.

The availability of ^{3}H-chlorpromazine enabled us to determine directly the rate of movement of chlorpromazine from plasma to an apolar environment at different pH values. As shown in Fig. 4, the rate at which ^{3}H-chlorpromazine moved into DCM was most rapid at pH 6.0, and considerably slower at pH 9.2 and pH 11.0. The data show, however, that if the extraction period is prolonged that essentially complete extraction occurs at pH values of 9.2. Because of the technical convenience of buffering plasma samples to this value, all phenothiazine extractions from plasma in our laboratory are routinely performed at this pH, but with the extraction period prolonged to 1 hour.

Using this extraction method and the GC technique described by Ballard and Tobin (1981), we were able to follow the plasma levels of acepromazine for 8 hours after intravenous administration of 0.3 mg/kg of acepromomazine maleate. As shown in Fig. 5, acepromazine levels at first dropped rapidly after intravenous injection, with an apparent A phase T½ of about 4.2 minutes. Thereafter, the plasma half-life of this drug fell more slowly, with an apparent B phase T½ of about 185 minutes. Acepromazine was not detectable in equine plasma for more than 8 hours after dosing.

The most sensitive pharmacological response to acepromazine that we discovered was the effect on acepromazine on hematocrit in horses. As shown in Fig. 6, doses of acepromazine of as little as 0.002 mg/kg produced a significant decrease in the hematocrit of horses, which effect lasted for several hours. These are remarkably small doses of acepromazine to produce pharmacological effects in a horse.

The next most sensitive pharmacological response to acepromazine was penile protrusion[3]. While doses of 0.004 mg/kg had no effect on penile protrusion, this response appeared at doses of about 0.01 mg/kg and was apparently maximal at doses of 0.4 mg/kg. Even at these large doses, however, this fairly sensitive pharmacological response did not last longer than about 10 hours. (Fig. 7).

The respiratory rate of horses is well known to be sensitive to acepromazine and Fig. 8 shows the response observed to increasing doeses of acepromazine. As with the penile response, about 0.04 mg/kg was required for a good effect, and 0.4 mg/kg produced the maximal response observed*.

The availability of a variable interval responding apparatus in our laboratory enabled us to test the effects of acepromazine on CNS function in our horses. In those trials, acepromazine was adminstered to these horses intravenously 10 minutes prior to each trial. As shown in Fig. 9, horses were relatively resistant to the central effects of acepromazine, about 0.4 mg/kg or the maximal dose tested being required for a significant inhibition in the responding rate of these animals.

Figure 10 shows a family of dose response curves for these responses. Half-maximal inhibition of hematocrit occurred at doses of about 1 mg/horse, while 5 mg/horse was required for penile protrusion and between 5 and 50 mg/horse for effects on variable interval responding and respiration.

In summary, these experiments have shown that acepromazine extracts very slowly from equine plasma, and that under the usual extraction conditions for basic drugs up to 1 hour extraction period is required. After its intravenous administration to horses, it has a plasma half-life of about 3 hours and was no longer detectable by our methodology at 8 hours post-dosing. Among pharmacological responses to acepromazine its depressant effect on the hematocrit was the most sensitive, requiring only about 1mg/horse for 50% inhibition. The next most sensitive response was penile protrusion, requiring about 5mg/horse, follwed by respiratory depression and inhibition of variable interval responding, both of which required about 5 to 50 mg/horse for 50% inhibition.

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FOOTNOTE

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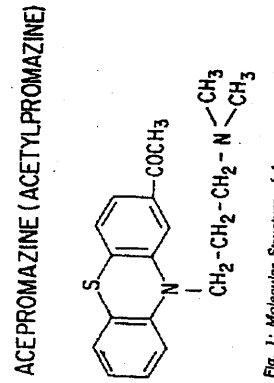

Fig. 1: Molecular Structure of Acepromazine (mol. wt. 326.47).

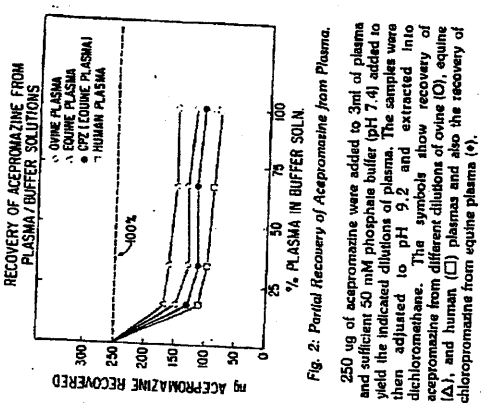

Fig. 2: Partial Recovery of Acepromazine from Plasma.

250 ug of acepromazine were added to 3ml of plasma and sufficient 50 mM phosphate buffer (pH 7.4) added to yield the indicated dilutions of plasma. The samples were then adjusted to pH 9.2 and extracted into dichloromethane. The symbols show recovery of acepromazine from different dilutions of ovine (O), equine (Δ), and human (□) plasmas and also the recovery of chloropromazine from equine plasma (•).

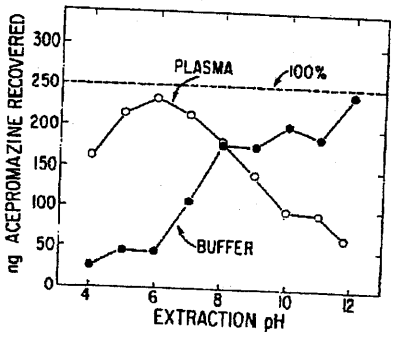

Fig. 3: Recovery of Acepromazine from Spiked Plasma and Buffer Samples at Different pH Values.

250 ug of acepromazine were spiked into 3 ml of equine plasma or water, buffered to the indicated pH, and then extracted into dichloromethane. The open circles (0) show recovery of acepromazine from plasma, while the solid circles (•) show recovery from buffer.

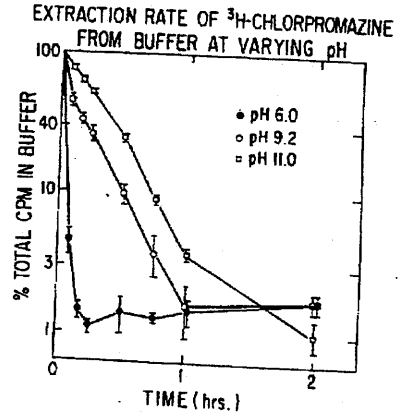

Fig. 4: Extraction Rate of Chlorpromazine from Buffer at Varying pH.

50 ug of ^{3}H-chlorpromazine were added to 5 ml of phosphate buffer at the indicated pH values. The symbols show the time course of disappearance of ^{3}H-chlorpromazine from the buffer solutions at pH 6.0 (solid circles), 9.2 (open circles), and pH 11.0 (open squares).

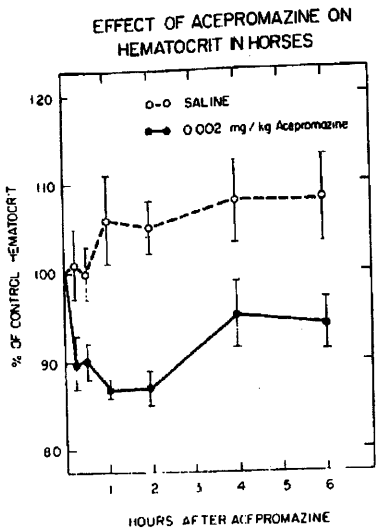

Fig. 6: Effect of Acepromazine on Hematocrit in Horses.

Four horses were dosed intravenously with 0.002 mg/kg of acepromazine in one trial and an equal volume of saline in another trial. The open circles (O) show the hematocrits of saline-treated animals, while the solid circle (•) show the effects of acepromazine. Hematocrits are expressed as a percent of the control (initial) hematocrit values. Zero time hematocrits in these horses averaged about 32.5%. All values are means — the standard errors of the means.

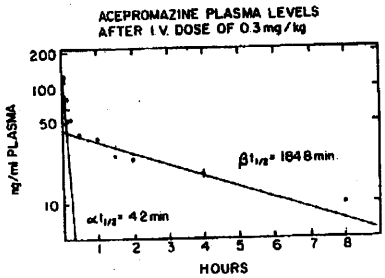

Fig. 5: Plasma Levels After Rapid Intravenous Injection of 0.3 mg/kg Acepromazine Maleate.

The data points (•) show plasma concentrations of acepromazine after rapid I.V. administration of 0.3 mg/kg. The B phase half-life was determined by computer analysis and found to be 184.8 min. The A phase half-life was also determined and found to be 4.17 min. All data points are means — standard errors of the means of determinations on 5 horses.

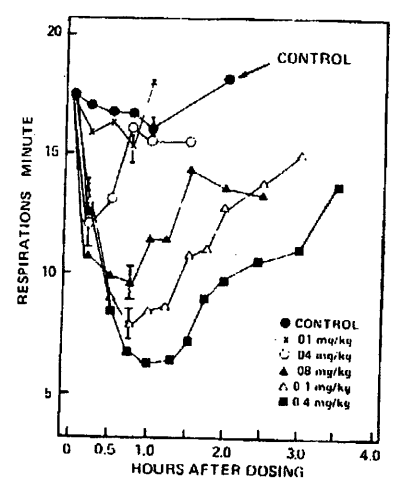

Fig. 8: Effects of Acepromazine I.V. on Respitory Rate in the Horse.

The closed circles (•) show the respiratory rate in control horses, while other symbols show the respiratory rates observed in these horses after the indicated doses of acepromazine I.V. All data points are the means of determinations on 4 horses and the vertical bars represent standard errors of the means.

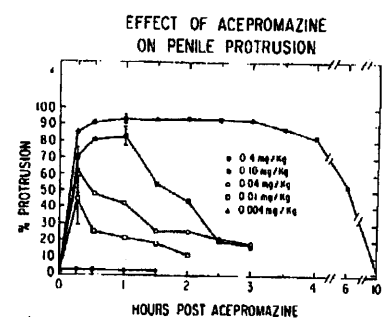

Fig. 7: Effect of Acepromazine on Penile Protrusion in Geldings.

Acepromazine at 0.4 mg/kg was administered intravenously to four geldings and the maximal length of penile protrusion measured. The symbols show the penile protrusion measured after each subsequent dosage of acepromazine and expressed as a percentage of the maximal protrusion seen in each horse.

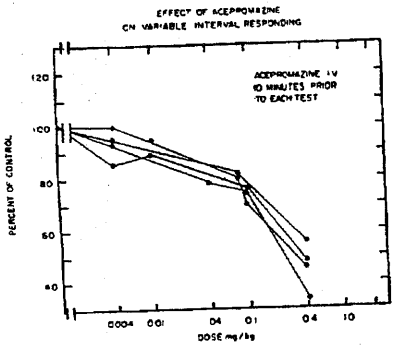

Fig. 9: Effect of Acepromazine on Variable Interval Responding.

Acepromazine was administered intravenously to 4 horses 10 min. prior to each trial. Closed circles (●) represent the response of each horse expressed as percent of saline control performance for the same horse.

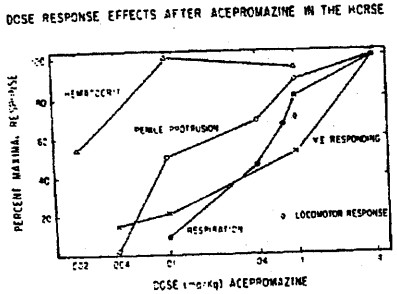

Fig. 10: Dose Response Effects After Acepromazine in the Horse.

The above graph shows the percent maximal observed response of 5 different pharmacological and behavioral parameters as related to intravenous dosage of acepromazine. Hematocrit effects are shown by open triangles (Δ), penile protrusion as open circles (0), respiration as closed circles (●), variable interval responding (X), and locomotor response as open diamonds (◊). Only one dosage level was used for locomotor response.

DISCUSSION

MASON: This is not so much a question to Dr. Tobin but a comment. Your report underlines the relationship between the veterinarian and analyst. On a number of occasions we have noted clinical symptoms of penile protrusion in horses. We have referred this matter to the laboratory on the basis that these were the clinical signs shown, suspecting the use of a tranquilliser. I think you have clarified a number of doubts I had about our laboratory, with apologies to our analyst.

REILLY: You were talking about 150 mg doses. Did you do any administrations of lower levels, say 5 mg. per horse, and were you able to detect the drug at those levels?

TOBIN: We did administrations as low as 1 mg for the pharmacological studies. There were no chemical studies at those levels. We consider ourselves fortunate to be able to detect it in plasma at the higher doses. We are doing some work on metabolites in urine, but that is a much slower proposition.

MELDRUM: Your graph showed penile protrusion at 5 mg per horse. We use this drug quite often for standing castrations and fail to get much response under about 20 mg. For the penis to be fully protruded you must use about 30 mg. What degree of penile protrustion do you get with a 5 mg dose?

TOBIN: It is about 50% at 0.01 mg per kg, a 5 mg per horse dose.

GERBER: Respiratory rates are of doubtful significance if they are above 16 or so at the start of observations. In our studies normally it is 8 to 12. The age of horses has some effect.

TOBIN: From my experience in our horses in Kentucky that are mature mares the rates are around 16. We have dosed horses with cocaine and got very clean dose reponse curves, and, in the case where exponentially the rate of decay of the respiratory rate changes as the dose increases, it always decayed back to about 16 breaths per minute.

fective when used in foals carrying various levels of related or unrelated maternal antibodies.

7. A comprehensive study of therapeutic agents routinely used in treatment of known foal pneumonia pathogens. Antibiotic evaluation should determine:
   a. The effectiveness of various antibiotics.
   b. The minimal inhibitory concentration needed.
   c. The length of time the antibiotic must be used to completely eradicate pathogen.
8. Evaluation of the effectiveness of various products, such as DMSO, Isoniazid and prostaglandins, that may be useful in the control of foal pneumonia. There is also a need to evaluate anthelmintics that may be effective against larval forms of helminths such as ascarids, strongyles and habronema.
9. Managerial research to improve the natural disease resistance of horses of all ages. This work should include:
   a. Establishment of the most disease-resistance-producing nutritional standards for all ages.
   b. Determination of the relative disease resistance of suckling foals and those fed other than their mothers' milk prior to weaning.
   c. Determination of the relative disease resistance of foals in relation to their weaning ages, and the influence of their post-weaning diets on disease resistance.
   d. Determination of the effects of various kinds of environmental and managerial stress upon foal health and resistance.
   e. Research into the selection of disease-resistant bloodlines.
   f. In the face of modern trends toward earlier weaning, studies are needed to more clearly evaluate nutritional levels afforded by mare's milk at various times of lactation.

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Pharmacology Review:

A Review of the Pharmacology of Reserpine in the Horse

—Tom Tobin, D.V.M., Ph.D., Kentucky Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, KY, 40506.

Reserpine is the principal alkaloid of a climbing shrub of the Apocynaceae family called *Rauwolfia serpentina*, which is indigenous to India and neighboring countries. It was described in ancient Hindu writings and medieval western medicine as the "insanity herb" and was widely used in traditional Indian medicine for treatment of hypertension, insomnia, and insanity.[12] These uses of the *Rauwolfia* plant went unnoticed by modern western medicine until 1931, when Sen and Bose described its use for treatment of psychoses and hypertension in Indian medical literature.[12] This report again attracted the attention of western investigators to *Rauwolfia*, and pure reserpine was isolated from the crude plant material in 1952. When reintroduced in this form, reserpine was the first of the modern major tranquilizers, and in the 1950s and early 1960s, was widely used in the treatment of psychoses and hypertension. It soon became apparent, however, that its use was associated with severe side effects. Thus, parkinsonism, severe depression, suicide, and seizures have been related to its use in humans, and reserpine is currently used only in occasional patients resistant to other forms of therapy.[12] In equines, however, reserpine is still widely used as a tranquilizer, because of its efficacy, difficulty of detection, and its long duration of action after a single dose.

Reserpine is a remarkably potent drug, and very small doses in the horse (<5 mg) can produce considerable biochemical and behavioral effects. Reserpine is active in such low doses, and is so difficult to detect, that it was first thought to be a "hit-and-run" drug, *i.e.* a drug which when administered produced a biochemical change which persisted for days after the drug was eliminated from the body.[2] This concept is now known to be incorrect, and reserpine is known to be very tightly bound at specific sites in the body, and remains bound at these sites for at least as long as it produces its pharmacological effects, and possibly for much longer.[1]

Reserpine produces its actions in the brain by greatly reducing the brain content of certain neurohormones. Among the neurohormones whose

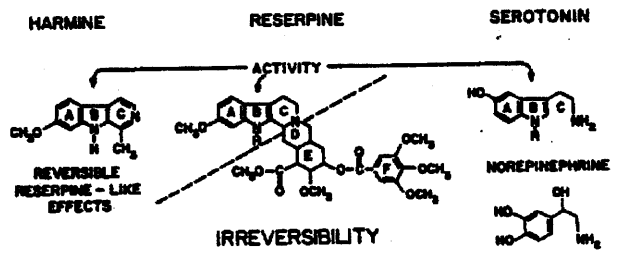

Figure 1. The structure of reserpine and related neurohormones. The A, B, C rings of the reserpine molecule are structurally related to the neurohormones norepinephrine and serotonin, whose storage in nerve terminals reserpine clocks. Harmine, which is structurally similar to the A, B, C rings of reserpine, produces the same pharmacological effects of reserpine, but its action is very short-lived. It therefore appears that the tight (irreversible) binding of reserpine and its prolonged action is associated with its D, E, F rings.

brain concentrations are reduced by reserpine are norepinephrine,[4] dopamine,[4] and serotonin,[16] and recent experimental work suggests that the behavioral effects due to reserpine are most closely linked to its depletion of brain norepinephrine,[5] although earlier work had suggested that serotonin was the neurohormone involved.[6] There appear to be good chemical reasons for this, because it turns out that one portion of the reserpine molecule (the A,B,C rings, Figure 1) is closely related structurally to norepinephrine and particularly to serotonin. Thus reserpine appears to displace norepinephrine and serotonin from their binding sites in nerve terminals, and in this way interferes with their function. Similar pharmacological and biochemical effects are also observed with harmine, a drug which is closely related structurally to serotonin (Figure 1), but whose effects do not last for more than a few hours.[12] It appears, therefore, that the D,E,F rings of the reserpine molecule are associated with the very tight binding of reserpine to its receptors, and it is this tight binding which gives reserpine its great effectiveness, potency, and its peculiarly long-lived actions.

While reserpine acts by depleting brain levels of catecholamines, it does so by depleting catecholamines from a storage granule which may account for some of the clinical characteristics of reserpine's actions. In catecholamine-containing neurons in the brain, the bulk of the catecholamines are found tightly complexed with ATP and Mg^{++} in granules (Figure 2).[13] It is these granular binding sites which are affected by reserpine. Reserpine binds tightly to these granules, and in so doing disrupts their structure and their ability to bind catecholamines. Because norepinephrine is an excitatory neurohormone in the brain, its depletion readily explains the calming or tranquilizing effect of reserpine in the horse. It has been calculated that each bound molecule of reserpine can cause the displacement of about 500 molecules of norepinephrine, which is then only very slowly replaced with newly synthesized norepinephrine. Other estimates

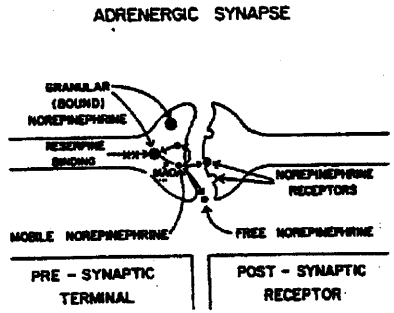

Figure 2. The site of action of reserpine on adrenergic synapses. Norepinephrine (or serotonin) is tightly bound in granules in the presynaptic terminal. Reserpine binds to these granules, disrupts them, and thus displaces the bulk of the neuronal norepinephrine. Normally, this released norepinephrine is metabolized by monoamine oxidase (M.A.O.), so it does not reach the postsynaptic receptor and result in central nervous system excitement. However, if monoamine oxidase has been blocked by a monoamine oxidase inhibitor, the released norepinephrine reaches the postsynaptic receptor causing excitement and the so-called "reserpine reversal."

suggest that only about 20 reserpine molecules are bound to each affected granule, further emphasizing the great potency of this drug.[1] The norepinephrine and catecholamines released by reserpine are usually hydrolyzed by monamine oxidase before they reach the postsynaptic receptors (Figure 2), and thus the release by reserpine of excitatory neurohormones is associated with minimal signs of central nervous system excitation in the horse.

This mechanism of action, however, leads to an unusual variation in the action of reserpine called "reserpine reversal." It turns out that if one administers a monamine oxidase inhibitor such as phenelzine or iproniazid to an animal prior to reserpine, all the catecholamines displaced from the granules are not metabolized and readily reach the postsynaptic membrane, where they cause central nervous system stimulation. Under these circumstances, reserpine can cause marked excitation instead of depression, giving rise to the name "reserpine reversal" for the effect.

Most of the experimental data on which this mechanism of action was developed is based on experiments on brain tissue and behavior in small laboratory animals.[12] However, it turns out that blood platelets also possess serotonergic granules and actively store serotonin[14] in much the same way as brain tissue. Because of this, in our studies on the actions of reserpine in the horse, we investigated the effects of reserpine on serotonin uptake in horse platelets as a biochemical correlate of the pharmacological effects of this drug in the horse.

When 10 mg of reserpine is administered intravenously (IV) to a horse, little change is seen for the first two hours. The first signs observed are usually sweating over the shoulder, back, stifle, and inguinal areas, at about three hours. Shortly thereafter the animal starts to pass considerably increased amounts of gas, and increased gastrointestinal motility and diarrhea commences. Slight ptosis (drooping of the eyelids) appears, increases with time, and remains one of the most enduring and sensitive indications of reserpinization. If the animal is male, penile extension starts at about five hours, is maximal (for this particular dose) at about 12 hours, and may be almost retracted by 24 hours.

At about four hours after dosing, the animal has a glazed appearance and by eight hours may be standing relatively immobile and apparently depressed. If the animal is particularly sensitive to reserpine (and there are suggestions that males are more sensitive to reserpine than females) it may show signs of acute colic and go down for a period. More commonly, however, the animal simply appears depressed and may appear so for up to 48 hours. By 60 hours postdosing, however, animals dosed with 10 mg of reserpine will appear clinically normal.

It is characteristic of the pharmacology of reserpine that animals sedated with this drug are easily aroused,[12] and Figure 3 shows a rather clear-cut demonstration of this effect in the horse. As pointed out previously,[17] the narcotic fentanyl produces a very clear-cut behavioral stimulation pattern in the horse. In Figure 3 a horse was challenged with fentanyl before and after treatment with reserpine. Despite the marked depression of spontaneous motor activity by reserpine, challenge with fentanyl after reserpine produced almost exactly the same motor response as before reserpine. Thus, though reserpine served to markedly reduce the spontaneous activity of the horse, it did not in any way affect the response of this animal to fentanyl.

In our hands, lower doses of reserpine produced correspondingly less marked pharmacological effects.

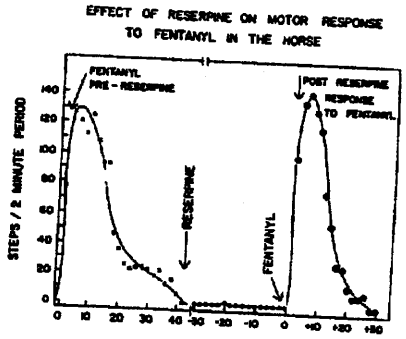

Figure 3. The effects of reserpine on spontaneous and fentanyl-induced motor activity in the horse. The left-hand portion of this figure (crosses, X - X) shows the marked increase in spontaneous motor activity induced in this horse by rapid IV injection of 5 mg Fentanyl Citrate. This animal was then treated with 10 mg reserpine IV, and the open circles (O - O) show the lack of spontaneous activity in this horse three hours after reserpine, the animal making one step in a 30-minute period. However, when again challenged with 5 mg of fentanyl, the animal responded as previously with no apparent diminution in response. Activity was measured by counting steps/2-minute period as described previously.[12]

A dose of 5 mg IV produced droopy eyelids at three hours with passage of gas and some diarrhea at about eight hours. A dose of 0.5 mg to a horse produced little observable change beyond the passage of some very loose stool at about 10 hours. At these low doses (0.5 to 1 mg) the animals were undistinguishable from untreated horses within 24 hours. These dose-response relationships agree well with some very early dose-response relationships reported in the horse by Earl.[8]

In experiments in small laboratory animals, reserpine shows similar calming effects, and while the animals may sleep, they are easily aroused and are responsive to external stimuli. Also, while spontaneous activity is decreased, motor coordination is not affected.[13] Our observations in the horse, as presented in Figure 3, correlate well with these reports.

In the peripheral nervous system most of the actions of reserpine are consistent with catecholamine depletion, and may be explained by a decrease in sympathetic tone. In the circulatory system reserpine produces hypotension and bradycardia, and these are the principal pharmacological effects of reserpine currently being utilized in human medicine.[13] In the gastrointestinal tract, reserpine causes increased frequency of defecation and diarrhea, due to decreased sympathetic and increased parasympathetic tone, effects readily observable in horses. Currently, reports in the literature of deaths due to reserpine are rare,[13] but the horse appears to be particularly susceptible.[4] Though Earl[8] reports statements that 5 mg can produce violent colic in a horse, we have routinely used 10 mg in our studies with no problems, so the lethal dose for the horse must be substantially above this. The recommended antidote to reserpine is methamphetamine,[2] which makes good pharmacological sense in view of the fact that the primary pharmacological action of reserpine is to deplete norepinephrine.

Since we could not sacrifice our horses for neurohormone assays, we elected to follow the biochemical effects of reserpine in these horses by studying the effects of reserpine on platelet serotonin.[7,14,15] It turns out that blood platelets have a serotonin uptake and storage mechanism similar to those found in serotonergic neurons in the brain, and this uptake mechanism is also sensitive to reserpine.[14] Analyzing the ability of horse platelets to take up radio-labeled serotonin before and after reserpine treatment, White and Tobin[19] found that this uptake was up to 75% inhibited six hours after a dose of 10 mg of reserpine, remained depressed for at least three days, and returned to control by seven days (Figure 4).

The most likely explanation for these effects is that reserpine is bound to the serotonergic granules in the platelets, which shows up as the inhibition of platelet serotonin uptake. This uptake was maximally depressed at six hours after an IV dose, and thereafter recovered slowly.[20] Because platelets in the horse live only about 14 days, in contrast with the lifetime existence of nerve cells in a horse, this is likely to be the shortest period for which the central effects of reserpine on brain norepinephrine and serotonin metabolism may be observed in the horse. It is also likely to be the shortest period for which reserpine persists in the body of the horse. It further turns out that horse platelets are surprisingly sensitive to reserpine, and doses of only 1 mg of reserpine or less produce an approximately similar effect.[20]

These actions of reserpine in the horse are quite consistent with its basic mechanism of action. Because

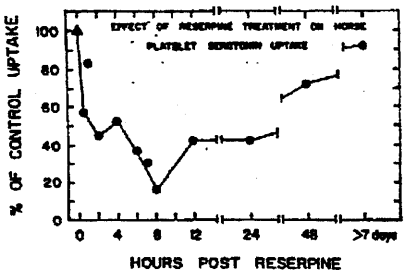

Figure 4. The effect of reserpine treatment on [^{3}H] serotonin uptake by equine platelets. The initial rates of [^{3}H] serotonin uptake were measured as described by White and Tobin.[19] The zero time value (solid triangle ▲) represents the initial rate of [^{3}H] serotonin uptake in platelets drawn from this horse prior to treatment with reserpine. Platelet uptake of serotonin is measured as nmx [^{3}H] serotonin/10^{11} platelets/min, and the control value is expressed as 100%. The solid circles (• - •) show the depression in the initial rate of serotonin uptake by platelets drawn from this horse at the indicated times after treatment with 5 mg of reserpine by rapid IV injection. These results are typical of experiments on a number of horses, and broadly similar results are seen after 10 mg and 1 mg of reserpine.

granular norepinephrine in the synapse is not directly available for neurotransmission, a lot of reserpine must be bound, and norepinephrine depleted from the granule, before pharmacological effects are seen. Thus, one might expect platelet serotonin uptake to be at least as sensitive, if not more sensitive, an indicator of reserpinization than the animal's behavior. Since the mobile pool of norepinephrine in the nerve terminals is not directly affected by reserpine, animals respond well to drugs or specific stimuli in a nearly normal manner, as was seen with fentanyl.

Although the plasma half-life of reserpine in the horse has not yet been determined, it is likely to be very long. The first reason for this is that reserpine is a highly lipid-soluble drug[13] which distributes very widely in the body. A lipid-soluble drug can get into every nook and cranny in the body, and it simply takes the body's metabolizing systems a long time to find and eliminate it. Secondly, reserpine is very tightly bound at its specific binding sites on the catecholamine storage granules.[2] It is not at this time clear whether or not this tightly bound reserpine is ever free again and is excreted, or even if this pool of reserpine is big enough to significantly affect the excretion pattern,[13] but its effect can only favor a longer half-life for this drug in the horse. Studying the plasma half-life of reserpine in humans, Maas and co-workers[9] reported a 271-hour (11-day!) half-life for reserpine in humans, and detected reserpine in plasma, urine, and feces 12 days after administration of 0.25 mg to humans. As pointed out earlier, there is no reason to suspect that reserpine will be any less persistent in the horse, and it may turn out that after a course of therapy reserpine will be detectable in the body of the horse for very long periods indeed.

Because reserpine is so persistent in the horse and has such a prolonged pharmacological action, it is possible to very accurately control the amount of reserpine in a horse, and the pharmacological effects attained. Thus daily administration of low-milligram doses of reserpine should allow the gradual accumulation of reserpine in the horse, and a gradual increase in the pharmacological effect. At any point during this slowly increasing effect, the effect may be maintained by simply reducing the dose rate by about 50% or more to a maintenance dose. If dosing is abruptly stopped, the drug effect will decay at the fastest possible rate, the same as that observed after an IV dose, though the pharmacological effects may take several days to dissipate and blood levels of the drug even longer.

Until very recently, no analytical test for the presence of reserpine in the horse was available, and because of this its use in competition horses could not be controlled. Recently, however, Sams and Huffman at Ohio State University have reported on a thin-layer screening method for detection of small doses of reserpine in the horse.[11] This is basically a micro-thin layer modification of a method described by Tripp and co-workers,[18] and it is reported to detect low levels of reserpine in equine plasma (but not urine) for several days after a dose of reserpine. Although this test represents a major advance in analytical testing for reserpine, it must be kept in mind that thin-layer chromatography does not chemically identify a substance. This lack of specificity can lead to the occurrence of false positives and thus to problems with its use as an unequivocal forensic test for reserpine.[a] Thin-layer chromatographic tests are not specific tests,[16] but merely tests which can eliminate compounds, *i.e.* tests which can prove the absence of a drug but not its presence. According to Stein *et al.*,[16] "If TLC is to be used for identification purposes, all possible alternative compounds, including derivatives and isomers, must be chromatographed and their Rf values found to be different from the unknown suspected material before a positive identification can be made."

The requirement for positive chemical identification is particularly important in the case of reserpine, since this drug is a plant product. Plants growing in the southeastern United States which have been reported to contain reserpine include the periwinkles, members of the genus *Vinca*, such as Vinca herbacea, and Vinca rosea. In addition, there are about ten or a dozen species of the Apocynaceae growing in the southeastern United States, but knowledge concerning the reserpine content of these plants is limited.[b] In view of the possibility of "positives" in equine plasma from reserpine or related alkaloids of botanical origin, particular care should be taken by analysts when testing for reserpine in equine plasma.

[a] Personal communications from: Dr. George Maylin and Dr. Jack Henion, NYS Racing and Wagering Board, Drug Testing and Research Program, Cornell University, Ithaca, NY, and Mr. John McDonald, Chief Chemist, Illinois Racing Commission, 160 N. LaSalle Street, Chicago, IL.

[b] Personal communication from Professor Trevor Robinson, Department of Biochemistry, University of Massachusetts.

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New Products

A brief summary of new products for the equine practitioner. Publication does not imply an endorsement by The Journal of Equine Medicine and Surgery.

A new Fiber Optic Annular Illumination System from Dolan-Jenner Industries, Inc., offers the benefits of high intensity, cool, shadowless light with no clutter of hot lamps, wires, or light pipes near the biological specimen, heat sensitive material, or microscope operator.

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A new surgical scrub that kills microorganisms more rapidly than hexachlorophene, and provides longer lethal activity than povidone-iodine, has just been announced by Fort Dodge Laboratories.

New Nolvasan Surgical Scrub is the latest in a long line of veterinary pharmaceuticals based on chlorhexidine diacetate, from Fort Dodge. Chlorhexidine diacetate has long been recognized for mildness combined with powerful antibacterial, antiviral and antifungal qualities.

In recent studies, Paul and Gordon compared new Nolvasan Surgical Scrub with other scrubs based on hexachlorophene and povidone-iodine. Cultures of *E. coli* and *Staph. aureus* were pipetted onto marked sites of clipped dog-skin. After two minutes of scrubbing, Nolvasan Surgical Scrub reduced the mean colony count of the bacteria lower than hexachlorophene and povidone-iodine—significantly lower than the hexachlorophene scrub.

After one hour, cultures of bacteria were again applied and incubated. The mean colony count showed that Nolvasan Surgical Scrub maintained high residual activity—higher than the other two scrubs against *E. coli*, but dramatically higher than povidone-iodine against *Staph. aureus*. Previous studies have shown that Nolvasan retains high antimicrobial activity even in the presence of blood and other organic matter, to a much greater extent than most other disinfecting agents.

(continued on page 444)

UNIVERSITY of KENTUCKY • COLLEGE of AGRICULTURE
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DEPARTMENT OF
VETERINARY SCIENCE
102 Animal Pathology Building
Lexington, Kentucky 40546-0076
(606) 257-0757

12 May 1983

David Vienna
A Corporation
510 C Street, N.E.
Suite 100
Washington, D.C. 20002

Dear Mr. Vienna:

Mr. (Mark) Paulus has stated in testimony that Kentucky has a mediocre equine drug testing laboratory. On what comparative bases Paulus makes this judgement, I do not know. The only reasonable basis must be in terms of "positive" drug findings within a given jurisdiction. This is the service which a drug testing laboratory performs for the governing Commission(s) within its jurisdiction.

Kentucky presents a unique situation in that the medication rule for the Thoroughbred (Kentucky State) Commission is liberal, whereas the medication rule for the Standardbred (Kentucky Harness) Commission is conservative. The laboratory, therefore, analyzes blood and urine samples representing two different policies. A "positive prohibited" drug finding for the Harness Commission might represent a "controlled" or "permitted" drug finding for the State Racing Commission. This is analogous to a "prohibited" phenylbutazone sample in Kentucky, in a Thoroughbred horse. As a result, it can be difficult to compare the percent of prohibited drugs detected among various jurisdictions because of the fact that a prohibited drug in, say New York, is in fact, a permitted drug in Kentucky. Despite the fact that many prohibited drugs in other jurisdictions are permitted drugs in Kentucky, the Kentucky Equine Drug Testing Laboratory finds considerable numbers of "positive prohibited" drugs each year.

All samples submitted to the Kentucky laboratory are "blind" samples, i.e. there is no indication of the permitted drugs contained in these samples. The permitted, as well as any prohibited drugs must be detected and reported daily to the Commission. A fair representation of the laboratory's effectiveness would be to tabulate the Commissions' submitted samples for a calendar year, indicate the total permitted drugs, the total prohibited drugs and the ratio of confirmed prohibited drugs to the total samples analyzed. Such a tabulation appears below. The data were acquired from the Annual Report(s) of the Equine Drug Research Program and the Equine Drug Testing Laboratory for the years 1976, 1977, 1978, 1979, 1980, 1981 and the 1982 data to be submitted to the Commission later in 1983. The years 1977 and 1978 include a single meet sponsored by the Kentucky Quarter Horse and Appaloosa ommission which operated under Thoroughbred medication rules.

Year	Total Submitted Samples	Total Permitted Drugs Found	Total Prohibited Drugs Found	Prohibited Drugs : Total Submitted Samples
1976	8821	4215	31	1:285
1977	9147	4888	39	1:234
1978	9273	4913	17	1:545
1979	8292	4063	21	1:395
1980	8945	3736	18	1:497
1981	9705	6015	6	1:1617
1982	8272	5217	48	1:172

From the above tabulation, a composite of effectiveness with regard to prohibited medication in the Kentucky laboratory can be drawn. In the seven full calendar years during which the laboratory has been active, 62,455 samples have been analyzed. 33,047 permitted drugs have been detected, in samples analyzed blind. 180 prohibited drugs have been reported. This is a seven year ratio of 1 prohibited drug per 347 samples tested. Also, many prohibited drugs in other jurisdictions are among the 33,047 permitted drugs found in Kentucky. I believe this is a very favorable accounting from a laboratory which Paulus has labelled "mediocre". It would be of interest to have a similar tabulation from other jurisdictions.

Kentucky has participated in the NARC Quality Assurance Program for about 8 months. Ohio State University is the Regional Laboratory with which Kentucky is affiliated. During this time one set of unknown samples has been analyzed - a true measure of a laboratory's competence with respect to its peer laboratories. With regard to Kentucky's performance on this set, the comment of Ohio State's Quality Assurance Officer was "Totally correct; excellent documentation; good job!"

Yours Sincerely,

J.W. Blake, PhD
Director of Equine Drug Testing Lab.

Rule 1.09. The Commission may designate one or more chemists, whether in or out of state, to receive specimens from the commission veterinarian and may execute contracts with said chemists to perform and report the results of routine examinations of body fluids for foreign substances, and to particpate in cooperative regional or national race testing and monitoring procedures and studies to improve testing procedures.

Ms. BOWMAN. May I ask something?

Mr. GEKAS. Yes, by all means.

Ms. BOWMAN. You leave me a little puzzled then, I guess. We have had testimony before us that there's a rather substantial rift between the HBPA and some of the other organizations.

For example on a hearing on April 27 of this year, Francis Ready, speaking on behalf of the U.S. Trotting Association, stated in written testimony that it is his belief that "the HBPA through its rigidity has lost its credibility in the judgment of many."

Can you give us some insight as to what prompted them to make such an uncomplimentary assertion?

Mr. VIENNA. Ms. Bowman, I can't speak for them or for that individual.

Ms. BOWMAN. You can't help us out on where that assertion may have come from?

Mr. VIENNA. No.

Mr. GEKAS. Dr. Tobin, you may proceed.

TESTIMONY OF DR. THOMAS TOBIN, KENTUCKY EQUINE DRUG RESEARCH PROGRAM, DEPARTMENT OF VETERINARY SCIENCE, UNIVERSITY OF KENTUCKY

Mr. Chairman, members of the subcommittee. My name is Thomas Tobin; I graduated in veterinary medicine from University College Dublin in 1964. I completed a doctorate in pharmacology-toxicology at the University of Toronto in 1970. I was a faculty member in the Department of Pharmacology at the Veterinary School at Michigan State University until 1974.

In 1975 I was invited to set up the Kentucky Equine Drug Research Program. In this program we study the detection, action, uses, and effects of medications in racing horses.

Since 1975 we have published two books and about 88 publications at the last count on this particular field.

I do this in association with a colleague of mine, Dr. Jerry Blake, who runs the Kentucky Equine Drug Testing Program and who actually does the racetrack testing for the State of Kentucky.

Mr. GEKAS. May I interrupt for just a moment to get the scene set up?

Dr. TOBIN. Certainly.

Mr. GEKAS. In Kentucky, is it a private contracting situation with the racing commission on the testing drug——

Dr. TOBIN. On the testing the university has a contract with the racing commissions—with both commissions. On the research side the commissions support my research program with funds from the parimutuel——

Mr. GEKAS. Thank you.

Dr. TOBIN. Both programs have completed considerable research on furosemide; in fact, most of the work on furosemide in literature has come from our laboratory and we are currently working on phenylbutazone.

Today I'm going to speak to the bleeder and Lasix; phenylbutazone, which we in the industry consider the horse's equivalent of aspirin. Then I'm going to speak about illegal medications, particularly medication control and the drug testing process.

I'm going to briefly refer to the Canadian drug testing system, which is under Federal control and is let to three private contract laboratories. I'm going to compare the performance of the Canadian system with what we have currently in place in the States.

I will refer very briefly to tests made by Mr. Paulus, who claimed that Kentucky has a mediocre drug testing program, our laboratory. I think when I finish some brief statements in that area, you will be satisfied that that is not the case.

I'm going to speak then to the problem of trace level or residue level determinations, because this will be a critical area if this bill is passed, and on these determinations will be made on whether horsemen go to jail or not, and that is an absolutely critical area. And then I will summarize.

I'm going to start and roll you back 300 years. If you take a thoroughbred horse and race him as fast as you can for about 1 mile and you look at him after that run, you'll find that about 1 to 2 percent of racehorses will trickle blood from the nose immediately after the race.

This has been known for well over 300 years. It's got nothing to do with medication, it's got nothing to do with the United States of America; it antedates the appearance of this Nation.

This is the classic bleeder. It's been known for a long time. Occasionally, a horse will die from this bleeding. He will bleed at the lungs and die. Now, this is rare, but it does occur.

It had long been taught when I was a young veterinarian in Ireland that some horses bled internally in the lungs and that it never appeared to trickle out of the nose, and these were called occult bleeders. And old veterinarians would speak about occult bleeders and as a young man I would say to myself, "Well, these guys don't know what they're talking about."

Comes the flexible fiberoptic endoscope, an endoscope which enables the veterinarian to look into the windpipe of a horse, and if you take these horses and run them a mile as fast as they can and then inspect their windpipes, postrace, you'll find that anywhere between 40 and 75 percent—and to the best of my knowledge, 75 percent is the highest figure that has been reported, of these horses will bleed in the windpipe, in the lungs postrace. They bring the blood up in the lungs, it comes to the pharynx, some of them will swallow it and some of them will let it trickle out the nose.

The trickling out the nose, the 2 percent, is the classic bleeder, and that was all we knew about bleeding until the late 1970's when this problem of lung bleeding and the fact that it's a much more prevalent problem was discovered.

So it's a widespread problem, Mr. Chairman; it's been with the thoroughbred as long as the thoroughbred—in fact, as long as the horse has been running, because it depends on the anatomic structure of his lungs.

When horses bleed in a race it's not a very pretty spectacle; they may slow down, they may stop, they may wreck up a field of horses. They put the life and limbs of jockeys and horses at risk.

For this reason, racing men have been searching for an effective treatment for bleeding for at least 300 years. The search goes on today and will continue.

The current drug of choice for the treatment of this problem is furosemide or Lasix. That is the drug that the veterinary profession recommends. It's their drug of choice at this time.

What is Lasix? Lasix is a very effective and very safe diuretic. It was developed for use in human medicine. It's about the fifth most commonly used drug in human medicine. And it's very safe, very effective. It wouldn't be used that widely if it wasn't safe.

It is specifically approved by the FDA for pulmonary congestion in horses. In this briefing book which you have with you there's a photocopy of the box in which Lasix is marketed and it says "pulmonary edema" (congestion), if I remember correctly, in horses. Pulmonary congestion is considered to be one of the causes of bleeding in horses.

Horses with atrial fibrillation who suffer pulmonary congestion have a much higher incidence of bleeding than normal horses. Clinical experience of equine veterinarians and horsemen suggest that furosemide is effective in the treatment of epistaxis or bleeders.

That is basically what it depends on. The clinical impression was created among equine veterinarians that it's effective and it went on from there.

Now to summarize, there is a clearcut pharmacological basis because it's an effective diuretic and affects pulmonary congestion in horses; there is a clear medical rationale because one can visualize circumstances under which it would be beneficial. There is regulatory approval from the FDA for its use in pulmonary congestion, and there's clinical evidence from horsemen and equine veterinarians that it is effective, and this includes university hospital studies.

These, in summary, are the reasons why the veterinary profession—the equine veterinarians—why furosemide is the drug of choice for use in horses.

Now I'm going to tell you what Lasix does in the horse. One of the reasons that it's a very safe drug in both humans and the horse is that it's very rapidly excreted, it's a very short-acting drug, and I want you to keep this in mind.

When you dose a horse with a dose of furosemide that's used to prevent—can you hear me clearly?

Mr. GEKAS. Yes.

Dr. TOBIN. Okay. When you dose a horse with a dose of Lasix which is used to prevent bleeding, only about 250 mg of the drug is given to a 1,000-pound horse intravenously into the jugular vein.

The formation, the increased formation of urine, the diuretic effect occurs very rapidly, and is essentially over within 1 hour.

Now the effect looks very dramatic. If I had a horse standing here in front of us and I put the furosemide into his jugular vein, then in about 5 minutes that horse would squat, lift his tail, and he'd void urine all over the carpet here. It would look like a lot of

urine to you. In fact, it would be 4 liters of urine and only about 2 percent of the body water in the horse. It's a relatively small fraction of the body water in the horse.

Drugs are distributed by and large through the body water or sometimes on a larger "volume" than the horse. Furosemide cannot—and I want to emphasize this very strongly—it cannot flush drugs or move significant quantities of drugs out of the body of the horse.

This misconception was repeated in the television program before you here today; that's quite incorrect. It moves 4 liters of fluid out of the horse. Whatever drug is in that 4 liters would possibly be moved out. There's 98 percent of the drug left in the horse for the analyst to find. The 2 percent is not a significant analytical difference.

So in a nutshell, it's a very safe short-acting drug, and it doesn't reduce the level of any drug in the body of the horse.

Now we come to the effects of furosemide on drug detection in urine. It does not affect—I just reemphasize it—it does not affect the detection of any drug in blood, it does not affect the detection of certain groups of drugs, basic, lipid soluble drugs in urine inasmuch as we know.

It will reduce the concentration of water soluble drugs and water soluble drug metabolites in urine for the brief period of the sharp diuretic effect after it's administered to the horse.

If the drug is given 4 hours prior to post time at the dose recommended by the American Association of Equine Practitioners, there is no interference whatsoever with the drug testing process.

In fact, there is some evidence from studies in my laboratory that under these circumstances the detection of certain narcotics—and we studied six narcotics, I believe you will find the paper in this briefing book—their detection is actually enhanced.

So there's no evidence for interference of testing. These studies are published from my laboratory, they're in the literature, they were repeated by a group, the Quality Assurance Program group recently, and their preliminary results confirm my results, and other work that I've done suggests that some drugs, their detection is enhanced.

Therefore if the dose recommended by the equine practitioners is used and if the time is 4 hours prior to post time there is no masking or diluting effect whatsoever.

Does Lasix affect the performance of horses——

Mr. GEKAS. That 4-hour period that you were talking about, Dr. Tobin, are you talking about prior to a race?

Dr. TOBIN. Prior to post. Post is—excuse me, that's a racing term. That's the actual start of the race.

If I use technical terms or racing terms or Irish phrases that you're unfamiliar with, please correct me.

A number of people have suggested that Lasix or furosemide will speed up or improve the performance of a horse. Studies with small number of horses, time trials at the University of Kentucky, similar studies at the Ohio State University and what we call a retrospective study of track times at Louisville Downs have shown no effects whatsoever of furosemide to improve the racing times of horses.

Now this is very important because it means that the horseman cannot use this drug to run horses hot and cold, run them on the drug, run them off the drug for a period and let the betting settle down, then run them on the drug when the performance is improved.

You cannot use furosemide or Lasix to do this because insofar as we can tell, it does not affect the performance of horses. Horsemen and equine veterinarians based on their experience are of the opinion that furosemide helps horses to run easier or easier in their wind or more reproducibly and more reliably, that is, truer to form.

And if a horse is a bleeder, he is likely to bleed again, so horsemen who own such horses or are training them have a keen desire to be allowed to use this medication.

Is Lasix toxic to horses? Lasix is a very safe drug in both humans and horses. The doses used in horses are small, the drug is rapidly excreted, there is no evidence of studies from my laboratory of toxic effects after single or repeated doses of the size used in racing horses.

Used on the racetrack in single, small and relatively infrequent doses, this drug is essentially nontoxic in horses.

In summary, I want to summarize. Bleeders are a common and very significant problem in racing horses; bleeders have been around for a long time. It leads to risk of life and limb of horses and jockeys. The treatment of choice in the veterinary profession at the moment is furosemide. It's safe, effective, it's approved by the FDA for use in horses. Properly used, it does not affect the detection of drugs in blood or urine. It does not mask. It will not move horses up or improve their performance.

I know of absolutely no evidence whatsoever to suggest that this treatment is cruel or inhumane. It's a rational therapeutic choice for a very common condition in horses. It may save the lives of both horses and jockeys.

I also wanted to say the wording in the bill refers to the drugging and numbing of racehorses. According to Webster, drugging means (1) to put a harmful drug in; (2) to stupefy or poison as with a drug; or (3) to administer something nauseating to. It's a very pejorative term.

Mr. Chairman, members of the subcommittee, nothing whatsoever that I have said about Lasix in any way meets the above definitions. To administer Lasix to a horse is in no way, shape or form, according to the general usage of the word, to "drug" a horse.

I have substantially more testimony; I see some questions in your mind.

Mr. GEKAS. Yes; I want to refer first to your summarization of the bleeding syndrome of the horse. Did you say at one point that the owner, after he should detect that his horse is a bleeder, that he should indeed apply the drug in order to help the horse?

Dr. TOBIN. I said it was the treatment of choice for this condition.

Mr. GEKAS. Does that imply that a bleeding horse runs and keeps running race after race; that it's possible for a horse who has bled and then treated runs again?

Dr. TOBIN. Yes; that's quite possible.

Mr. GEKAS. I mean, how soon?

Dr. TOBIN. Inasmuch as—in jurisdictions where Lasix is approved, he would be running the normal number of starts per year, which would be 8 to 10 per year, which would be 1 every 6 weeks, just off the top of my head.

Mr. GEKAS. What troubles me is that if the horse is a bleeder, as you say it's been known for 300 years as such, does that not affect his performance, the horse's performance?

Apparently not; they keep running them for the number of races a horse that normally any other horse is running.

Dr. TOBIN. It will affect his performance if the jurisdiction chooses to ban the bleeders, which some English jurisdictions do. If they do not, and if he is allowed access to therapeutic medication, he will run a normal racing career.

Mr. GEKAS. Does it trouble you at all that bleeders run again and again?

Dr. TOBIN. Mr. Chairman, horses race on horseshoes; it's been that way for a long time. It protects their feet, it's part of the art and craft of horsemanship, and it's one of the advances—therapeutic medication is an advance in this area.

Mr. GEKAS. I was just wondering about the risk of death to a horse if it becomes known that it's a bleeder and——

Dr. TOBIN. The risk of death is very small. The risk of pulling up is a more likely problem. I want to emphasize that until 5 years ago, as far as we knew, only about 1 to 2 percent were bleeders. We know now that it's a much more widespread problem and is related to the architectural structure of the lungs of the horse. It's part and parcel of being a horse.

Mr. GEKAS. But the main assertion that you are presenting is that the drug in question here is one that is therapeutic and curative of the bleeding problem.

Dr. TOBIN. It's the recommended medication by the profession at the moment for this condition.

Mr. GEKAS. And it does not act as a stimulant or as an enhancement for the capabilities of the horse in a particular race?

Dr. TOBIN. No. We conducted scientific studies and tested that hypothesis and it did not show any signs of stimulation.

Mr. GEKAS. All right. Mr. DeWine.

Mr. DEWINE. No questions.

Mr. GEKAS. Mr. McCollum.

Mr. MCCOLLUM. Not at this time.

Mr. GEKAS. Counsel.

Ms. BOWMAN. Thank you, Mr. Chairman. Dr. Tobin, you make reference in your testimony to the proper use of Lasix and well-run medication programs. The testimony before us has indicated that it's not as much the use of drugs as the problem with misuse.

Let me read you a quote from James Kramon, an attorney who handles jockey injury cases. He appeared before the subcommittee on December 15 of last year. He stated that the problem is not whether the drugs have therapeutic value, the problem is that the more incompetent to run the horse becomes, the more used becomes the——

Dr. TOBIN. Could you slow down a little? I'm trying to follow you.

Ms. BOWMAN. I'm sorry, let me try again. "The problem is not whether the drugs have therapeutic value, the problem is that the

more incompetent to run the horse becomes, the more used becomes the drug. The quantities around the racetracks are enormous. The drugs are given to people who have no license to administer them at all. The horses aren't examined properly for the use of the drugs."

Would you care to comment?

Dr. TOBIN. I don't see what that has to do with furosemide at all.

Ms. BOWMAN. My point is that our problem seems to be not as much the kind of thing you envision, the careful administering at the proper time by the proper person of the drug, but the use of the various drugs by individuals who are not licensed to use them at the wrong times and the wrong amounts.

Dr. TOBIN. If you allow me to continue my testimony, I believe I'll deal with that question.

Mr. GEKAS. I was incorrect in assuming that we were going to cover his entire testimony in our questioning. There's more to come. Go ahead.

Ms. BOWMAN. Then I'll wait.

Mr. GEKAS. Do you have any question as to what's already been covered? Yes?

Mr. HUTCHISON. Yes. Doctor, you stated that by giving Lasix 4 hours prior to post time, no masking or drug dilution effects or interference is encountered.

At what point prior to post does Lasix have that masking effect?

Dr. TOBIN. In my studies, the effects are essentially completely gone by 3 hours prior to post. I would say that 2 hours priors to—within 2 hours ahead of post the effect is beginning to be significant.

If you give the drug at minus 4 hours, that's 4 hours before post, the interference effect is maximal at about 15 to 20 minutes after you give the drug—from the top of my head, about 60 to 75 percent gone by 1 hour, about 80 to 90 percent by 2 hours. Essentially gone by 3 hours; by 4 hours has no effect, by 4 hours and 40 minutes when you pull the urine sample post race, there's actually an enhancement of drug detection.

Mr. HUTCHISON. Do I understand you correctly to say then that administered at the proper time Lasix could have a masking effect?

Dr. TOBIN. Administered at the proper time—if you define the proper time as the recommended dose with administration times as recommended by the AAEP, it has no masking effects whatsoever and will actually enhance the detection of some drugs.

Mr. HUTCHISON. But I thought I understood your answer to be when I asked at what point will it have a masking effect, and you said approximately 2 hours there would be a masking effect and then it's worn off a certain percentage as the time goes on.

I understood that to mean that if you administered Lasix 2 hours before post, there would be a masking effect; you're not saying that?

Dr. TOBIN. If you administered Lasix 2 hours before post, the urine sample will be taken at 2 hours and 45 minutes, so there will be little or no masking effect at that time.

Mr. HUTCHISON. Or administered 1 hour before post?

Dr. TOBIN. Yes, you could have a small effect then.

Mr. HUTCHISON. And if the dosage were larger than the recommended dosage that you're talking about, would there also be a masking effect?

Dr. TOBIN. That's correct.

Mr. HUTCHISON. And one other question. You state, using your definition from Webster, that this legislation would not have an impact on the use of Lasix; do I understand you correctly? If this legislation were enacted, it would not prohibit in any way the administration of Lasix.

Dr. TOBIN. The definition of drugging in this legislation is extremely broad. It's not in accordance with the usage of the word in my profession or in my discipline, which is pharmacology. Or in fact with the regularly accepted definitions in the English language.

This legislation is choosing to redefine the English language, which I guess is your privilege.

Mr. HUTCHISON. Well then, let me just ask you directly. Do you view the word "drugging" as used in the legislation as encompassing the use of Lasix?

Dr. TOBIN. The definition of drugging——

Mr. HUTCHISON. Used in the legislation.

Dr. TOBIN. Yes. Oh, absolutely. It encompasses everything. You have the legislation in front of you; it encompasses everything you might find in horse urine.

Mr. HUTCHISON. OK. Then your statement about "according to Webster drugging means these three things," you were really quarreling with the use of the word drugging in there as being inappropriate as applied to Lasix, because it's a pejorative term and to your mind the administration of Lasix does not have bad effects and warrant a pejorative term.

Dr. TOBIN. The administration of Lasix does not meet the commonly accepted definitions in my profession of drugging.

Mr. HUTCHISON. Thank you.

Mr. GEKAS. Shall we go on to phenylbutazone or whatever your next part is?

Dr. TOBIN. The next drug I wish to speak to, phenylbutazone, which is a nonsteroidal anti-inflammatory drug.

Phenylbutazone is a member of the nonsteroidal anti-inflammatory group of drugs. Aspirin is the principal, and again the oldest member, of this group of drugs that you are familiar with.

If you wish to keep phenylbutazone in the horse in perspective, it is useful to think of it as being basically horse aspirin.

Phenylbutazone and aspirin have little effect, in essence no effect, on normal pain perception. It is the hypersensitivity to pain associated with the inflammatory process which responds to treatment by phenylbutazone and by aspirin.

This hypersensitivity to pain associated with inflammation, such as sunburn, is caused by prostaglandins. Prostaglandin concentrations in tissues increase and the inflamed areas become hypersensitive.

In inflamed tissues prostaglandin concentrations increase and the inflamed areas become hypersensitive. Phenylbutazone and aspirin block prostaglandin biosynthesis, reduce tissue hypersensitivity and normalize pain perception in the affected area.

They leave the area with normal pain perception, they do not numb—I repeat, they do not numb any area in the body of the horse.

I'm going to give you some comparisons with other drugs to keep it in perspective. It's not thought out to be a stimulant in horses; it has no more ability to stimulate an unusual performance in a horse than aspirin has in you or me. Phenylbutazone is also quite clearly and distinctly different from the local anesthetics.

A local anesthetic is a drug that's injected at a specific point in the body along a nerve or else directly into a joint. It numbs a specific area in the body. You lose pain perception. You also lose proprioception and proprioception is the awareness I have sitting in front of you here that my legs are crossed underneath this table. I can't see them, but I can feel them, there's feedback from the legs and I know where they are.

If I block my legs out with a local anesthetic, I would lose that perception. My leg would be numbed with respect to pain and with respect to awareness of where it was.

These drugs—phenylbutazone does not do this. It does not numb, it does not affect proprioception. Blockage of proprioception in a racehorse can lead to a misstep and a breakdown, and that's why the use of these agents is illegal in horseracing.

For comparative purposes, as you well know, in human athletics the use of local anesthetic blocks is quite legal and permissible.

It is important to remember that phenylbutazone has no effect on normal pain perception——

Mr. GEKAS. Will you suspend for a moment?

Dr. TOBIN. Certainly, Mr. Chairman.

Mr. GEKAS. Let's find out what's going on.

[Pause.]

Mr. GEKAS. You may proceed. At an appropriate time we may have to suspend to allow the members to go vote; it might be within the next 5 minutes.

Dr. TOBIN. OK. Phenylbutazone is completely different from the narcotic analgesics. These are the morphine-like drugs. These agents act on the central nervous system in the brain to reduce the perception of pain there.

They act to reduce the horse's pain threshold below normal, and in addition they are a quite powerful stimulant in racehorses.

Such a horse would meet my profession's definition of being drugged.

Phenylbutazone has no depressant or tranquilizing effects. The principal pharmacological factor of phenylbutazone like aspirin is to normalize an inflamed tissue and has very little effects outside an area of inflammation.

I want to speak to you very briefly about phenylbutazone and breakdowns. This is one of the major charges against the drug.

Because it reduces the hypersensitivity to pain found in inflamed tissues, it is been argued that a horse treated with this drug will run on injured joints and tendons and be more likely to breakdown.

Now, first off this argument ignores the fact that in the articular cartilages of horses, the surfaces on which they actually run, there is no pain perception.

Phenylbutazone cannot affect pain perception there, because in the actual surfaces of the joints themselves there is no pain perception.

The great bulk of resistance to joint movement is based on the soft tissues: your skin, your tendons and so forth, around the joint.

Soft-tissue resistance to joint movement is about 99 percent of the resistance in an inflamed joint. Phenylbutazone removes this, reduces this, and gives the horse back complete use of himself. This is the manner in which it restores normal performance.

Another important factor, and this is very common in the racing horse, that while a horse is protecting a sore limb, he may overload the sound limb, he will put his weight and more than normal on the sound limb and may cause a breakdown of the sound limb.

By allowing a horse to run evenly, this is a way in which phenylbutazone can actually reduce the instance of breakdowns.

The State of California legalized phenylbutazone at the end of 1970. Between 1970 and 1978, there was no increase in the instance of breakdowns in racing horses in California which require their destruction. These are severe breakdowns and ones which should be carefully examined before the animal was put down.

Studies by the Colorado Racing Commission veterinarian, Dr. Gene Bierhaus, showed that over 9 years the probability of a horse having an accident which required his destruction was not any greater if the horse was on phenylbutazone than if it was not.

Pilot studies from the University of Pennsylvania at Keystone Racetrack show that the incidence of breakdown in this area was not significantly different from that in New York or New Jersey, both States which had stringent restrictions on the use of phenylbutazone.

These studies, but most particularly the California studies, appear to suggest that with a well-run medication program the rate of breakdowns is not increased by the use of phenylbutazone; in fact, you can recalculate the California data and show, if you're statistically inclined, that as the use of phenylbutazone increases, the incidence of breakdowns actually dropped slightly.

Phenylbutazone and masking. This is one of the major charges made against this drug. I want to emphasize that there are no published scientific studies and no reports of experimental studies anywhere in the scientific literature that show that masking by phenylbutazone is a forensic problem. There are no such studies.

The nature of the drug testing process is such that the masking of stimulants, depressants, narcotic analgesics, the so-called hard medications in racing—these what the chemists call basic drugs or medications—is such that these agents are separated out in the testing process and the potential for phenylbutazone to interfere with the detection of these agents is small.

Very recently, this spring, from our laboratories, studies have shown that the concentrations of oxyphenbutazone, which is a major metabolite of phenylbutazone and is the one most commonly regarded as being a potential masking agent, the concentrations of this agent in horse urine are not determined by the horseman. They are determined by the PH or the acidity of the horse urine.

If the urine of a horse is what we call basic, which has a very low acidity, the concentration of oxyphenbutazone in the urine is

extremely high. This is controlled by the horse and not by the horseman.

Mr. GEKAS. Will the witness suspend for a moment?

Dr. TOBIN. Certainly.

Mr. GEKAS. I yield back the gavel to the Chairman with the suggestion that we suspend for the moment and perhaps recess to an appropriate time to allow the members to go back to vote and invite the witnesses to have a cup of coffee, then come back later.

Mr. Conyers. So ordered.

Mr. FRANK. Until what time?

Mr. CONYERS. Well, you're running it.

Mr. GEKAS. This hearing is now recessed until 12:45.

[Recess.]

Mr. CONYERS. The subcommittee will come to order. We'll continue our discussion and I'd like to particularly welcome Caldwell Butler, our former member of the Judiciary Committee who served with us through some of the most trying periods of our country's history.

I am delighted that he is here with us and particularly welcome him back to the committee.

I think the witnesses were still in the midst of their testimony.

Mr. BUTLER. Mr. Chairman, at the time we recessed, I had completed the portion of my statement, Mr. Vienna had completed his statement, and Dr. Tobin was about a third of the way through here.

Mr. CONYERS. All right. That's a great way to resume again.

Dr. TOBIN. Mr. Chairman, I was discussing the allegations about masking by phenylbutazone. I had arrived at the point of discussing oxyphenbutazone, which is a major metabolite of phenylbutazone and which is the metabolite most commonly accused of being a masking agent.

I pointed out that recent research from the University of Kentucky had shown or has shown the concentration of this metabolite in equine urine is not determined by the horseman, it's not determined by the dose of phenylbutazone, it's determined by the variability in the physiology of the horse and what we call the PH or the hydrogen ion concentration of urine.

The hydrogen ion concentration is low. If the horse has a basic urine, the horse will dump an unusually large amount of oxyphenbutazone in the urine. This is a random event and is no way controlled by the horseman, so a horseman cannot purposely use phenylbutazone to mask other drugs.

That's a function of the physiology of the horse.

I wanted to point out that Kentucky, as you know, allows use of phenylbutazone. I want to introduce into the record—it should have been with my testimony but I have it here, the number of positive calls per year in Kentucky compared for example with New York and Canada, which do not allow the use of phenylbutazone.

Our positive call rate per year in Kentucky, Mr. Butler, I have these tables with me; I'm not sure whether they have them in front of them, should I——

[Pause.]

Dr. TOBIN. That's a more elaborate table; this is an abbreviated version which draws attention particularly to the comparison between Kentucky and New York and Canada. Kentucky calls, 2.9 positives per 1,000 urine tested on the average over the last number of years. New York calls, 0.08 per 1,000 from our figures, and that includes the number of phenylbutazone which you don't call in Kentucky.

Canada, which has no control medication whatsoever, has a positive call rate for hard drugs of 0.06 per 1,000; California, 0.4 per 1,000.

In Kentucky we are of the opinion that our regulations on phenylbutazone in no way interfere with the efficacy of our testing process and we believe that these figures support that position very strongly.

I know of no scientific evidence which suggests that masking by phenylbutazone interferes with drug detection. I believe that masking is not a scientific issue but rather a political or what we would call a pseudoissue.

Detailed scientific studies on masking by phenylbutazone are now underway at the University of Kentucky.

Phenylbutazone: its effect on performance. Phenylbutazone is not thought to change a horse's innate ability to race. By relieving inflammation, it may enable him to race nearer to his maximum capability.

It unquestionably will aid horsemen in fielding a racing sound horse. It unquestionably will aid the horse to run a better, more comfortable race.

I want to make this point perfectly clear; if any of us in this room want to compete in the human Olympics, we could compete on any medication that we wished, except we could not use a stimulant, we could not use a narcotic analgesic, we could not use an anabolic steroid.

Medications such as phenylbutazone or furosemide would be entirely legal and permissible and would not result in any ruling against an athlete on them.

The use of drugs such as phenylbutazone is entirely legal and ethical in human sports medicine. There is no reason therefore to think that this type of medication is any less ethical or any less humane when used in horses.

To summarize, phenylbutazkne is a nonsteroidal anti-inflammatory drug of the same general class and the same pharmacological activity as aspirin.

Its primary pharmacological action is to normalize an inflamed tissue. It has no effects to reduce normal pain perception or numb any tissue in the body.

It will reduce the hypersensitivity to pain in an inflamed tissue, but it leaves that tissue and other tissues with normal pain perception.

Its use from California statistics, from a study at the University of Pennsylvania, does not appear to be associated with an increased instance of breakdowns.

There are no studies, there is no scientific evidence that it makes testing for illegal drugs less effective; is thought to enable a horse to perform up to its innate ability; and enables horsemen to feel

that racing sound horses and use of this type of medication is entirely legal, ethical and humane in human sports medicine.

There is no reason to think that it is otherwise in veterinary medicine.

Again, to go back to my point about drugging, drugging refers to a harmful drug, a poisoning drug, or to administer something nauseating.

Nothing whatsoever that I have said about phenylbutazone meets any of these definitions in any way, shape or form.

Phenylbutazone does not drug a horse in the commonly accepted term in my profession in the English language. Phenylbutazone does not numb a horse.

I am going to speak, I have laid out for you the basic pharmacology of the two medications that are in major dispute in this bill. I am going to speak now to the other aspects of this bill, which is the control of illegal medication.

All racing jurisdictions in the United States and all racing organizations are committed to preventing the use of stimulants, depressants, narcotic analgesics, local anesthetics, and tranquilizers in racing horses.

The stimulant drugs and narcotic analgesics are banned because for other reasons they make a horse unsafe to ride, they are unnaturally stimulating, and they may affect the outcome of a race. Such a horse would be drugged.

Depressants and tranquilizers are banned because they may be used to reduce the performance of a horse and thus affect the outcome of a race. Such a horse would also be drugged.

Local anesthetics can block pain perception and proprioception from a limb. This may cause the horse to misstep and break down. Such a horse would clearly be numbed.

Use of these agents in racing is illegal and is strictly controled by chemical testing of blood and urine samples.

Racing has the longest history of medication control of any human endeavor that I know of.

In 1910, testing for drugs in racing horses was introduced in Europe. It's the most extensive range of drug testing that I know of in any human endeavor.

The scope of testing is wide. Dr. Maylin testified that he tests for approximately 900 to 1,000 drugs. He has these standards in his laboratories and with a possible range—up to 30,000 chemicals using a computer system and a mass spectrometer.

The range of testing is wide. Drug testing depends on the testing of blood and urine samples from racing horses. The best drug coverage is obtained by testing both blood and urine.

If the blood is drawn and tested prerace, this process is called prerace testing. Many medications, and particularly the illegal medications, are not readily detectable in prerace blood samples.

Because of this, prerace blood testing must always be run in conjunction with postrace urine testing for optimal—indeed, Mr. Chairman, for satisfactory drug coverage.

The combination of prerace blood and postrace urine testing offers the best drug coverage and prevents the running of some illegally medicated horses.

I want to emphasize that exactly the same drug coverage is afforded by postrace blood and urine testing. You draw a blood sample immediately after the race, a urine sample within 45 minutes when the horse voids. This is the system that Kentucky uses and it gives us the same drug coverage that prerace testing would.

In Kentucky you could calculate that each positive costs us about $7,000. To install prerace testing would cost us at least $300,000 more, and if we picked up 10 horses per year as prerace positives, each prerace positive would cost us at least $30,000.

The average purse raced for in Kentucky in 1981 was about $4,000.

Prerace testing is clearly technically feasible for some drugs. Its efficacy and particularly its cost effectiveness is debatable.

I want to briefly speak about the Canadian testing system because it directly relates to legislation before this committee. In Canada, drug testing is directly under the control of Agriculture Canada, that is, the Canadian Civil Service.

The contract for testing is "let" to private laboratories. There is one in Vancouver, one in Toronto, and one in Montreal. They test about 80,000 samples per year, predominantly urine samples.

The cost—and this includes the research cost—is about U.S. $40 per sample. They only test blood if urine is not available.

They're putting in a research facility in Ontario which, including startup costs, they reckon will have a budget of about $700,000 a year.

Now, the positive call rate—and I define this as a Kentucky positive call rate for hard drugs—of this system, the Canadian system which is rather close to what this bill would mandate, a Civil Service-controled system, is about 0.6 samples per 1,000 samples tested.

That's in every 2,000 urines approximately, ballpark figures, they find one positive.

This call rate compares very unfavorably with Kentucky's rate of 2.9 per thousand. And if you compare the Canadian call rate with call rates throughout the United States in the table that's in my testimony, you'll find that most American States have "Positive" call rates above that of Canada.

There is no evidence, therefore, that a drug testing system supervised directly by a federal goverment is any more effective than the current situation in the United States.

The cost of testing are relatively much greater in Canada than most U.S. laboratories.

We charge about $20 per sample in Kentucky——

Mr. CONYERS. I apologize, Dr. Tobin, here we go again.

Let's take a short recess and as soon we we get back, you can continue.

Dr. TOBIN. Thank you, Mr. Chairman.

[Recess.]

Mr. CONYERS. The committee will come to order. We apologize once again, Dr. Tobin, for the interruption. I find your testimony very interesting. Please continue.

Dr. TOBIN. Thank you, Mr. Chairman.

The costs of testing are relatively much greater in Canada than in most U.S. laboratories. The cost per sample in Kentucky and its

approximation, I think is actually slightly less—is about $20 per sample.

The Canadian system at that price does not include prerace testing which is mandated in H.R. 1694. The Canadian system does not include quantitative testing of drugs in blood which will be necessary to regulate the next provision of the bill which I will speak to, which is the determination of trace levels.

Costs for a based testing system as mandated by H.R. 1694 would inevitably be much higher—I would estimate two to three times higher—than the current Canadian testing costs.

It would be an extremely expensive bill because the Canadian testing costs do not include prerace testing and they do not include quantitative determinations which would be necessary under the bill before this group.

The effectiveness of the current U.S. testing programs—the attached tables shows an analysis of the positive call rates of 18 U.S.-racing States and Canada.

Of these States, as I review this rapidly, Arizona, Arkansas, Colorado, Rhode Island, Pennsylvania, Nebraska, Maine, Michigan, Kentucky, Massachusetts, New York, South Dakota, Idaho, Delaware, Montana, and West Virginia—that was the great bulk of the States responding to our queries had higher positive call rates than the Canadian system.

California and New York, at 0.4 and 0.8 positive per thousand were somewhat less than—actually New York is slightly more than the comparable call rates but they're clearly comparable.

The only interpretation possible from this table is that drug testing in U.S. jurisdictions compares very favorably with drug testing in the federally run Canadian program.

I want to skip over Mr. Paulus' statement just to say that we have a high positive call rate in Kentucky, we believe our laboratory does a very good job, we have a research program that has published about 80 papers and 2 books—we believe it does a good job and we do not believe that Mr. Paulus' allegations that Kentucky has a mediocre laboratory is correct in any way.

As I pointed out, we have positive call rates much higher than the New York and Canadian with which he directly compared us.

In summary, postrace testing of blood and urine samples offers exactly the same drug coverage as prerace and postrace testing at considerably less cost.

The federally controlled medication control program in Canada is less efficient and more expensive than most or all major U.S. drug testing programs. This bill mandates pre-race testing and quantitation of residue levels. This process will be enormously more costly than the current method and, from the Canadian example, less effective.

The last thing I want to speak to is the problem of establishing trace levels. The bill says that the Administrator may, by regulation, establish permissible trace levels of substances foreign to the natural horse that he determines to be innocuous.

This simple sentence defines the critical decisions that the Administrator will have to make that will send innocent horsemen to jail. He will send innocent horsemen to jail because there does not

currently exist any research base upon which to determine these trace levels.

Even when this research base is developed, the metabolism of the horse will introduce a substantial element of randomness into the drug levels—appearing in the urine or blood.

Because of this, the Administrator will have to coldly decide how many innocent horsemen he is going to jail and he will jail these horsemen because the art of analytical chemistry is not such that analytical chemists can tell when a drug was administered.

For example, phenylbutazone has been detected in horse blood for up to 9 days after the last dose and long after its therapeutic effects have ceased.

The critical question therefore is, What is a trace level and what is a therapeutic amount of each medication?

This is a question the Administrator will have to answer for each medication drug and foreign substance that his chemists find in horse urine.

Criminal prosecutions will hang on the outcome of these decisions. Therefor, the basis for the Administrator's decision must be solid and scientifically defensible.

Further, because each drug is unique, the data will have to be obtained for each drug individually in the horse.

When will the Administrator get this information? The Administrator will have to generate this information himself because the information is not presently available for any equine medication.

To give you an idea of how complex this problem is, I will tell you what the Administrator will have to do for phenylbutazone, the most commonly used equine medication.

Now one way that he can set a trace level is to determine the amount of phenylbutazone in the blood at, let us say, 24 hours after the last dose.

If he takes this approach, however, he will find that his experiments provide him with not just one single level but with a complete range of levels.

It will give him basically a bell curve, a normal distribution. The Administrator will have to determine where in this bell curve he's going to cut off arbitrarily and any horseman who falls outside that level will become a felon, he will be in violation although there will be statistical probability that he will violate that level even though he has strictly complied with the medication rules.

It is inevitable that some horsemen who strictly obey the rules will accidentally go over the trace level and become felons. That's one way that he can do it; he can say, "I'm going to cut the dose off for 24 hours, determine the range of blood levels." He can cut off the bell curve wherever he feels like and say "I'm going to send—penalize the top 2 percent," and that's one way of handling that problem.

On the other hand, the Administrator may choose to determine at what blood level the pharmacological effect of phenylbutazone disappears. He may then use this information to determine what is a trace level of phenylbutazone in the horse.

To do this he will actually have to study the actions of phenylbutazone on lameness in horses. He will have to acquire some natu-

rally lame horses so that he can actually measure the therapeutic effects of phenylbutazone.

This has not been done in horses or, to my knowledge, in any other species. They are technically very challenging experiments.

When he has worked out how he is going to measure lameness, he will then be able to give them phenylbutazone and measure the antilameness effects. He will then have to measure the blood and urine levels of phenylbutazone in these horses so that he can know what is an effective level of phenylbutazone and what a trace level of phenylbutazone is.

This will be a critical decision for the Administrator. He will have to decide on a logical and effective way to approach it and do it. This is a very substantial undertaking. It requires the acquisition of horses, the services of veterinarians expert in biomechanics and analytical chemistry. It requires the development of methods like high-speed cinematography to measure and quantitate lameness and drug effects.

Such studies for phenylbutazone alone would take at least 1 year and likely longer. These experiments will then have to be repeated for each of the many nonsteroidal anti-inflammatory drugs in the horse. There are probably 5 or 6 of those, and certainly 20 or 30 available in this country.

Then the Administrator will have to study the effects of combinations of these drugs so that he will be able to rule in circumstances where more than one of these particular drugs is found in the bloodstream of a horse.

He will have to work with the blood levels of drugs and not with the urinary levels as is now common in racing chemistry. The reason for this is that blood and urinary levels of drugs do not correlate very well.

For example, the urinary level of oxyphenbutazone, which I mentioned earlier, a major metabolite of phenylbutazone, can vary up to 1,000-fold from its blood levels, depending only on whether the urine is acidic or basic. That's the physiology of the horse.

The basic message here is that with urine testing, the physiology of the horse can vary the urine levels so much that it's very difficult to draw any quantitative conclusions whatsoever.

There are 4,000 drugs, Mr. Chairman, in common use in North America today. There are 63,000 chemicals in common use. Many of these will show up in horse urine and each one will have to have a trace level determined for it. This is a huge task and will require the addition of a substantial research unit to the regulatory structure.

The Administrator will not be able to adapt information from other species. This is because each animal species is by and large unique in its handling of drugs.

Doses are different from species to species, the blood levels at which drug responses occur are different. The metabolism and pharmacokinetics are different.

If the Federal Government wishes to get into the business of regulating equine medication, it will have to get into the business of researching medication in a very substantial way indeed.

In summary, Mr. Chairman, there is no evidence whatsoever that the use of phenylbutazone or Lasix leads to the drugging or

numbing of horses. Passage of this bill will ban these valuable therapeutic medications.

Use of these agents is accepted and humane in human sports medicine. They are just as acceptable and humane in veterinary medicine. Passage of this bill will discriminate against the horse.

Testing for illegal medications in the United States is economic and effective, the more so when compared to the federally run Canadian system. Passage of this bill is not likely to improve testing in the United States.

Prerace testing gives exactly the same drug coverage as postrace blood and urine testing. Passage of this bill will give the same coverage at greatly increased costs.

The determination of methodologies and approaches to the problem of trace levels is a major undertaking. Well led, well financed, and productive research terms are in place in a number of States and working on these problems. Development of this research base is not likely to be accelerated by H.R. 1694.

And finally, and the one thing I want to leave you with, if H.R. 1694 is passed by Congress and if it's vigorously enforced, the lack of an effective method of determining and enforcing trace levels, which is a critical part of this bill, may well result in sending innocent horsemen to jail.

Thank you very much, Mr. Chairman.

[The prepared statement follows:]

Testimony Prepared for

The United States Congress

The Committee on the Judiciary

and

The Subcommittee on Criminal Law

Presented by

Thomas Tobin

Kentucky Equine Drug Research Program
Department of Veterinary Science
University of Kentucky

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Mr. Chairman, members of the subcommittee, my name is Thomas Tobin. I graduated in veterinary medicine from University College Dublin in 1964 and completed a PhD in Pharmacology-Toxicology at the University of Toronto in 1970. Thereafter, until 1975, I was a faculty member in the Department of Pharmacology, Michigan State University, East Lansing, Michigan. In 1975, I was invited to set up the Kentucky Equine Drug Research Program and I have been at the University of Kentucky since 1975. Currently, I am Professor of Veterinary Science and a Professor of Toxicology at the University of Kentucky. My research program is an independent research program funded by the Kentucky State Racing and Harness Racing Commissions. Since 1975, this program, in conjunction with my colleague, Dr. Jerry Blake and the Kentucky Equine Drug Testing Program, has published two books and about 85 research papers on the detection, actions, uses, and effects of drugs in racing horses. Both programs have completed considerable research on furosemide or Lasix® in horses and are currently working on research concerning phenylbutazone in horses.

Mr. Chairman, today I will speak to:

1. The Bleeder, and Furosemide or Lasix.
2. Phenylbutazone, the horses' equivalent of aspirin, and its effects in racing horses.
3. The Illegal Medications.
4. Medication Control and The Drug Testing Process.
5. The Canadian federally controlled testing system: A comparison with the United States System.
6. The effectiveness of current United States testing systems.
7. Testimony by Mr. Paulus that Kentucky has "a mediocre drug testing laboratory."
8. The problem of trace levels or residue determinations in racing horses.
9. Summary and Conclusion.

THE BLEEDER

1. If you take a Thoroughbred horse, run him a mile at top speed, you will find that about 1-2% of horses will trickle blood from the nose post-race.
2. This is the classic bleeder - known 300 years or more. Occasionally a horse will die from bleeding, though this is rare.
3. It has long been thought that some horses bled in lungs without blood appearin at the nose. These were called "Occult Bleeders".
4. The Flexible Fiberoptic Endoscope became available in veterinary medicine in the late 1970's. Using this instrument, it was found that:
   (a) 1-2% of horses drip blood from nose
   (b) 40-70% of all horses show signs of blood in the windpipe
   (c) About 10-15% show substantial amounts of blood in the windpipe
   (d) About 10-15% show flecks of blood in the windpipe
   (e) About 20-30% show in-between amounts of blood in the windpipe
5. This work showed that bleeding into lungs and bringing blood up into the windpipe is very common in horses post-race
6. When horses bleed in a race, they may stop and wreck a field of horses. This results in risks to life and limb for horses and jockeys.
7. There has long been a search for a drug which will help bleeders.
8. Current drug of choice of the veterinary profession for bleeders is furosemide. It has been approved for use in racing horses by some Racing Commissions.

FUROSEMIDE - LASIX®

1. A very effective and safe diuretic in both horses and humans.
2. Fifth most commonly prescribed drug in human medicine and there is no reason to think that it is any less safe or effective in the horse.
3. Furosemide is specifically approved by the FDA for use in pulmonary congestion in the horse.
4. Because pulmonary congestion is a likely cause of bleeding in the horse, there is direct medical rationale for the use of furosemide or Lasix® in bleeders.
5. Horses with atrial fibrillation, who suffer pulmonary congestion, have a much higher incidence of bleeding than normal horses.
6. Clinical experience of equine veterinarians and horsemen suggests that furosemide is effective in the treatment of epistaxis or bleeders.
7. Based on this evidence, there is a clearcut pharmacological basis, a clear medical rationale, regulatory approval, and clinical evidence from both university hospital studies and equine practice to support the use of furosemide in the prophylaxis of epistaxis.

EFFECTS OF LASIX® IN HORSES

1. One of the reasons that Lasix® is a very safe drug is that it is a very short acting drug.

2. When a horse is dosed with Lasix® to prevent bleeding, only 250 mg of the drug is given IV into the jugular vein.

3. The formation of urine (the diuretic effect) peaks within 15 minutes and is essentially over within one hour.

4. About 4 liters of extra urine are formed and voided, usually within one hour of drug administration.

5. While this effect looks quite dramatic, one must remember that this is only about 2% of the body water in the horse.

6. Because of this, Lasix® is unable to move or flush more than 2% of any drug out of the body of a horse. This amount is forensically insignificant.

7. Furosemide is therefore a very safe, short acting drug and it does not reduce the level of any drug in the body of a horse.

8. In a nutshell, Lasix® does not flush drugs out of the bloodstream of the horse.

LASIX® AND DRUG DETECTION: THE "MASKING" EFFECT

1. Lasix® is not known to affect the detection of any drug in blood.

2. Lasix® does not reduce the concentration of basic, lipid soluble drugs in urine.

3. Lasix® will reduce the concentration of some water soluble drugs and drug metabolites in equine urine.

4. Because the action of Lasix® is short lived, these dilution effects are over within about three hours, if the dose of furosemide is the dose recommended (250 mg) by the AAEP.

5. By giving Lasix four hours prior to post time, at the anti-epistaxis dose, no masking or drug dilution effects or interference with drug testing is encountered.

6. In studies at the University of Kentucky, it was found that the detection of six narcotics was not affected and that there was some evidence for enhancement of the detection of these drugs four hours after furosemide administration.

7. Preliminary studies by Doctor Maylin in the NASRC Quality Assurance Program have shown that with the recommended dose and time constraints, furosemide does not affect the detection of the following drugs: cocaine, caffeine, lidocaine, apomorphine, phenylbutazone, fentanyl, oxymorphone, clenbuterol, flunixin.

8. Therefore, if the dose of furosemide is 250 mg IV and it is given four hours prior to post-time, furosemide does not "interfere" or mask drug detection and may enhance it.

DOES LASIX® AFFECT THE PERFORMANCE OF HORSES? DOES IT "SPEED" HORSES UP?

1. Studies at the University of Kentucky, the Ohio State University and a study of track times at Louisville Downs Racetrack all show that Lasix does not improve the performance of horses.

2. Therefore, horsemen cannot use Lasix® to "move horses up" or improve their performance. Therefore, Lasix® cannot be used to run horses "hot" and "cold".

3. Horsemen and equine veterinarians, based on their experience, are of the opinion that Lasix® helps horses run "easier" or "easier in their wind" and makes them run more reproducibly and reliable, i.e. true to form.

4. If a horse is a bleeder, he is likely to bleed again. Horsemen who own such horses have a keen desire to be permitted to run them on Lasix®.

IS LASIX TOXIC TO HORSES?

1. Lasix® is a very safe drug, in both humans and horses.

2. Doses used in horses are small and the drug is rapidly excreted.

3. No evidence of toxic effects after single or repeated doses.

4. As used on the racetrack, in single,small, isolated doses, this drug is essentially nontoxic in horses.

SUMMARY

1. Bleeders are a common and very significant problem in racing horses.

2. Bleeders lead to risks to life and limb of horses and jockeys.

3. The treatment of choice is furosemide.

4. Furosemide is safe, effective and approved by FDA for use in horses.

5. Properly used, it does not affect the detection of drugs in blood or urine; does not "mask".

6. Does not "move horses up".

7. There is absolutely no evidence to suggest that this treatment is cruel or inhumane to horses.

8. Lasix® is a rational therapeutic choice for a very common condition in horses. Its use may save the lives of both horses and jockeys. Denial of horsemen the right to use this drug in racing horses is illogical in terms of human endeavor and inhumane to the horses.

"DRUGGING" OF HORSES BY FUROSEMIDE

According to Webster, drugging means (1) to put a harmful drug in (2) to stupefy or poison with or as with a drug; (3) to administer something nauseating to.

Mr. Chairman, members of the subcommittee, nothing whatsoever that I have said about Lasix meets any of the above definitions. To administer Lasix to a horse is in no way, shape or form the "drugging" of a horse.

PHENYLBUTAZONE: ITS EFFECTS IN RACING HORSES

A. PHENYLBUTAZONE: A NON-STEROIDAL ANTI-INFLAMMATORY DRUG.

1. Phenylbutazone is a member of the non-steroidal anti-inflammatory group of drugs. Aspirin is the principal member of this group of drugs that you are familiar with.

2. In general, if you wish to keep phenylbutazone in the horse in perspective, it is useful to think of it as being basically horse aspirin.

3. Phenylbutazone and aspirin have little effect on normal pain perception. It is the hypersensitivity to pain associated with the inflammatory process which responds to phenylbutazone and aspirin.

4. The hypersensitivity to pain associated with inflammation (such as a sunburn) is caused by prostaglandins. Prostaglandin concentrations in tissues increase and the inflamed area becomes hypersensitive.

5. Phenylbutazone and aspirin block prostaglandin biosynthesis, reduce tissue hypersensitivity, and normalize pain perception in the affected area.

6. They leave the area with normal pain perception. They do not numb any area in the body of a horse.

PHENYLBUTAZONE: COMPARISON WITH OTHER DRUGS

1. Phenylbutazone is not thought to be stimulant to horses. It has no more ability to stimulate an unusual performance in a horse than aspirin has in you or I.

2. Phenylbutazone is quite different from local anesthetics. These drugs are injected at specific points or into specific areas and "numb" specific areas. Local anesthetics block both pain and proprioception completely. Proprioception is the horse's awareness of where his leg is and what it is doing. Blockade of proprioception can lead to a misstep and a breakdown.

3. It is important to remember that phenylbutazone has no effect on normal pain perception or normal proprioception and is thus completely different from the local anesthetics.

4. Phenylbutazone is completely different from the narcotic analgesics. These agents act in the central nervous system (brain) to reduce the perception of pain. They act to reduce a horse's pain threshold, and, in addition, they are quite powerfully stimulant in racehorses. Such a horse would be "drugged".

5. Phenylbutazone is therefore clearly distinct from local anesthetics which numb specific areas, and narcotics which suppress pain perception in the brain and stimulate horses.

6. Phenylbutazone has no depressant or tranquilizing effects in horses.

7. The principal pharmacological effect of phenylbutazone is to normalize an inflamed tissue and it has very little effect outside the area of inflammation.

PHENYLBUTAZONE AND BREAKDOWNS

1. Because phenylbutazone reduces the hypersensitivity to pain found in inflamed tissues, it has been argued that horses treated with this drug will run on injured joints and tendons and be more likely to break down.

2. This argument ignores the fact that there is no pain perception in the actual joint surfaces of horses. Phenylbutazone, therefore, cannot affect pain perception directly from joint surfaces, since there is none.

3. The great bulk of the resistance to joint movement is based in the soft tissues around a joint. Soft tissue resistance to joint movement in an inflamed joint is markedly reduced by phenylbutazone. This is the manner in which phenylbutazone restores normal performance.

4. Another important factor is that while protecting a hypersensitive limb, a horse may overload a sound limb. In allowing a horse to run evenly on both limbs, phenylbutazone may, under some circumstances, reduce the incidence of breakdowns.

5. The state of California legalized phenylbutazone at the end of 1970. Between 1970 and 1978, over which period the use of phenylbutazone steadily increased, the number of horses suffering breakdowns which required destruction of the animal remained relatively constant. Other analyses of this data suggest that the incidence of breakdowns actually decreased as the use of phenylbutazone increased.

6. Studies by the Colorado Racing Commission veterinarian, Dr. Gene Bierhaus, showed that over nine years the probability of a horse having an accident which required his destruction was not any greater if the horse was phenylbutazone-treated.

7. Pilot studies at the University of Pennsylvania found that the risk of breakdowns was 1 per 206 horses at risk, a rate of breakdown not higher than the incidence in New York and New Jersey, states which restricted the use of phenylbutazone.

8. These studies, but most particularly the California studies, appear to suggest that with a well run medication program the rate of breakdowns is not increased.

PHENYLBUTAZONE AND MASKING

1. There are no published studies and no reports of experimental studies that show that "masking" by phenylbutazone is a significant forensic problem.

2. The nature of the drug testing process is such that "masking" of stimulants, depressants, narcotic analgesics, tranquilizers and depressants and local anesthetics by phenylbutazone or its metabolites is small.

3. Oxyphenbutazone is the major metabolite in equine urine which causes masking problems. Its concentration in horse urine is controlled by the horse, not by the horseman.

4. Kentucky allows horses to run on phenylbutazone. New York and Canada do not. The positive call rate for illegal drugs in Kentucky is substantially greater than that in New York and Canada. "Masking" is therefore not a problem in Kentucky when compared with the New York experience.

5. I know of no scientific evidence which suggests that "masking" by phenylbutazone significantly interferes with drug detection. I believe that "masking" is not a scientific issue, but a political or, rather, a pseudo issue.

6. Detailed scientific studies on masking by phenylbutazone are now underway at the University of Kentucky.

PHENYLBUTAZONE: ITS EFFECT ON PERFORMANCE

1. Phenylbutazone is not thought of as changing a horse's innate ability to race.

2. By relieving inflammation, it may enable him to race nearer to his maximum capability.

3. It unquestionably will aid the horseman to field racing sound horses. It unquestionably will aid the horse to run a better, more comfortable race.

4. The use of drugs such as phenylbutazone is entirely ethical and legal in human sports medicine. There is no reason, therefore, to think that this type of medication is any less ethical and humane when used in horses.

PHENYLBUTAZONE: A SUMMARY

1. Phenylbutazone is a nonsteroidal anti-inflammatory drug of the same general class as aspirin.

2. Its primary pharmacological action is to normalize inflamed tissues.

3. It has no effects to reduce normal pain perception, or "numb" any tissues in the body.

4. Its use does not appear to be associated with an increased incidence of breakdowns.

5. There is no evidence that it makes testing for illegal drugs less effective.

6. It is thought to enable a horse to perform up to his innate ability and it enables horsemen to field racing sound horses.

7. Use of this type of medication is entirely legal, ethical and humane in human sports medicine. There is absolutely no reason to think that it is otherwise in veterinary medicine.

THE DRUGGING OF HORSES

According to Webster, drugging means (1) to put a harmful drug in, (2) to stupefy or poison with or as with a drug, (3) to administer something nauseating to.

Mr. Chairman, members of the subcommittee, nothing whatsoever that I have said about phenylbutazone meets any of the above definitions. To administer phenylbutazone to a horse is in no way, shape or form the "drugging" or numbing of a horse.

THE ILLEGAL MEDICATIONS

1. All racing jurisdictions in the United States and all racing organizations are committed to preventing the use of:

   (a) Stimulants

   (b) Depressants

   (c) Narcotic analgesics

   (d) Local anesthetics

   (e) Tranquilizers

   in racing horses.

2. Stimulant drugs and narcotic analgesics are banned because, among other reasons, they make a horse unsafe to ride, unnaturally stimulate him, and may affect the outcome of the race. Such a horse would be drugged.

3. Depressants and tranquilizers are banned because they may be used to reduce the performance of a horse and thus affect the outcome of a race. Such a horse would be drugged.

4. Local anesthetics can block pain perception and proprioception from a limb. This may cause the horse to misstep and break down. Such a horse would be numbed.

5. Use of these agents in racing is illegal and strictly controlled by chemical testing of blood and urines.

MEDICATION CONTROL IN HORSE RACING

1. Horse racing has the longest history (since 1910) and the most extensive range of drug testing of any human endeavor. The scope of testing is wide, with 500-1,000 drugs common and a possible range of 10,000 drugs.

2. Drug testing depends on the testing of blood and urine samples from racing horses. Best drug coverage is obtained by testing both blood and urine.

3. If the blood is drawn and tested pre-race, the process is called pre-race testing.

4. Many medications, particularly among the illegal medications, are not readily detectable in pre-race blood testing.

5. Because of this, pre-race blood testing must always be run in conjunction with post-race urine testing for optimal drug coverage.

6. The combination of pre-race blood and post-race urine testing offers the best drug coverage and prevents the running of some illegally medicated horses.

7. Exactly the same drug coverage is afforded by post-race blood and urine testing. This is the system that Kentucky uses.

8. In Kentucky, each positive costs about $7,000. to fund. To install pre-race testing would cost at least $300,000. extra per year. This works out at an estimated cost of about $30,000. per pre-race positive. The average purse raced for in Kentucky in 1981 was $4,186.

9. Pre-race testing is clearly technically feasible for some drugs. Its efficacy and particularly its cost effectiveness is debatable.

THE CANADIAN TESTING SYSTEM: COST EFFECTIVENESS OF A CIVIL SERVICE CONTROLLED NATIONAL TESTING SYSTEM

1. In Canada, drug testing is directly under the control of Agriculture Canada, i.e. the Canadian Civil Service.

2. The contract for the testing is "let" to private laboratories; one in Vancouver, one in Toronto, and one in Montreal.

3. About 80,000 samples/year are tested by these laboratories.

4. The cost is about $40.00 (US) per sample tested for post-race urine testing (blood is only tested if urine is not obtainable).

5. A research facility with a budget (including start-up costs) of about $700,000. a year is being established in Ontario.

6. The positive call rate (Kentucky positive) of this system, very close to what this bill would mandate, is about 0.6 per 1,000 samples tested. This call rate compares unfavorably with Kentucky's 2.9/1,000 rate.

7. There is no evidence, therefore, that a drug testing system supervised directly by a federal government is any more effective than the current situation.

8. The costs of testing are relatively much greater in Canada than in most U.S. laboratories. The cost/sample is $20.00 in Kentucky.

9. The Canadian system does not include pre-race testing, mandated in HR 1694.

10. The Canadian system does not include quantitative testing of drugs in blood which will be necessary to regulate the next provision of the bill that I will speak to, which is the determination of trace levels.

11. Costs for a based testing system mandated by HR 1694 would inevitably be much (2-3 times) higher than the current Canadian testing costs.

EFFECTIVENESS OF THE CURRENT UNITED STATES TESTING PROGRAMS

1. The attached table shows an analysis of the positive call rates of 18 U.S. racing states, and Canada.

2. Of these states:

Arizona
Arkansas
Colorado
Rhode Island
Pennsylvania
Nebraska
Maine
Michigan
Kentucky
Massachusetts
New York
South Dakota
Idaho
Delaware
Montana
West Virginia

all had positive call rates for hard or illegal medications equal or greater than that of Canada.

3. California and New York, at 0.4 and 0.8 positives/thousand, were less than but clearly comparable with Canadian call rate for positives.

ALL HORSES

State	$\bar{x}$ Positive Call Rate	± S.D.	N	Years
Arizona	0.6	±0.4	5	(1978-82)
Arkansas	2.5	±2.7	5	(1977-81)
California	0.4	±0.1	7	(1975-81)
Canada	0.6	±0.2	5	(1978-82)
Colorado	1.3	±0.5	6	(1976-81)
Rhode Island	0.6	±0.7	5	(1974-78)
Pennsylvania	2.2	±0.4	3	(1979-81)
Washington	0.3	±0.3	5	(1977-81)
Nebraska	0.9	±0.7	5	(1978-82)
Maine	1.7	-	8	(1975-82)
Michigan	0.6	±0.3	4	(1977,78,80,82)
Kentucky	2.9	±1.6	7	(1975-81)
Massachusetts	2.2	±1.5	7	(1975-81)
South Dakota	6.0	±6.8	6	(1976-81)
Idaho	2.3	±4.0	3	(1979-81)
Delaware	4.0	±1.6	5	(1978-82)
Montana	1.6	±1.9	6	(1976-81)
New York*	0.8** (0.4)***	±0.3	4	(1978-81)
West Virginia	2.2**	±1.1	6	(1976-81)

*Pre- and post-race bloods and urines
**Includes Kentucky Permitted Positives
***Estimated positive call rate in New York for Kentucky Thoroughbred positives

As part of a study on phenylbutazone in horses by the Kentucky Equine Drug Research Program, a survey of the "positive call" rates in various U.S. racing juridsictions was undertaken in November, 1982. On our request the race horse medication statistics were provided by most of the state racing commissions or the authorized testing laboratories. These statistics included the number of urine samples analyzed, lists of positives called, and the medication rules for each jurisdiction in each year. For each state, each year, the positives were categorized into Kentucky prohibited (stimulents, depressants, narcotics, tranquilizers, local anaesthetics) and Kentucky permitted (NSAI's, diuretics, antibiotics, steroids) drugs. A "positive call rate" of Kentucky prohibited positives per one thousand analyzed urine samples was calculated for each year and a mean over the years reported was generated for each jurisdiction.

4. The only interpretation possible from this table is that drug testing in U.S. jurisdictions compares very favorably with the federally run Canadian program.

TESTIMONY BY MR. PAULUS THAT THE STATE OF KENTUCKY HAS A MEDIOCRE LABORATORY

The following documents are submitted in rebuttal:

1. Letter from Dr. J. W. Blake, Director of the Kentucky Equine Drug Testing program.
2. Letter from Dr. Thomas Tobin, Kentucky Equine Drug Research Program.
3. Table showing that Kentucky has a much higher "positive call" rate than the testing laboratories mentioned in the quote.
4. Attention is drawn to the previous table where Kentucky has one of the highest positive call rates.
5. Publication lists from the Kentucky Equine Drug Testing and Research Programs.
6. Based on this information, we know of no objective data to support this comment by Mr. Paulus.

SUMMARY: CURRENT MEDICATION CONTROL

1. Post-race testing of blood and urine samples offers exactly the same drug coverage as pre-race and post-race testing at considerably less cost.

2. The federally controlled medication control program in Canada is less efficient than most and more expensive than all major United States drug testing programs.

3. This bill mandates pre-race testing and quantitation of residue levels. This process will be enormously more costly than the current method and, from the Canadian example, less effective.

ESTABLISHING TRACE LEVELS

The administrator may, by regulation, establish permissible trace levels of substances foreign to the natural horse that he determines to be innocuous."

1. This simple sentence defines the critical decisions that the administrator will have to make that will send innocent horsemen to jail. The administrator will send innocent horsemen to jail because:

2. There does not currently exist any research base on which to determine these trace levels.

3. Even when this base is developed, the metabolism of the horse will introduce a substantial element of randomness into it.

4. Because of this, the administrator will have to coldly decide how many innocent horsemen he will jail.

5. The administrator will jail innocent horsemen because the state of the art of analytical chemistry is such that analytical chemists cannot tell when a drug was administered.

6. For example, phenylbutazone has been detected in horse blood for up to nine days after the last dose, long after its therapeutic effects have ceased.

A critical question, therefore, is what is a "trace" and what is a therapeutic amount of each medication. This is a question that the administrator will have to answer for each medication, drug, and foreign substance that his chemists find in horse urine. Criminal prosecutions will hang on the outcome of these decisions. Therefore, basis for the administrator's decision must be solid and scientifically defensible. Further, because each drug is unique, the data will have to be obtained for <u>each</u> drug <u>individually</u> in the horse.

Where will the administrator get this information? The administrator will have to generate this information himself because this information is not presently available for any equine medication. To give you an idea of how complex this problem is, let me tell you what the administrator will have

to do the enforce the regulations concerning phenylbutazone, the most commonly used equine medication, and the horse's equivalent of aspirin.

One way for an administrator to set a trace level is to determine the amount of phenylbutazone in the blood of a horse at, let us say, 24 hours after the last dose. If he takes this approach however, he will find that his experiments provide him with not just a single level but a range of levels. In determining the actual "trace" level that he is going to work with, the administrator will have to decide how many innocent horsemen he is going to convict. Only when he has made this decision can he go ahead and set the level. This is because the random variability in both horses and drug testing in horses make it inevitable that some horsemen who strictly obey the rules will accidentally go over the "trace" level and become felons.

On the other hand, the administrator may choose to determine at what blood level the pharmacological effect of phenylbutazone disappears in horses. He may then use this information to determine what is a trace level of phenylbutazone in the horse. To do this, he will have to actually study the actions of phenylbutazone on lameness in horses. First, the administrator will have to acquire some naturally lame horses so that he can actually measure the therapeutic effects of phenylbutazone. This has never been done in horses or, to my knowledge, in any other species. These are technically very challenging experiments. When he has worked out how he is going to measure lameness in horses, he will then be able to give them phenylbutazone and measure its anti-lameness effects. Then he will have to measure the blood and urine levels of phenylbutazone in these horses so that he can know what is an effective level of phenylbutazone and what is a trace level of phenylbutazone.

This is a substantial undertaking. It requires the acquisition of horses, the services of veterinarians expert in biomechanics, and analytical chemists. It requires the development of methods, likely high-speed cinematography, to measure and quantitate lameness and drug effects in horses. Such studies, for phenylbutazone alone, would take at least one year and likely longer. These experiments than would have to be repeated for each of the many non-steroidal anti-inflammatory drugs in the horse. Then the administrator would have to study the effects of combinations of these drugs so that he would be able to rule in circumstances where more than one of a particular type of drug was found in the bloodstream of a horse.

The administrator will have to work with blood levels of drugs and not with urinary levels as is now common in racing chemistry. The reason for this is that blood and urinary levels of drugs do not correlate very well. For example, urinary levels of oxyphenbutazone, a major metabolite of phenylbutazone in horse urine, can vary up to about 1,000-fold from its blood levels, depending only on whether the urine is acidic or basic. Because of this poor relationship between blood and urinary levels of drugs, one cannot use trace levels of drugs in urine for regulatory purposes. Traditional methods of regulating phenylbutazone use, by quantitating phenylbutazone and its metabolites in urine, are far too variable to serve a valid regulatory function.

There are about 4,000 drugs and 63,000 chemicals in common use in North America. Many of these will show up in horse urine and each one will have to be tested and a "trace" level established. This is a huge task and will require the addition of a substantial research unit to the regulatory structure.

It will not be possible for the administrator to simply adapt information from other species to the horse. This is because each species is, by and large, unique in its handling of drugs. Doses are different from species to species, the blood levels at which drug responses occur are different, and the metabolism and pharmacokinetics of drugs are different in the horse. If the Federal government wishes to get into the business of regulating equine medication, it will have to get into the business of researching medication in horses in a very substantial way.

SUMMARY

1. There is no evidence whatsoever that the use of phenylbutazone or Lasix leads to the "drugging" or "numbing" of horses. Passage of this bill will ban these valuable therapeutic medications.

2. Use of these agents is accepted and humane in human sports medicine. They are just as acceptable and humane in veterinary medicine. Passage of this bill will discriminate against horses.

3. Testing for illegal medications in the United States is economic and effective, the more so when compared with the federally run Canadian system. Passage of this bill is not likely to improve testing in the United States.

4. Pre-race testing gives exactly the same drug coverage as post-race blood and urine testing. Passage of this bill will give the same coverage at greatly increased costs.

5. Determination of methodologies and approaches to the problem of trace levels is a major undertaking. Well led, well financed and productive research teams are in place in many states and working on these problems. Development of this research base is unlikely to be accelerated by HR 1694.

6. If HR 1694 is passed by Congress and vigorously enforced, the lack of an effective method of determining and enforcing trace levels will result in the sending of innocent horsemen to jail.

Mr. CONYERS. You're not a lawyer, you're a doctor, right?

Dr. TOBIN. That's correct.

Mr. CONYERS. How do you know who's going to jail under the bill? Can you show me something that leads you to suggest that that might happen? Innocent persons.

Dr. TOBIN. Absolutely, Mr. Chairman. The section on drugging states—do we have a copy of the bill with us?

It's all embracing. It says quite clearly that the detection of anything in the horse will be an offense, unless the administrator determines that it is not.

That is the area of my expertise, Mr. Chairman, the determination of trace levels of metabolites in horses. And I can tell you that the exactitude of the science is not such that I would not wish to be at risk under this bill.

Mr. CONYERS. Well, you're a doctor but not a lawyer.

Dr. TOBIN. Mr. Chairman——

Mr. CONYERS. And your telling me who's going to jail is not a medical conclusion, it's a legal conclusion. Don't you think that's fair?

Dr. TOBIN. I'm not telling you specifically who's going to jail. I can tell you——

Mr. CONYERS. But you did, that's exactly what my problem is, not only who's going to jail but that innocent horsemen are going to jail. Now that's enough to scare the pants off of even people in Detroit that like horses.

Dr. TOBIN. Well, Mr. Chairman, if I've succeeded in doing that, I've succeeded in catching the attention of this committee, and that is what I set out to do.

The determination of trace levels is an area of substantial dispute. How to approach this problem is technically not clear cut, the variability in the actual measurement of the detection of drugs, the simple measurement of drugs in racehorse blood and urine is substantial. The amount of variability that the horse, the horse himself, the metabolism of the horse, can put into this system will completely negate the efforts of the most careful horseman.

Because of this, it is my opinion that it would be inevitable that if you have substantial penalties and it's vigorously enforced, that you will have innocent people going to jail.

I do not believe that the regulatory system, the scientific methodology for the setting up of these trace levels is perfect.

Mr. CONYERS. Well, have you talked to the lawyer——

Dr. TOBIN. It is, indeed, far from it.

Mr. CONYERS [continuing]. And the Congressman at the witness table?

Dr. TOBIN. I have given you my expert opinion as a pharmacologist and a veterinarian and a person experienced in the detection of drugs in horses on the drafting of this bill. I am speaking as a scientist.

If I look beyond that, I come to that conclusion. I now defer to the attorney——

Mr. CONYERS. I didn't ask you to do all that. I just asked you to answer the question.

Dr. TOBIN. If you could restate the question, I will do my best.

Mr. CONYERS. Have you talked to the lawyer and Congressman at the witness table—yes or no?

Dr. TOBIN. Yes, I have.

Mr. CONYERS. All right. Now if he were telling me about the medical conclusions involved in this bill, I would be in a state of slight shock.

Here is an experienced lawyer, someone probably acquainted with the horsing industry and sport, a Member of Congress, may have been a judge in addition to other things, and then I get from the medical expert what is going to happen to innocent horsemen drug into the Federal court.

Now that, sir, is beyond your range of expertise, with all due respect.

Now, Caldwell Butler is going to tell me about innocent horsemen getting pulled into the Federal court and sent to the slammer. I can take it. But I am not quite sure that there is anything in your experience or background, other than your opinion, which has certainly been spread on the record—if you haven't said that at least six times during the short time that I was here, that innocent horsemen are going to jail—I don't know how many other times you said it, but that is not a medical conclusion.

And I just want to make the record clear that I appreciate that fact.

Now, I would like to defer to Congressman Butler to tell me about all the innocent people that will be prosecuted unfairly because that is what he is here for.

Mr. BUTLER. No; that is not why I am here, Mr. Chairman, but I am here to assist in the preparation of the testimony and make sure the record is complete in this regard.

And I think you have to recognize from the testimony of Dr. Tobin that when we get to determining what a trace level is that it is an inexact science and that if the Administrator has got to determine what trace levels determine guilt or innocence, then we create a problem of enforcement.

Of course, if you are convicted under the legislation, then you are not an innocent person, and I guess we have to agree with that. The people who would not expect to be—who in all innocence were using these medications for therapeutic purposes could wind up in violation of this law, and that disturbs me.

I think we have to defer to the scientific basis for Dr. Tobin, and it is true that his opinion has been spread on the record extensively today, but I think that is the standing he has in his profession.

I sincerely believe that when we agreed to try to put this testimony together we undertook to do what we could to make the record balanced, and it was the most appropriate thing in the world to bring him here to testify because he has got a standing like no other person, I think, in that profession.

Mr. CONYERS. Well, I quite agree with you about him spreading his opinion fully on the record. That is also a policy of this chairman——

Mr. BUTLER. Yes, and we appreciate——

Mr. CONYERS [continuing]. To allow the widest latitude in witnesses presenting their testimony, their views, and their opinion, and your legal conclusions are equally welcome, too.

There is nothing that says that you have to be a lawyer or a Congressman to appreciate what can happen to a person in court.

It does seem to me, though, that this matter would become a question for the jury or the judge, and for me to assume in every jurisdiction they would be anxiously waiting to violate the difficult medical questions that you so eloquently described here and that they would be anxious to send innocent horsemen to the slammer, that is something that would weigh heavily on my conscience for the rest of my life if this were ever to happen.

We have a number of people that are already behind bars in America that claim that there was some failure of the process. So this would be the thing that I would severely dread, and perhaps that is why I am starting out our discussion on this very sensitive point.

Mr. BUTLER. Mr. Chairman, three witnesses have prepared their testimony. I recognize now that we want to get into questions next. So rather than complete my testimony, I would just ask if you would allow me to file my statement in full and make that a part of the record.

Mr. CONYERS. I am sorry, Caldwell. I thought that you had gone first and that this was——

Mr. BUTLER. I had gone first. I was sandwiching them in, but I have some 20 pages of additional testimony which—you have been patient with us.

Mr. CONYERS. Well, maybe after my questioning you may want to come back before the committee or work with us in whatever way you choose.

Mr. BUTLER. All right, for the present, could all of our prepared statements be filed and made a part of the record?

Mr. CONYERS. Exactly, without objection.

Mr. BUTLER. There is one other issue that came up during the course of the questioning by counsel with reference—that we probably ought to clarify, and that has to do with the activities of the Horseman's Benevolent and Protective Association with reference to the guidelines, and so I would simply say to you that we have the statement of Mr. Flint, who is not here today, who is directed to that point, and we would like that also to be filed and made a part of the record.

[The prepared statement of Mr. Flint follows:]

STATEMENT OF EDWARD FLINT, NATIONAL PRESIDENT, HORSEMEN'S BENEVOLENT & PROTECTIVE ASSOCIATION

For the past several years, the therapeutic medication issue has been the subject of much debate within the sport of racing. The Horsemen's Benevolent & Protective Association (HBPA) has been participating in this debate in an effort to come to a consensus with regard to reasonable regulation.

This issue is therapeutic medication. It is not stimulants, not depressants, not narcotics, and the HBPA has been and remains unalterably opposed to the use of such drugs.

The HBPA does not advocate the use of medication on raceday, other than for therapeutic medications prescribed by a licensed, practicing veterinarian. The HBPA is also convinced that the use of these medications is not harmful or inhumane to the horse or harmful to the betting public. We support a thorough pre-race veterinary examination of all horses entered in a race. We do not believe that the best interests of racing are served if unsound horses are permitted to race.

Change is a slow and painful process. The HBPA and the National Association of State Racing Commissioners (NASRC) have been hesitant to back away from previously stated positions. But the HBPA position on medication has substantially softened.

In January, 1982, the HBPA Board of Directors adopted a proposed medication rule—a rule which it believes can lead to agreement within racing. Already, the rule has formed the basis for extensive industry discussion. This proposed rule is similar to the current guidelines of the NASRC and to the existing rules in many racing jurisdictions. The HBPA has been working closely with the NASRC in an effort to resolve the differences which, in HBPA's opinion, are not significant.

Since appearing before this Subcommittee in September, 1982, HBPA has made even greater progress. The HBPA is working with other segments of the industry, and I am hopeful that the industry will reach an agreement by the end of this year.

One area in which the HBPA and NASRC are in basic agreement is in their support of a quality assurance program for racing laboratories. Any rule that cannot be enforced is unfair. Therefore, the HBPA has urged racing commissions from all States to adopt a quality assurance program such as the one drafted by HBPA. This proposal is more strict than that of NASRC.

The HBPA is prepared to continue its financial support, with the States, the Thoroughbred Racing Association (TRA), and other interested groups of the research programs which will answer many of the unresolved questions relating to this issue. In fact, the HBPA previously has contributed many thousands of dollars for such research at such respected institutions as the University of Pennsylvania's New Bolton Center and the Veterinary College at the University of Kentucky.

The HBPA has proposed and helped finance studies which will determine whether there are "masking" or "interference" capabilities in both furosemide and phenylbutazone. When definitions and protocols are agreed upon, the HBPA will help finance research to determine the pharmacological effects of these drugs. NASRC

representatives have already given a positive response to this proposal. Based on this and also on othe research projects which the HBPA is sponsoring, we believe that the controversy of the past few years will end shortly. The HBPA believes that the questions raised concerning the use of these therapeutic medications should be answered scientifically.

All of these actions are part of the internal industry efforts that are, indeed, leading to a consensus, which I feel is close at hand.

Mr. BUTLER. As you know, there is not total agreement with reference to this. The HBPA and the National Association of Commissioners have not gotten together entirely, but they are approaching a consensus, and certainly the HBPA has moved a great deal toward arriving at this agreement.

Now I am here as a lawyer and not as a medical man or a veterinarian, so if you want to know the specifics of how close they are together, then I think Dr. Tobin could tell you about that.

Mr. CONYERS. We have here, Dr. Tobin, some quotations from some medical journals that have guided us in the past, and I would like to solicit your views about them.

One witness stated:

The logical treatment for horses suffering from severe pulmonary hemorrhage is rest from racing, and Dr. Cook emphasizes in his book the breaking of blood vessels can be prevented if the stress which produces the hemorrhage is removed—if the horse is taken out of the training. Trainers are often reluctant to follow this advice, but a method of emphasizing the need for at least some rest from racing is to ask the trainer what advice he or she would give an athlete's son who coughed up blood from the lungs after a 200-meter hurdle race.

"Unfortunately, just as trainers"—this is the witness now going on, and I have provided you with a copy of this testimony

Unfortunately, just as trainers attempt to keep the musculoskeletal cripples racing by the use of butazolidine, they also strive to keep their pulmonary cripples racing through the use of lasix—practices not only cruel, but also appear to be based on misconceptions and untenable economics.

The number of horses observed bleeding on the racetracks in California actually increased by 20 percent after lasix was permitted by the California Horseracing Board.

With the above statistics, lasix could hardly be considered a drug effective in the prevention of pulmonary hemorrhage nor a drug necessary for the economic survival of horseracing.

While the U.S. Food and Drug Administration has approved lasix as a diuretic, it has never approved lasix for purpose of treating horses hemorrhaging from the lung. Furthermore, there has been no evidence to date that proves that lasix significantly prevents the occurrence of pulmonary hemorrhage, nor is there one iota of evidence to substantiate that lasix cures pathological condition in the lungs which causes this hemorrhaging.

In fact, according to the Arizona Racing Commission, it reported that the number of horses which have suffered bleeding during a race has been reduced substantially since lasix was banned in Arizona in April 1981.

Are there any statements there that strike your attention that would warrant your comment?

Dr. TOBIN. Well, my first question would be, can you give me the date of the Cook reference, Dr. Cook's quotation? Can you give me the date when that was made?

Mr. CONYERS. I have no way. I have never seen the book—Equine Veterinary Journal.

Dr. TOBIN. Yes. That is a periodical that is published in Britain, and it is an equine journal.

I do not recall the date on that, but if my recollection is correct that is at least—I can't give you the date, but I am quite sure that

it antedates the development of the fibryoptic endoscope and the realization that pulmonary hemorrhage is a much more common event or occurrence in the racing horse than it was when Dr. Cook made that statement.

I do not know whether he would be comfortable with that statement today. I do know that—my best opinion on that is that it is quite a dated statement.

Mr. CONYERS. I see.

Dr. TOBIN. If you would like me to continue, I would like to just, first of all, point out that studies at the University of Kentucky and the Ohio State University has shown that lasix has no effect on the racing performance of horses.

Now, and I believe this is Mr. Baker's comment—if his basic hypothesis is correct that furosemide will keep a pulmonary cripple running—and that is his statement there—one would expect to see some improvement in performance.

That is not so. In controlled studies at the University of Kentucky, at the Ohio State University, and in a retrospective study of horses actually running on the track, there was no evidence whatsoever of an improvement in performance.

If it is indeed a miracle drug, if it is indeed able to do what Mr. Baker says it does, keep pulmonary cripples running, this is not the result that one would expect. That is a clearcut indication from scientific information that his hypothesis that he has thrown out with no basis whatsoever is probably incorrect. It is certainly not supported by the data.

Mr. CONYERS. Well, he certainly didn't throw it out. He started off quoting what you claim to be now an outdated medical statement, although you apparently concede that it was accurate at the time it was made. I assuming that you are correct——

Dr. TOBIN. No; I can see that the clinical observations on which he based it—the perception of the clinical events has since changed. He might wish to change his statement based on our newer knowledge about furosemide since he made that.

Mr. CONYERS. Well, let's not worry about him so much. What about the number of horses observed bleeding at the racetracks in California increased by 20 percent after lasix?

Dr. TOBIN. Mr. Conyers, I think that clearly shows—excuse me, Chairman Conyers—that clearly shows that horsemen in California are rational men, and when they are permitted to use a therapeutic medication when it is approved they will come forward with their bleeders and say I have a bleeder. When they are not allowed a medication, there is no advantage to them to say they have a bleeder.

That simply shows that California horsemen are rational and if they are permitted to use a medication, a therapeutic medication for a certain condition, they will come forward with their horses.

In the absence of permission to do that——

Mr. CONYERS. In other words, they are to be commended for their conduct?

Dr. TOBIN. I believe that if you can commend any man for rational behavior, I think one should. This is rational behavior.

Mr. CONYERS. All right.

Well, then Cook's statement is outdated in the journal. So we will set that aside.

I just want to read this again to make sure, because I had looked upon this as critical commentary, and now I find out I have been misunderstanding it.

The witness said:

> Unfortunately, just as trainers attempt to keep their cripples racing by the use of butazolidine, they also strive to keep their pulmonary cripples racing through the use of lasix. This practice is not only cruel, but appears to be based on clinical misconception and untenable economics.
>
> The number of horses observed bleeding on the racetracks in California actually increased by 20 percent after lasix was permitted by the California Horseracing Board.
>
> From the above statistics, lasix could hardly be considered a drug effective in the prevention of pulmonary hemorrhage nor a drug necessary for the economic survival of horseracing.

OK. We commend them for that, their conduct, and find nothing wrong with it because they are perfectly rational men.

Let me go to the U.S. Food and Drug approval of lasix as a diuretic but not for the purpose of treating horses for hemorrhaging lungs, and you have the statement in front of you.

Dr. TOBIN. Which one, Mr. Chairman?

Mr. CONYERS. Page 39.

Dr. TOBIN. Which line?

Mr. CONYERS. Third paragraph.

Dr. TOBIN. I am sorry, if you could read it, it would help me find it.

Mr. CONYERS [reading]:

> While the U.S. Food and Drug Administration has approved lasix as a dieretic it has never approved it for the purpose of treating horses hemorrhaging.
>
> Furthermore, there has been no evidence to date that proves that lasix significantly prevents the occurrence of pulmonary hemorrhage, nor is there one iota of evidence to substantiate that lasix cures pathological condition in the lungs which causes this hemorrhaging.
>
> In fact, according to the Arizona Racing Commission, it reported that the number of horses that have suffered bleeding during a race has been substantially reduced since lasix was banned.

So I would like you to comment about the Drug Administration's approval, the findings at Arizona if you have any impressions about that at all.

Dr. TOBIN. Well, my impressions about Arizona would be the converse of the California situation. The drug was banned. It was eliminated. So people who had bleeders took them out of the jurisdiction in Arizona and brought them elsewhere.

Again, it is a natural and logical and rational result. If you ban a medication, people who have horses who need that therapeutic medication will take them elsewhere.

Mr. CONYERS. Well, maybe they won't run them.

Dr. TOBIN. That is also a possibility, Mr. Chairman.

Mr. CONYERS. Well, I am glad I thought of that.

I mean, it is really kind of silly to present me with sort of ultimate factual—

Dr. TOBIN. I didn't present you with these statistics, Mr. Chairman. Somebody else did.

Mr. CONYERS. Yes, but it was your thought that they would be run somewhere else rather than not run at all. I guess—or maybe that is based on your experience.

Dr. TOBIN. I said they would be taken—I do not recall my precise statement. It possibly is run or taken somewhere else. They will move from that racing jurisdiction.

Mr. CONYERS. And to another?

Dr. TOBIN. It depends on the choice of the horseman.

Mr. CONYERS. I see.

Well, let me read you something else and solicit your view.

According to Dr. Arthur Patterson, Equine Veterinary Officer for the U.S. Food and Drug Administration,

> There is no doubt some trainers are using lasix to mask other illicit drugs that may be administered before a race. Whether lasix actually prevents bleeders is iffy. The real interest is in flushing and masking illegal drugs.

Your comment, if any.

Dr. TOBIN. Well, again, if you look at the date on that particular statement, I think it dates—actually, it is here in, unless I am much mistaken, Mr. Baker's book. It dates from 1978, OK.

I started my research program at the University of Kentucky in 1975. About that time I started to publish my research results. Unless I am much mistaken again, I don't believe you were in the room when I testified about the pharmacological actions of furosemide.

I testified that the actions of furosemide were very brief. When it is given in the dose and by the recommended route by the American Association of Equine Practitioners, the amount of urine produced is small. It is about four liters. It is a relatively small proportion of the body water of the horse. It is about 1 to 2 percent.

That is all the drug that furosemide can move out of the body of the horse. It cannot significantly flush drugs out of a horse.

I have been making this statement repeatedly, and in the television program that we had here earlier today, that misstatement was made again. Furosemide does not significantly reduce the blood level of any drug test to date, and we have no reason to believe that it would.

To the best of my knowledge, that statement is in error, and we have substantial scientific evidence in the record before you to say that that is so.

With respect to the concentrations of drugs in urine, if the dose is the dose used in the treatment of epistaxis, if it is given by the correct route, research has shown at the University of Kentucky, and research which has been repeated in the quality assurance program has shown that at four hours, at more than 4 hours after administration of the drug there is no significant masking or drug dilution effect.

Work in my laboratory has shown that in fact the detection of some narcotic analgesics is enhanced if furosemide is administered under these conditions.

Does that answer your question?

Mr. CONYERS. Well, has anyone ever challenged this statement?

Dr. TOBIN. It has been challenged. I would be very happy to present you—counsel has a copy of my book. You

lenged in large black type in the copy of my book, which is to your left.

Mr. CONYERS. Well, I am talking—I realize you are challenging it, and I would assume you have written it, but the point is this, sir. If this has been a mistake since 1978, we would welcome the evidence to point out that it is wrong.

Dr. TOBIN. The scientific evidence is in the binder that has been presented to the committee this morning.

Mr. CONYERS. And the scientific evidence is compiled by whom, since I haven't had an opportunity to read it?

Dr. TOBIN. The bulk of the work that is published in the scientific literature has come out of my laboratory. I understand that Dr. Maylin testified for this committee at similar circumstances, and in his studies he also found no effects of furosemide under the conditions that I mentioned.

I believe if you check the record you will find that in it.

Mr. CONYERS. Well, the point that I am working toward is this. Here is an official of the U.S. Food and Drug Administration making a statement that apparently is widely refuted, or at least by you and one other person, and it has been there since 1978 according to yourself, and I am just trying to piece together the logic how a statement that would have this large of an impact on an industry would not be brought more prominently into dispute than me holding hearings to listen to the different medical testimony.

Dr. TOBIN. You are in a much better position than I, Mr. Chairman. I am a professor at the University of Kentucky. I publish my science, and that is basically what I do.

I put it in the book that I wrote to review this field. The fact that I guess I had to come here in person to testify as to my findings shows that the process is functioning.

Mr. CONYERS. Well, my impression is that you do a lot of testifying. Maybe that is incorrect.

Dr. TOBIN. It is a matter of opinion, Mr. Chairman.

Mr. CONYERS. Well, let me get into a few more statements here that we need to examine. Here is another textbook quotation, Equine Medicine and Surgery.

> The horse should not be trained while being given an anti-inflammatory drug because it will not protect the leg as it would if pain were experienced normally.

In another textbook in which butazolidine is referred to:

> In many cases it is used to alleviate symptoms of lameness without allowing sufficient rest for the healing of the part. In this case additional damage is done to the joint while the horse goes on with the racing workout. This eventually leads to a complete degeneration of the joint.

Now, it seems to me that in all these cases plus the examples I have quoted before, we are talking about the improper use, and you keep talking about the approved and the appropriate amount of use of the drug, and I think we may be missing each other in that respect.

Could that be part of the problem?

We are talking about abuse of the drug, which is what gives rise to the Federal Government's concern about this. It is not, as you stated, that we would ban the use of the drug entirely, which is not the point of this legislation.

Dr. TOBIN. Mr. Chairman, perhaps I can accelerate the process. Unless I am much mistaken, that edition of Equine Medicine and Surgery was the second edition, which was—these comments have been spoken to in the testimony, and I will just read from the testimony.

Mr. CONYERS. Please don't. Just tell me if you disagree with them or if you agree with them.

Dr. TOBIN. I disagree with Mr. Baker, and I was going to draw your attention to the current edition, a quote from the current edition of Equine Medicine and Surgery, which says about nonsteroidal anti-inflammatory drugs:

> These products may also be used prophylactically since they do not impair healing or immunity. They are useful to control postsurgical pain and swelling and may prevent the soreness and injury associated with training.
>
> Phenylbutazone administered 23 hours before time trials has improved performance, perhaps due to the relief of subclinical lameness.
>
> In a controlled study, Naproxin, which is closely related to phenylbutazone and a similar drug, prevented training complications. Intermittent, two times to three times weekly, administration of similar products resulted in apparent clinical benefit.

In essence, the author—and this is the current edition, the most recent edition of Equine Medicine and Surgery, states that these agents prevent soreness and injury associated with training or, no doubt, Mr. Chairman, with racing, because racing and training are essentially similar.

Another work, Dr. Doyne Hamm, has shown that when young quarterhorses in training and racing were treated with Naproxin, another nonsteroidal anti-inflammatory drug, there were less interruptions of training and racing in the nonsteroidal anti-inflammatory treated group.

For example, during the training season, the untreated group lost about 13 percent of their training time due to musculoskeletal disorders while the Naproxin-treated horses lost only about 3 percent.

That is a substantial difference. This study, in essence, showed that the use of this medication in training and racing horses enabled them to train and race more effectively.

That study—it is cited here from—I would say it is a 1978 to 1979 study, considerably more recent than—again, I am guessing the quotation that this refers to, since it is not cited here, which I think is 1972 to 1973.

Mr. CONYERS. So you don't agree with what O.R. Adams said about butazolidine that we quote here in his book "Lameness in Racehorses?"

Dr. TOBIN. Mr. Chairman, he did not specifically name butazolidine. He mentioned anti-inflammatory drugs in general.

I have not read that quote. I do not know whether he refers to steroidal or nonsteroidal anti-inflammatory drugs.

Mr. CONYERS. Well, it says in the witness testimony that it was in reference to butazolidine.

Dr. TOBIN. Again, I would have to accept the witness with that. And all I can say is the Adams book—do we have a date on the Adams' book—the bulk of the work that Mr. Baker cites, the books were published in the early 1970's. They relate, then, to work completed in the late 1960's.

Our perceptions today, with a decade of experience with these drugs, is somewhat different.

Mr. CONYERS. What about Merck Veterinary Manual?

Dr. TOBIN. Merck Veterinary Manual is compiled by veterinarians working in the Merck—I assume in the Merck Drug Co.—by reviewing other works. They have no direct experience, and they are not, to the best of my knowledge, practicing veterinarians.

It is a compilation.

Mr. CONYERS. So you don't bother to agree or disagree with them?

Dr. TOBIN. With the Merck Manual, I—it is a——

Mr. CONYERS. Well, let me just make the statement without reference to the manual itself.

"Anti-inflammatory treatment combined with continued training and racing will accelerate the degenerative process within the knee of horses."

Dr. TOBIN. It depends on the type of anti-inflammatory drug.

Mr. CONYERS. I see.

Dr. TOBIN. And as near as we can tell, with the nonsteroidal anti-inflammatory drugs this is not a problem at the moment.

I quoted the Hamm study which showed that in actual fact the training careers of the horses was improved, and the racing careers.

Mr. CONYERS. What about the Journal of American Veterinary Medical Association? They sound pretty reputable.

Dr. TOBIN. Can you give me a date on that study?

Mr. CONYERS. No.

Dr. TOBIN. If it is—can you give me the quote?

Mr. CONYERS. Yes. You have it in front of you on page 37.

The quote is:

Because of the ability of phenylbutazone to reduce inflammation and alleviate pain, thoroughbreds otherwise unable to compete remain in training and race successfully. This has led to the indiscriminate use of the drug at many racetracks.

Dr. TOBIN. That is dated February 15, 1970, Mr. Chairman. It is in the introduction of the paper.

When a person introduces a scientific paper, you feel free to pull hypotheses or statements out of the literature, out of the air that you are setting up for a study. It is a statement in the introduction to the study. It is a hypothetical statement by these people as to a possibility.

What the conclusions from that study were I do not know.

Mr. CONYERS. Do you agree with the hypothetical conclusion? Do you admit to its possibility of being correct?

Dr. TOBIN. The ability of phenylbutazone to reduce inflammation and alleviate pain within the constraints that I told you—the hypersenstivity pain is correct.

"Thoroughbreds otherwise unable to complete training have remained in training"—I have no scientific information whatsoever on that. I can't give you an opinion one way or the other.

"Indiscriminate use of drugs at the racetrack"—I cannot comment on that either.

Mr. CONYERS. Well, that is precisely why we have the bill before us. Doctor. We are not talking about the trainers and the managers

and owners that use it in discriminate amounts. There would be no point in us even coming here.

We are talking about the abuse, and that is why we have put together some very modest proposals for their control and also nationalizing them so that we won't have these incredibly different standards that frequently exist from track to track.

Well, tell me a little bit about your activities. You say you don't testify much, and I was beginning to think that you do quite a bit of this.

Dr. TOBIN. With respect to?

Mr. CONYERS. Well, why don't you tell me? I mean, I would like to now begin an inquiry into your background connection and activities with the horseracing industry.

Dr. TOBIN. I am a professor at the University of Kentucky.

Mr. CONYERS. What else?

Dr. TOBIN. I run a research program on drugs and racehorses.

Mr. CONYERS. What else?

Dr. TOBIN. Those are my primary avocations. I write and consult on drugs and racing horses.

Mr. CONYERS. Do you have a research fund—a research program funded by Kentucky State Racing and Harness Racing Commission?

Dr. TOBIN. Yes—I do not have that. I work for the University of Kentucky, and I work in that program.

Mr. CONYERS. Well, that is another one of your activities?

Dr. TOBIN. That was my major activity.

Mr. CONYERS. That is your major activity?

Dr. TOBIN. Yes.

Mr. CONYERS. Do you teach?

Dr. TOBIN. Yes, I do.

Mr. CONYERS. All right. Tell me about the courses.

Dr. TOBIN. I teach in—basically, I teach in toxicology, which is——

Mr. CONYERS. And which ones are you teaching presently?

Dr. TOBIN. I am not teaching presently.

Mr. CONYERS. Not teaching presently.

What about some other research projects that you have worked with connected with the racing industry?

Dr. TOBIN. I have had money from the United States Harness—the Harness Tracks Association to work on reserpine. I have had money from the Horseman's Benevolent Protective Association for specific research on furosemide. I have had money from the U.S. Department of Agriculture to study endotoxin shock in horses.

Those would be the major moneys that have come into me for research.

Mr. CONYERS. I have got 12 that constitute a million dollars—in excess of a million dollar's worth of money coming in.

Dr. TOBIN. That is correct.

Mr. CONYERS. Is that correct?

Dr. TOBIN. That is correct.

Mr. CONYERS. Well, you left out a few then? But I can understand, you probably wouldn't remember them all.

Dr. TOBIN. You have them——

Mr. CONYERS. Let me just see if this is correct: College of Veterinary Medicine, MSU; Michigan Heart Association; the MUCIA grant—I am not aware of what that is.

Dr. TOBIN. That was a grant to assist Indonesian students, or to educate Indonesian students.

Mr. CONYERS. What about the Kentucky Equine Research Foundation grant?

Dr. TOBIN. That would be my—I believe—can you give the quick figure on that?

Mr. CONYERS. Yes, $900,000.

Dr. TOBIN. That would be my support from the racing commissions. That would be a cumulative sum over the years of support from the racing commissions.

Mr. CONYERS. From 1973 to 1981?

Dr. TOBIN. 1973 would be a misprint.

Mr. CONYERS. I see. What is correct?

Dr. TOBIN. 1975.

Mr. CONYERS. I would like to find out a little bit more about these. I probably won't be able to do it today.

What about the U.S. Trotting Association Detection of Reserpine Administration in Horses? Do you remember that grant?

Dr. TOBIN. Absolutely. It resulted in the publication of two scientific papers.

Mr. CONYERS. Are you under only one grant at the moment or more than one?

Dr. TOBIN. At the moment I have the money from the racing commission, and I have a specific grant from the equine drug research council to research masking by phenylbutazone in racing horses.

Mr. CONYERS. What other States have you operated in?

Dr. TOBIN. Have I taught in or—I have been a professor at Michigan State University. Prior to that I was a doctoral candidate research associate at the University of Toronto.

Mr. CONYERS. Where else?

Dr. TOBIN. Prior to that I was a veterinary student in Ireland.

Mr. CONYERS. I have got a letter that bears some explanation, if you would be willing. It is from the former Secretary of the Department of Health and Human Services, formerly Richard Schweiker.

It is dated March 13, 1981, and it is with reference to yourself in connection with the post of Director of the Bureau of Veterinary Medicine for the Food and Drug Administration, in which he wishes to bring to your attention—this is a letter—I am sorry, I should have said from the Secretary; it is to him from the chairman of the West Virginia Racing Commission.

We wish to bring to your attention Dr. Tobin's role in obstructing the efforts of the West Virginia Racing Commission in controlling the use of drugs in thoroughbred racing in this state, which has reached scandalous proportions.

In 1980, this Commission followed the lead of our sister state Pennsylvania and the national trend and banned the use of drugs in thoroughbreds being raced. Before our rule could go into effect, however, under the West Virginia Administrative Procedures Act approval of the Legislative Rulemaking Committee was required.

At the hearing Dr. Tobin appeared as the paid representative of the Horseman Drug Lobby, and by the skilled use of innuendo and half-truths was able to prevent the implementation of our rule.

We are still battling in the West Virginia Legislature to control the serious problem of drug abuse, as Dr. Tobin's influence is still being felt there.

It is my understanding that he has played a similar role in Kentucky.

The failure of state racing commissions to deal with this problem is giving much momentum to the movement to enact federal legislation in this area. Dr. Tobin and others like him will pave the way for this legislation.

We would hope that you would not see fit to make this appointment of Dr. Tobin. It would be most unfortunate for one who has shown such little concern for a serious problem with drug abuse in horses to be placed in control of all drugs used in animals.

From the then-chairman of the racing commission in West Virginia.

Did this result in the appointment not being made, or could it have played some part?

Dr. TOBIN. Well, I certainly can't answer that, Mr. Chairman. I can tell that that might not have helped. [Laughter.]

I didn't mean to be facetious. I think it is an indication of the professional respect in which I am held by my colleagues that I was considered as a candidate for this post. I was one of the finalists for this post in, I believe, an earlier period, about 1978 or 1979.

I must confess I had no feeling that I had irritated the good William D. Watson. So I wouldn't care to characterize it, but he is obviously disturbed.

All I can say, Mr. Chairman, is that I have put my information, my science in the scientific literature. I have put it in books. I have never said anything in front of a group that I have been disturbed about.

The other thing that I would want to say is I did not play a similar role in Kentucky. In Kentucky I cannot appear as a paid representative, as I am not a paid representative here.

The University of Kentucky has paid my fare up here because of comments made about the quality of the Kentucky drug testing program and because they are proud of the research program that I run, as I am.

This is a controversial area, Mr. Chairman, and tempers run high, and it appears that I have unfortunately borne the brunt of this gentleman's ill will.

That is all I can respond to that.

Mr. CONYERS. Well, I don't characterize this as something personal or that he was being high-tempered about it. Perhaps he was. You may know him; I don't.

Dr. TOBIN. I had the pleasure of having dinner with the gentleman once in Kentucky shortly before I was invited down to discuss these drugs in West Virginia. I haven't seen him since.

[Pause.]

Mr. CONYERS. Mr. DeWine.

Mr. DEWINE. Thank you, Mr. Chairman.

Doctor, the chairman has asked you about your testimony, and there has been some inference, I guess, that you testify quite a bit. I am just curious about that.

In the last year how many times have you testified?

Dr. TOBIN. I testified at a hearing in front of the racing commission in Arizona in, I believe, either February or March. My testimony was excluded. They maneuvered to exclude my testimony.

I testified——

Mr. DEWINE. I can't let that one go. Why? I am sorry.

Dr. TOBIN. Well, it is very simple, Mr. Chairman. They are measuring oxyphenbutazone. They are calling positives in Arizona on urinary levels of phenylbutazone and oxyphenbutazone.

I have just submitted a paper for publication which shows that the levels of oxyphenbutazone, the major metabolites in equine urine, varies from a thousandfold and quite independently of when the drug was administered to the horse.

Mr. DEWINE. Well, Doctor, you mean they did not pay attention to your testimony?

Dr. TOBIN. No, no. They knew——

Mr. DEWINE. Or do you mean they excluded it?

Dr. TOBIN. I shall have to give you my interpretation, OK, of the events if you will accept that.

Mr. DEWINE. Sure.

Dr. TOBIN. I am left with the impression that they knew if I went on and testified they would have to revise the rule. I understand that what they did was committed a reversible error, excluded my testimony, and then they could ahead and hold the hearing when I wasn't there. It is a long way from Kentucky to Arizona.

Mr. DEWINE. Right. Doctor, how many other times have you testified? Just give me the number. I am just——

Dr. TOBIN. I don't keep lists. I am just going back through my mind.

Prior to that, the previous year, in Puerto Rico——

Mr. DEWINE. Doctor, excuse me. I am not trying to pick on you. I really am not. Just give me a rough idea how many times you testify.

Dr. TOBIN. Two to three times a year.

Mr. DEWINE. That is all I want to know.

Dr. TOBIN. This is my second——

Mr. DEWINE. Doctor, I am not making any inference to the fact that you have testified. It had just been brought out by the chairman. I just wanted to get on the record how many times you have testified. It is of no great significance to me, frankly.

There have been—your testimony—and I apologize for missing part of your testimony. I heard the earlier part of your testimony. I heard the latter part of your testimony. You may have covered some of these questions, and, again, I apologize. I apologize to the Chair if I have covered something that you have already talked about.

The two drugs that you have talked about—would you have any idea how many States presently allow their use or disallow their use, however you prefer to answer it?

Dr. TOBIN. I would have to make a rough——

Mr. DEWINE. Just a guess, Doctor. I am not going to hold you to the exact figure. I am just trying to get a feel for this.

Dr. TOBIN. Approximately 50 percent, and I should really pass on that question. I do not have those statistics.

Mr. DEWINE. Does anybody at the table know that, roughly?

[No response.]

I think it is an important question because when we are talking about making a Federal policy and a Federal law about a particular area I think it is of importance to know what the States are already doing.

Mr. CONYERS. Counsel says that it is probably around 50 percent.

Mr. DEWINE. OK.

Mr. CONYERS. She is checking it.

Mr. DEWINE. We are in basic agreement, then. Thank you, Mr. Chairman.

Doctor, you have had a chance to—I take it from your prior testimony that you have had a chance to actually look at the language of the bill?

Dr. TOBIN. Yes.

Mr. DEWINE. Are there other drugs besides the two that you have mentioned here that would be excluded by the bill that are in common usage today in the racing industry?

Dr. TOBIN. The principal problem with the bill, as it is written, is it excludes everything. For example——

Mr. DEWINE. Excuse me, let me follow that though if I can.

Dr. TOBIN. OK.

Mr. DEWINE. I understand that it excludes everything.

Are there some things that are in common use today that are of—at least in your opinion and the opinion of some of the States—of benefit that would be excluded by this?

Dr. TOBIN. Well, there is a whole group—when I speak about phenylbutazone, I speak of what pharmacologists call the aspirin group of drugs, and there is a whole group of those, and at least four or five which are essentially equivalent to phenylbutazone. They would be banned.

They are, in essence, no different from phenylbutazone or, indeed, no different from aspirin. They would all be banned.

Mr. DEWINE. And roughly 50 percent of the States allow those today?

Dr. TOBIN. Well, I could not give you a figure on what proportion of States allow those. I just have no idea.

I know that California specifically permits some of these, the drugs which are equivalent to phenylbutazone.

Mr. VIENNA. Mr. DeWine?

Mr. DEWINE. Yes, sir.

Mr. VIENNA. We have a chart that I would be pleased to give to you. We obtained it, I believe, from the National Association of State Racing Commissioners. It is dated, but it will give you an idea of just what is permitted and what is not.

If I might, sir, give this to you now?

Mr. DEWINE. Yes, sure. I would appreciate that.

Mr. VIENNA. Thank you.

Mr. DEWINE. Doctor, while I am taking a look at that, have you had the opportunity—I note from your testimony this morning that you are originally from Ireland, is that correct?

Dr. TOBIN. That is correct.

Mr. DEWINE. Have you been involved in, or are you familiar with the racing industry, let's say, in Ireland?

Dr. TOBIN. Broadly, yes.

Mr. DEWINE. How do the laws in Ireland compare with the laws in this country concerning the use of drugs?

Dr. TOBIN. They are quite different.

Mr. DEWINE. How are they different? And just give me the broad strokes. You are talking to a layman here who does not understand a lot of technical——

Dr. TOBIN. In essence, in Europe—excuse me, I am sorry for interrupting.

Mr. DEWINE. Go ahead.

Dr. TOBIN. In essence, in Europe medication is banned completely.

Mr. DEWINE. It is banned?

Dr. TOBIN. No traces of nothing in your horse's urine.

Mr. DEWINE. Is that right?

Dr. TOBIN. That is correct.

Mr. DEWINE. Can you shed any historical light on why that is and why we have a different practice in this country?

Dr. TOBIN. The structure of racing is very different in Europe. In England and Ireland, it is much more social. The horse is much more of a status symbol, believe it or not, in Europe than it is here. Racing is on the grass. Racing is straight. It is not on oval tracks.

You have far more entries for every race than you have slots. So they have an excess of horses racing.

Under those circumstances, you can run good racing with no traces of medications in urine. You do not, however, get around the problem of trace levels. They have had a consistent and very severe problem with traces of theobromine, which is a metabolite of caffeine, showing up in horse urines.

What happens is a trace of theobromine gets into the horse's food from cattle food. Going through the mixing apparatus, it gets into the horse food. It shows up in urine. They lose purses.

In plain simple terms, it is a nuisance, but it activates this whole regulatory process. Horsemen get penalized. They lose purses. For a horseman to lose a purse, in particular in the stakes race, is a very severe penalty, indeed, because he loses the value of the—breeding value of the animal of being a stakes-winning horse.

Have I——

Mr. DEWINE. Yes, you have.

Dr. TOBIN [continuing]. Rambled on a little too much?

Mr. DEWINE. No, no, no.

Dr. TOBIN. OK.

Mr. DEWINE. No, no, I appreciate the answer.

Is that true on the Continent as well?

Dr. TOBIN. Between you and I and this table, they are not quite as strict in Continental Europe, but that is the rule that is on the books.

Mr. DEWINE. But Ireland completely bans it?

Dr. TOBIN. In Great Britain—in thoroughbred racing in Ireland, England and France and Germany, in fact in Western Europe, is nominally drug-free and is kept analytically so. If the chemist finds anything in urine, he will call it improper.

Mr. DEWINE. In your answer to my question, though, why the difference, how can they do it and we don't do it, is that they have more horses. Is that it?

Dr. TOBIN. They have more horses.

Mr. DEWINE. There are more potential——

Dr. TOBIN. They race them—they have more horses entering. They race them on grass tracks. They race them on straight tracks. The whole tradition and the way horsemanship is handled over there is different.

Mr. DEWINE. I don't want to extrapolate too far with your statement, but would it be a fair statement to say that they are able to do that because they can exclude horses that aren't healthy?

In other words, they can just—if the horse isn't prime, they don't run the horse?

Dr. TOBIN. The structure of the industry is quite different over there. That would be the basic way to answer your question, and they can and do successfully run a racing——

Mr. DEWINE. Well, would you comment on my comment? Would you comment on my question? Is that true or not?

Do you want me to repeat the statement?

Dr. TOBIN. Yes. A note came up to me they run far fewer races, which is also true.

Can you roll the question by me again?

Mr. DEWINE. Sure.

Dr. TOBIN. I apologize, I got distracted.

Mr. DEWINE. No, no, and I don't want to infer anything that you are not saying——

Dr. TOBIN. Yes.

Mr. DEWINE [continuing]. But I want to make a statement, and I want you to comment on the statement basically yes or no and then explain your answer if you could.

Dr. TOBIN. Yes, OK.

Mr. DEWINE. I get the feeling from your testimony that what you are saying is that the reason that in Ireland they can have a rule of no use of drugs is they have more entrants, which means that they do not have to run horses, or, to put it another way, they only run horses that are in prime shape. If a horse has a problem, it doesn't run.

Dr. TOBIN. That is not true at all, Mr. DeWine.

Mr. DEWINE. OK, then explain what you meant by the number of entrants making a difference.

Dr. TOBIN. The structure of racing is different, but they have the same problem with bleeders, and there has been consistent friction in that they have tried to get approval for furosemide there.

The reason that that has not been approved is that the structure—the governing body in racing in Great Britain and Ireland is extremely conservative. It is the Jockey Club in England, the Turf Club in Ireland. That is one reason.

There is pressure from the horsemen to use these medications. It does not occur. They do not permit it.

Your basic question, the thrust of your question to me, is that if you can take the top of the crop, you can pick better horses. I would have to say to you that under the conditions of North American racing, which is quite different, this is not so.

Spectacular Bid, which was a very successful racehorse in this country, would probably have had difficulty in racing under European rules.

Mr. DEWINE. Why?

Dr. TOBIN. The structures of the industries are different.

Mr. DEWINE. Why?

Dr. TOBIN. Partly because the structure of the tracks, the grass horses versus the track horses. I am getting quite outside my expertise when I begin to discuss the actual structure of the industry.

Mr. DEWINE. I understand. I guess I am intrigued or maybe bothered by the answer that in Ireland they don't use drugs and they seem to be doing all right.

Dr. TOBIN. The other question that I can't answer for you is quite how rigorous the testing is there and what they get by with. I don't have as good a feel for that as I do for this country.

Mr. DEWINE. Is there any movement in Ireland that you are aware of—and I realize that—I don't know how often you get back there, but I realize that your familiarity is here now, but is there any movement to change that policy and to use drugs there?

Dr. TOBIN. Well, there is pressure from the horsemen, yes, but it has not been effective so far.

Mr. DEWINE. Thank you, Mr. Chairman.

Mr. CONYERS. Well, gentlemen, it has been a long afternoon, and we really are probably terminating earlier than we ought to. There has been quite a bit of comment and expert opinion and also non-expert opinion ventured here.

I am grateful to all of you for giving so generously of your time. It is always a pleasure to see my colleague in the Congress, former colleague, Caldwell Butler.

Mr. Vienna, you have been very helpful.

Dr. Tobin, you have been generous. We have been a little bit detailed, and I hope not exceedingly rough on you, but you appear to be able to take the give and take of these kinds of discussions very well.

Thank you.

Mr. Vienna, you wanted to make a clarifying statement?

Ms. BOWMAN. Are we clear on the record on the question I asked now?

Mr. VIENNA. I thought I cleared that up, yes.

Ms. BOWMAN. OK.

Mr. CONYERS. So we thank you again very much.

Mr. BUTLER. Thank you, sir.

Dr. TOBIN. Thank you, Mr. Chairman Conyers.

[Prepared statements of Caldwell Butler, David Vienna, and Dr. Thomas Tobin follow:]

STATEMENT OF CALDWELL BUTLER
BEFORE THE HOUSE JUDICIARY CRIMINAL JUSTICE SUBCOMMITTEE
MAY 18, 1983

Mr. Chairman, Members of the Subcommittee on Criminal Justice, my name is Caldwell Butler, I am a practicing attorney in the city of Roanoke, Virginia.

I have recently been employed by the Horsemen's Benevolent & Protective Association as its special counsel. You will recall from earlier testimony on behalf of the Association on September 30, 1982, that this organization is comprised of the 50,000 owners and 5,000 trainers of thoroughbred racing horses who are the persons upon whom this legislation could impact most directly.

I am accompanied by Dr. Thomas Tobin who is here at the invitation of the Subcommittee. Dr. Tobin is a veterinarian and pharmocologist and one of the foremost experts in those fields. I want to emphasize that Dr. Tobin is appearing at his own expense, and he is receiving no remuneration for his time or expenses from the Horsemen's Benevolent & Protective Association.

I am also accompanied by Mr. David Vienna who is the Washington representative of the Horsemen's Benevolent & Protective Association. Mr. Vienna is a former investigator with the United States Senate Permanent Subcommittee on Investigations.

Testimony Prepared for

The United States Congress

The Committee on the Judiciary

and

The Subcommittee on Criminal Law

Presented by

Thomas Tobin

Kentucky Equine Drug Research Program
Department of Veterinary Science
University of Kentucky

May 18, 1983

Mr. Chairman, members of the subcommittee, my name is Thomas Tobin. I graduated in veterinary medicine from University College Dublin in 1964 and completed a PhD in Pharmacology-Toxicology at the University of Toronto in 1970. Thereafter, until 1975, I was a faculty member in the Department of Pharmacology, Michigan State University, East Lansing, Michigan. In 1975, I was invited to set up the Kentucky Equine Drug Research Program and I have been at the University of Kentucky since 1975. Currently, I am Professor of Veterinary Science and a Professor of Toxicology at the University of Kentucky. My research program is an independent research program funded by the Kentucky State Racing and Harness Racing Commissions. Since 1975, this program, in conjunction with my colleague, Dr. Jerry Blake and the Kentucky Equine Drug Testing Program, has published two books and about 85 research papers on the detection, actions, uses, and effects of drugs in racing horses. Both programs have completed considerable research on furosemide or Lasix® in horses and are currently working on research concerning phenylbutazone in horses.

Mr. Chairman, today I will speak to:

1. The Bleeder, and Furosemide or Lasix.
2. Phenylbutazone, the horses' equivalent of aspirin, and its effects in racing horses.
3. The Illegal Medications.
4. Medication Control and The Drug Testing Process.
5. The Canadian federally controlled testing system: A comparison with the United States System.
6. The effectiveness of current United States testing systems.
7. Testimony by Mr. Paulus that Kentucky has "a mediocre drug testing laboratory."
8. The problem of trace levels or residue determinations in racing horses.
9. Summary and Conclusion.

THE BLEEDER

1. If you take a Thoroughbred horse, run him a mile at top speed, you will find that about 1-2% of horses will trickle blood from the nose post-race.
2. This is the classic bleeder - known 300 years or more. Occasionally a horse will die from bleeding, though this is rare.
3. It has long been thought that some horses bled in lungs without blood appearin at the nose. These were called "Occult Bleeders".
4. The Flexible Fiberoptic Endoscope became available in veterinary medicine in the late 1970's. Using this instrument, it was found that:
   (a) 1-2% of horses drip blood from nose
   (b) 40-70% of all horses show signs of blood in the windpipe
   (c) About 10-15% show substantial amounts of blood in the windpipe
   (d) About 10-15% show flecks of blood in the windpipe
   (e) About 20-30% show in-between amounts of blood in the windpipe
5. This work showed that bleeding into lungs and bringing blood up into the windpipe is very common in horses post-race
6. When horses bleed in a race, they may stop and wreck a field of horses. This results in risks to life and limb for horses and jockeys.
7. There has long been a search for a drug which will help bleeders.
8. Current drug of choice of the veterinary profession for bleeders is furosemide. It has been approved for use in racing horses by some Racing Commissions.

FUROSEMIDE - LASIX®

1. A very effective and safe diuretic in both horses and humans.
2. Fifth most commonly prescribed drug in human medicine and there is no reason to think that it is any less safe or effective in the horse.
3. Furosemide is specifically approved by the FDA for use in pulmonary congestion in the horse.
4. Because pulmonary congestion is a likely cause of bleeding in the horse, there is direct medical rationale for the use of furosemide or Lasix® in bleeders.
5. Horses with atrial fibrillation, who suffer pulmonary congestion, have a much higher incidence of bleeding than normal horses.
6. Clinical experience of equine veterinarians and horsemen suggests that furosemide is effective in the treatment of epistaxis or bleeders.
7. Based on this evidence, there is a clearcut pharmacological basis, a clear medical rationale, regulatory approval, and clinical evidence from both university hospital studies and equine practice to support the use of furosemide in the prophylaxis of epistaxis.

EFFECTS OF LASIX® IN HORSES

1. One of the reasons that Lasix® is a very safe drug is that it is a very short acting drug.

2. When a horse is dosed with Lasix® to prevent bleeding, only 250 mg of the drug is given IV into the jugular vein.

3. The formation of urine (the diuretic effect) peaks within 15 minutes and is essentially over within one hour.

4. About 4 liters of extra urine are formed and voided, usually within one hour of drug administration.

5. While this effect looks quite dramatic, one must remember that this is only about 2% of the body water in the horse.

6. Because of this, Lasix® is unable to move or flush more than 2% of any drug out of the body of a horse. This amount is forensically insignificant.

7. Furosemide is therefore a very safe, short acting drug and it does not reduce the level of any drug in the body of a horse.

8. In a nutshell, Lasix® does not flush drugs out of the bloodstream of the horse.

LASIX® AND DRUG DETECTION: THE "MASKING" EFFECT

1. Lasix® is not known to affect the detection of any drug in blood.

2. Lasix® does not reduce the concentration of basic, lipid soluble drugs in urine.

3. Lasix® will reduce the concentration of some water soluble drugs and drug metabolites in equine urine.

4. Because the action of Lasix® is short lived, these dilution effects are over within about three hours, if the dose of furosemide is the dose recommended (250 mg) by the AAEP.

5. By giving Lasix four hours prior to post time, at the anti-epistaxis dose, no masking or drug dilution effects or interference with drug testing is encountered.

6. In studies at the University of Kentucky, it was found that the detection of six narcotics was not affected and that there was some evidence for enhancement of the detection of these drugs four hours after furosemide administration.

7. Preliminary studies by Doctor Maylin in the NASRC Quality Assurance Program have shown that with the recommended dose and time constraints, furosemide does not affect the detection of the following drugs: cocaine, caffeine, lidocaine, apomorphine, phenylbutazone, fentanyl, oxymorphone, clenbuterol, flunixin.

8. Therefore, if the dose of furosemide is 250 mg IV and it is given four hours prior to post-time, furosemide does not "interfere" or mask drug detection and may enhance it.

DOES LASIX® AFFECT THE PERFORMANCE OF HORSES? DOES IT "SPEED" HORSES UP?

1. Studies at the University of Kentucky, the Ohio State University and a study of track times at Louisville Downs Racetrack all show that Lasix does not improve the performance of horses.

2. Therefore, horsemen cannot use Lasix® to "move horses up" or improve their performance. Therefore, Lasix® cannot be used to run horses "hot" and "cold".

3. Horsemen and equine veterinarians, based on their experience, are of the opinion that Lasix® helps horses run "easier" or "easier in their wind" and makes them run more reproducibly and reliable, i.e. true to form.

4. If a horse is a bleeder, he is likely to bleed again. Horsemen who own such horses have a keen desire to be permitted to run them on Lasix®.

IS LASIX TOXIC TO HORSES?

1. Lasix® is a very safe drug, in both humans and horses.

2. Doses used in horses are small and the drug is rapidly excreted.

3. No evidence of toxic effects after single or repeated doses.

4. As used on the racetrack, in single,small, isolated doses, this drug is essentially nontoxic in horses.

SUMMARY

1. Bleeders are a common and very significant problem in racing horses.

2. Bleeders lead to risks to life and limb of horses and jockeys.

3. The treatment of choice is furosemide.

4. Furosemide is safe, effective and approved by FDA for use in horses.

5. Properly used, it does not affect the detection of drugs in blood or urine; does not "mask".

6. Does not "move horses up".

7. There is absolutely no evidence to suggest that this treatment is cruel or inhumane to horses.

8. Lasix® is a rational therapeutic choice for a very common condition in horses. Its use may save the lives of both horses and jockeys. Denial of horsemen the right to use this drug in racing horses is illogical in terms of human endeavor and inhumane to the horses.

"DRUGGING" OF HORSES BY FUROSEMIDE

According to Webster, drugging means (1) to put a harmful drug in (2) to stupefy or poison with or as with a drug; (3) to administer something nauseating to.

Mr. Chairman, members of the subcommittee, nothing whatsoever that I have said about Lasix meets any of the above definitions. To administer Lasix to a horse is in no way, shape or form the "drugging" of a horse.

PHENYLBUTAZONE: ITS EFFECTS IN RACING HORSES

A. PHENYLBUTAZONE: A NON-STEROIDAL ANTI-INFLAMMATORY DRUG.

1. Phenylbutazone is a member of the non-steroidal anti-inflammatory group of drugs. Aspirin is the principal member of this group of drugs that you are familiar with.

2. In general, if you wish to keep phenylbutazone in the horse in perspective, it is useful to think of it as being basically horse aspirin.

3. Phenylbutazone and aspirin have little effect on normal pain perception. It is the hypersensitivity to pain associated with the inflammatory process which responds to phenylbutazone and aspirin.

4. The hypersensitivity to pain associated with inflammation (such as a sunburn) is caused by prostaglandins. Prostaglandin concentrations in tissues increase and the inflamed area becomes hypersensitive.

5. Phenylbutazone and aspirin block prostaglandin biosynthesis, reduce tissue hypersensitivity, and normalize pain perception in the affected area.

6. They leave the area with normal pain perception. They do not numb any area in the body of a horse.

PHENYLBUTAZONE: COMPARISON WITH OTHER DRUGS

1. Phenylbutazone is not thought to be stimulant to horses. It has no more ability to stimulate an unusual performance in a horse than aspirin has in you or I.

2. Phenylbutazone is quite different from local anesthetics. These drugs are injected at specific points or into specific areas and "numb" specific areas. Local anesthetics block both pain and proprioception completely. Proprioception is the horse's awareness of where his leg is and what it is doing. Blockade of proprioception can lead to a misstep and a breakdown.

3. It is important to remember that phenylbutazone has no effect on normal pain perception or normal proprioception and is thus completely different from the local anesthetics.

4. Phenylbutazone is completely different from the narcotic analgesics. These agents act in the central nervous system (brain) to reduce the perception of pain. They act to reduce a horse's pain threshold, and, in addition, they are quite powerfully stimulant in racehorses. Such a horse would be "drugged".

5. Phenylbutazone is therefore clearly distinct from local anesthetics which numb specific areas, and narcotics which suppress pain perception in the brain and stimulate horses.

6. Phenylbutazone has no depressant or tranquilizing effects in horses.

7. The principal pharmacological effect of phenylbutazone is to normalize an inflamed tissue and it has very little effect outside the area of inflammation.

PHENYLBUTAZONE AND BREAKDOWNS

1. Because phenylbutazone reduces the hypersensitivity to pain found in inflamed tissues, it has been argued that horses treated with this drug will run on injured joints and tendons and be more likely to break down.

2. This argument ignores the fact that there is no pain perception in the actual joint surfaces of horses. Phenylbutazone, therefore, cannot affect pain perception directly from joint surfaces, since there is none.

3. The great bulk of the resistance to joint movement is based in the soft tissues around a joint. Soft tissue resistance to joint movement in an inflamed joint is markedly reduced by phenylbutazone. This is the manner in which phenylbutazone restores normal performance.

4. Another important factor is that while protecting a hypersensitive limb, a horse may overload a sound limb. In allowing a horse to run evenly on both limbs, phenylbutazone may, under some circumstances, reduce the incidence of breakdowns.

5. The state of California legalized phenylbutazone at the end of 1970. Between 1970 and 1978, over which period the use of phenylbutazone steadily increased, the number of horses suffering breakdowns which required destruction of the animal remained relatively constant. Other analyses of this data suggest that the incidence of breakdowns actually decreased as the use of phenylbutazone increased.

6. Studies by the Colorado Racing Commission veterinarian, Dr. Gene Bierhaus, showed that over nine years the probability of a horse having an accident which required his destruction was not any greater if the horse was phenylbutazone-treated.

7. Pilot studies at the University of Pennsylvania found that the risk of breakdowns was 1 per 206 horses at risk, a rate of breakdown not higher than the incidence in New York and New Jersey, states which restricted the use of phenylbutazone.

8. These studies, but most particularly the California studies, appear to suggest that with a well run medication program the rate of breakdowns is not increased.

PHENYLBUTAZONE AND MASKING

1. There are no published studies and no reports of experimental studies that show that "masking" by phenylbutazone is a significant forensic problem.

2. The nature of the drug testing process is such that "masking" of stimulants, depressants, narcotic analgesics, tranquilizers and depressants and local anesthetics by phenylbutazone or its metabolites is small.

3. Oxyphenbutazone is the major metabolite in equine urine which causes masking problems. Its concentration in horse urine is controlled by the horse, not by the horseman.

4. Kentucky allows horses to run on phenylbutazone. New York and Canada do not. The positive call rate for illegal drugs in Kentucky is substantially greater than that in New York and Canada. "Masking" is therefore not a problem in Kentucky when compared with the New York experience.

5. I know of no scientific evidence which suggests that "masking" by phenylbutazone significantly interferes with drug detection. I believe that "masking" is not a scientific issue, but a political or, rather, a pseudo issue.

6. Detailed scientific studies on masking by phenylbutazone are now underway at the University of Kentucky.

PHENYLBUTAZONE: ITS EFFECT ON PERFORMANCE

1. Phenylbutazone is not thought of as changing a horse's innate ability to race.

2. By relieving inflammation, it may enable him to race nearer to his maximum capability.

3. It unquestionably will aid the horseman to field racing sound horses. It unquestionably will aid the horse to run a better, more comfortable race.

4. The use of drugs such as phenylbutazone is entirely ethical and legal in human sports medicine. There is no reason, therefore, to think that this type of medication is any less ethical and humane when used in horses.

PHENYLBUTAZONE: A SUMMARY

1. Phenylbutazone is a nonsteroidal anti-inflammatory drug of the same general class as aspirin.

2. Its primary pharmacological action is to normalize inflamed tissues.

3. It has no effects to reduce normal pain perception, or "numb" any tissues in the body.

4. Its use does not appear to be associated with an increased incidence of breakdowns.

5. There is no evidence that it makes testing for illegal drugs less effective.

6. It is thought to enable a horse to perform up to his innate ability and it enables horsemen to field racing sound horses.

7. Use of this type of medication is entirely legal, ethical and humane in human sports medicine. There is absolutely no reason to think that it is otherwise in veterinary medicine.

THE DRUGGING OF HORSES

According to Webster, drugging means (1) to put a harmful drug in, (2) to stupefy or poison with or as with a drug, (3) to administer something nauseating to.

Mr. Chairman, members of the subcommittee, nothing whatsoever that I have said about phenylbutazone meets any of the above definitions. To administer phenylbutazone to a horse is in no way, shape or form the "drugging" or numbing of a horse.

THE ILLEGAL MEDICATIONS

1. All racing jurisdictions in the United States and all racing organizations are committed to preventing the use of:

   (a) Stimulants

   (b) Depressants

   (c) Narcotic analgesics

   (d) Local anesthetics

   (e) Tranquilizers

   in racing horses.

2. Stimulant drugs and narcotic analgesics are banned because, among other reasons, they make a horse unsafe to ride, unnaturally stimulate him, and may affect the outcome of the race. Such a horse would be drugged.

3. Depressants and tranquilizers are banned because they may be used to reduce the performance of a horse and thus affect the outcome of a race. Such a horse would be drugged.

4. Local anesthetics can block pain perception and proprioception from a limb. This may cause the horse to misstep and break down. Such a horse would be numbed.

5. Use of these agents in racing is illegal and strictly controlled by chemical testing of blood and urines.

MEDICATION CONTROL IN HORSE RACING

1. Horse racing has the longest history (since 1910) and the most extensive range of drug testing of any human endeavor. The scope of testing is wide, with 500-1,000 drugs common and a possible range of 10,000 drugs.

2. Drug testing depends on the testing of blood and urine samples from racing horses. Best drug coverage is obtained by testing both blood and urine.

3. If the blood is drawn and tested pre-race, the process is called pre-race testing.

4. Many medications, particularly among the illegal medications, are not readily detectable in pre-race blood testing.

5. Because of this, pre-race blood testing must always be run in conjunction with post-race urine testing for optimal drug coverage.

6. The combination of pre-race blood and post-race urine testing offers the best drug coverage and prevents the running of some illegally medicated horses.

7. Exactly the same drug coverage is afforded by post-race blood and urine testing. This is the system that Kentucky uses.

8. In Kentucky, each positive costs about $7,000. to fund. To install pre-race testing would cost at least $300,000. extra per year. This works out at an estimated cost of about $30,000. per pre-race positive. The average purse raced for in Kentucky in 1981 was $4,186.

9. Pre-race testing is clearly technically feasible for some drugs. Its efficacy and particularly its cost effectiveness is debatable.

THE CANADIAN TESTING SYSTEM: COST EFFECTIVENESS OF A CIVIL SERVICE CONTROLLED NATIONAL TESTING SYSTEM

1. In Canada, drug testing is directly under the control of Agriculture Canada, i.e. the Canadian Civil Service.

2. The contract for the testing is "let" to private laboratories; one in Vancouver, one in Toronto, and one in Montreal.

3. About 80,000 samples/year are tested by these laboratories.

4. The cost is about $40.00 (US) per sample tested for post-race urine testing (blood is only tested if urine is not obtainable).

5. A research facility with a budget (including start-up costs) of about $700,000. a year is being established in Ontario.

6. The positive call rate (Kentucky positive) of this system, very close to what this bill would mandate, is about 0.6 per 1,000 samples tested. This call rate compares unfavorably with Kentucky's 2.9/1,000 rate.

7. There is no evidence, therefore, that a drug testing system supervised directly by a federal government is any more effective than the current situation.

8. The costs of testing are relatively much greater in Canada than in most U.S. laboratories. The cost/sample is $20.00 in Kentucky.

9. The Canadian system does not include pre-race testing, mandated in HR 1694.

10. The Canadian system does not include quantitative testing of drugs in blood which will be necessary to regulate the next provision of the bill that I will speak to, which is the determination of trace levels.

11. Costs for a based testing system mandated by HR 1694 would inevitably be much (2-3 times) higher than the current Canadian testing costs.

EFFECTIVENESS OF THE CURRENT UNITED STATES TESTING PROGRAMS

1. The attached table shows an analysis of the positive call rates of 18 U.S. racing states, and Canada.

2. Of these states:

Arizona
Arkansas
Colorado
Rhode Island
Pennsylvania
Nebraska
Maine
Michigan
Kentucky
Massachusetts
New York
South Dakota
Idaho
Delaware
Montana
West Virginia

all had positive call rates for hard or illegal medications equal or greater than that of Canada.

3. California and New York, at 0.4 and 0.8 positives/thousand, were less than but clearly comparable with Canadian call rate for positives.

ALL HORSES

State	$\bar{x}$ Positive Call Rate	± S.D.	N	Years
Arizona	0.6	±0.4	5	(1978-82)
Arkansas	2.5	±2.7	5	(1977-81)
California	0.4	±0.1	7	(1975-81)
Canada	0.6	±0.2	5	(1978-82)
Colorado	1.3	±0.5	6	(1976-81)
Rhode Island	0.6	±0.7	5	(1974-78)
Pennsylvania	2.2	±0.4	3	(1979-81)
Washington	0.3	±0.3	5	(1977-81)
Nebraska	0.9	±0.7	5	(1978-82)
Maine	1.7	-	8	(1975-82)
Michigan	0.6	±0.3	4	(1977,78,80,82)
Kentucky	2.9	±1.6	7	(1975-81)
Massachusetts	2.2	±1.5	7	(1975-81)
South Dakota	6.0	±6.8	6	(1976-81)
Idaho	2.3	±4.0	3	(1979-81)
Delaware	4.0	±1.6	5	(1978-82)
Montana	1.6	±1.9	6	(1976-81)
New York*	0.8** (0.4)***	±0.3	4	(1978-81)
West Virginia	2.2**	±1.1	6	(1976-81)

*Pre- and post-race bloods and urines
**Includes Kentucky Permitted Positives
***Estimated positive call rate in New York for Kentucky Thoroughbred positives

As part of a study on phenylbutazone in horses by the Kentucky Equine Drug Research Program, a survey of the "positive call" rates in various U.S. racing juridsictions was undertaken in November, 1982. On our request the race horse medication statistics were provided by most of the state racing commissions or the authorized testing laboratories. These statistics included the number of urine samples analyzed, lists of positives called, and the medication rules for each jurisdiction in each year. For each state, each year, the positives were categorized into Kentucky prohibited (stimulents, depressants, narcotics, tranquilizers, local anaesthetics) and Kentucky permitted (NSAI's, diuretics, antibiotics, steroids) drugs. A "positive call rate" of Kentucky prohibited positives per one thousand analyzed urine samples was calculated for each year and a mean over the years reported was generated for each jurisdiction.

4. The only interpretation possible from this table is that drug testing in U.S. jurisdictions compares very favorably with the federally run Canadian program.

TESTIMONY BY MR. PAULUS THAT THE STATE OF KENTUCKY HAS A MEDIOCRE LABORATORY

The following documents are submitted in rebuttal:

1. Letter from Dr. J. W. Blake, Director of the Kentucky Equine Drug Testing program.
2. Letter from Dr. Thomas Tobin, Kentucky Equine Drug Research Program.
3. Table showing that Kentucky has a much higher "positive call" rate than the testing laboratories mentioned in the quote.
4. Attention is drawn to the previous table where Kentucky has one of the highest positive call rates.
5. Publication lists from the Kentucky Equine Drug Testing and Research Programs.
6. Based on this information, we know of no objective data to support this comment by Mr. Paulus.

SUMMARY: CURRENT MEDICATION CONTROL

1. Post-race testing of blood and urine samples offers exactly the same drug coverage as pre-race and post-race testing at considerably less cost.

2. The federally controlled medication control program in Canada is less efficient than most and more expensive than all major United States drug testing programs.

3. This bill mandates pre-race testing and quantitation of residue levels. This process will be enormously more costly than the current method and, from the Canadian example, less effective.

ESTABLISHING TRACE LEVELS

The administrator may, by regulation, establish permissible trace levels of substances foreign to the natural horse that he determines to be innocuous."

1. This simple sentence defines the critical decisions that the administrator will have to make that will send innocent horsemen to jail. The administrator will send innocent horsemen to jail because:

2. There does not currently exist any research base on which to determine these trace levels.

3. Even when this base is developed, the metabolism of the horse will introduce a substantial element of randomness into it.

4. Because of this, the administrator will have to coldly decide how many innocent horsemen he will jail.

5. The administrator will jail innocent horsemen because the state of the art of analytical chemistry is such that analytical chemists cannot tell when a drug was administered.

6. For example, phenylbutazone has been detected in horse blood for up to nine days after the last dose, long after its therapeutic effects have ceased.

A critical question, therefore, is what is a "trace" and what is a therapeutic amount of each medication. This is a question that the administrator will have to answer for each medication, drug, and foreign substance that his chemists find in horse urine. Criminal prosecutions will hang on the outcome of these decisions. Therefore, basis for the administrator's decision must be solid and scientifically defensible. Further, because each drug is unique, the data will have to be obtained for <u>each</u> drug <u>individually</u> in the horse.

Where will the administrator get this information? The administrator will have to generate this information himself because this information is not presently available for any equine medication. To give you an idea of how complex this problem is, let me tell you what the administrator will have

to do the enforce the regulations concerning phenylbutazone, the most commonly used equine medication, and the horse's equivalent of aspirin.

One way for an administrator to set a trace level is to determine the amount of phenylbutazone in the blood of a horse at, let us say, 24 hours after the last dose. If he takes this approach however, he will find that his experiments provide him with not just a single level but a range of levels. In determining the actual "trace" level that he is going to work with, the administrator will have to decide how many innocent horsemen he is going to convict. Only when he has made this decision can he go ahead and set the level. This is because the random variability in both horses and drug testing in horses make it inevitable that some horsemen who strictly obey the rules will accidentally go over the "trace" level and become felons.

On the other hand, the administrator may choose to determine at what blood level the pharmacological effect of phenylbutazone disappears in horses. He may then use this information to determine what is a trace level of phenylbutazone in the horse. To do this, he will have to actually study the actions of phenylbutazone on lameness in horses. First, the administrator will have to acquire some naturally lame horses so that he can actually measure the therapeutic effects of phenylbutazone. This has never been done in horses or, to my knowledge, in any other species. These are technically very challenging experiments. When he has worked out how he is going to measure lameness in horses, he will then be able to give them phenylbutazone and measure its anti-lameness effects. Then he will have to measure the blood and urine levels of phenylbutazone in these horses so that he can know what is an effective level of phenylbutazone and what is a trace level of phenylbutazone.

This is a substantial undertaking. It requires the acquisition of horses, the services of veterinarians expert in biomechanics, and analytical chemists. It requires the development of methods, likely high-speed cinematography, to measure and quantitate lameness and drug effects in horses. Such studies, for phenylbutazone alone, would take at least one year and likely longer. These experiments than would have to be repeated for each of the many non-steroidal anti-inflammatory drugs in the horse. Then the administrator would have to study the effects of combinations of these drugs so that he would be able to rule in circumstances where more than one of a particular type of drug was found in the bloodstream of a horse.

The administrator will have to work with blood levels of drugs and not with urinary levels as is now common in racing chemistry. The reason for this is that blood and urinary levels of drugs do not correlate very well. For example, urinary levels of oxyphenbutazone, a major metabolite of phenylbutazone in horse urine, can vary up to about 1,000-fold from its blood levels, depending only on whether the urine is acidic or basic. Because of this poor relationship between blood and urinary levels of drugs, one cannot use trace levels of drugs in urine for regulatory purposes. Traditional methods of regulating phenylbutazone use, by quantitating phenylbutazone and its metabolites in urine, are far too variable to serve a valid regulatory function.

There are about 4,000 drugs and 63,000 chemicals in common use in North America. Many of these will show up in horse urine and each one will have to

be tested and a "trace" level established. This is a huge task and will require the addition of a substantial research unit to the regulatory structure.

It will not be possible for the administrator to simply adapt information from other species to the horse. This is because each species is, by and large, unique in its handling of drugs. Doses are different from species to species, the blood levels at which drug responses occur are different, and the metabolism and pharmacokinetics of drugs are different in the horse. If the Federal government wishes to get into the business of regulating equine medication, it will have to get into the business of researching medication in horses in a very substantial way.

SUMMARY

1. There is no evidence whatsoever that the use of phenylbutazone or Lasix leads to the "drugging" or "numbing" of horses. Passage of this bill will ban these valuable therapeutic medications.

2. Use of these agents is accepted and humane in human sports medicine. They are just as acceptable and humane in veterinary medicine. Passage of this bill will discriminate against horses.

3. Testing for illegal medications in the United States is economic and effective, the more so when compared with the federally run Canadian system. Passage of this bill is not likely to improve testing in the United States.

4. Pre-race testing gives exactly the same drug coverage as post-race blood and urine testing. Passage of this bill will give the same coverage at greatly increased costs.

5. Determination of methodologies and approaches to the problem of trace levels is a major undertaking. Well led, well financed and productive research teams are in place in many states and working on these problems. Development of this research base is unlikely to be accelerated by HR 1694.

6. If HR 1694 is passed by Congress and vigorously enforced, the lack of an effective method of determining and enforcing trace levels will result in the sending of innocent horsemen to jail.

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Pharmacology Review: The Nonsteroidal Anti-Inflammatory Drugs. I. Phenylbutazone.

Thomas Tobin, D.V.M., Ph.D.

Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, Kentucky 40546.

The anti-inflammatory drugs in use in equine medicine fall into two principal categories, the corticosteroids, which we have dealt with previously[1] and all the others. These other drugs are known collectively and logically as nonsteroidal anti-inflammatory drugs[a] (NSAID).[2] The original member of this group is aspirin. The second oldest is phenylbutazone, which was introduced into human medicine around the time of World War II and shortly thereafter into veterinary medicine. Phenylbutazone was widely used in human medicine for a number of years,[16] but it soon became apparent that its use was associated with a small incidence of specific and serious toxicities.[6] Because phenylbutazone has caused deaths in humans from aplastic anemia and agranulocytosis, its use in human patients is now restricted. With the realization of the therapeutic limitations of the corticosteroid derivatives and phenylbutazone, and the huge market for a good anti-inflammatory drug, drug companies have researched the nonsteroidal anti-inflammatory field thoroughly in the last 10 years. The upshot of this has been that a large number of new nonsteroidal anti-inflammatory drugs have been developed and have recently become available in both human and equine medicine.

The nonsteroidal anti-inflammatory drugs all share a number of properties which seem to be required for their anti-inflammatory action (Figure 1). All are acidic drugs, with a pKa of 4.5 or less.[2,3] The acidic nature of these drugs means that they are all between 95% and 99% bound to plasma proteins. Because of this, drugs of this group do not pass into saliva, and saliva testing is essentially useless for detection of this important group of drugs. Their acidic nature also seems to be important for their action and has led to

[a] Members of the nonsteroidal anti-inflammatory group of drugs include aspirin, ibuprofen, naproxen, flufenamic acid, tenamic acid, mefenamic acid, indomethacin (the most potent), niflumic acid and meclofenamic acid. All these drugs share the same basic mechanism of action and the same general pharmacology. Miscellaneous nonsteroidal anti-inflammatory drugs currently used in veterinary medicine include orgotein and hyaluronic acid. Because the mechanism of action of these agents is quite distinct from the antiprostaglandin agents they are usually not thought of as typical NSAID's.

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ARE:

(1) HIGHLY ACIDIC DRUGS

(2) HIGHLY PROTEIN BOUND IN PLASMA

(3) DIFFICULT TO DETECT IN PLASMA

(4) ACT BY INHIBITING PROSTAGLANDIN SYNTHESIS

(5) ARE LIKELY TO BE DILUTED IN URINE BY FUROSEMIDE

ARQUEL (SHOWN BELOW) IS A SIMPLE NONSTEROIDAL ANTI-INFLAMMATORY DRUG.

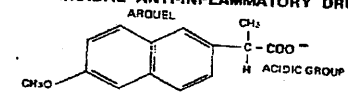

Figure 1. Properties of nonsteroidal anti-inflammatory drugs.

suggestions that their acidity enables them to accumulate in inflamed tissues, which also tend to be acidic.[2] This accumulation would then allow them to have more effect in inflamed areas than in normal tissue. However, these agents also tend to accumulate in the stomach, small intestine and kidney,[1] and we will see later that the toxicities produced by these agents are usually associated with these tissues (Figure 2). Also, because prostaglandins are involved in the generation of fever, all these drugs are antifever or antipyretic agents (Figure 2).

Among the nonsteroidal anti-inflammatory agents, phenylbutazone remains the most popular and widely used in equine medicine. After its introduction into equine medicine, phenylbutazone established itself as a very effective anti-inflammatory agent. It soon made its appearance at the racetrack, and pressure from horsemen for permission to use it during racing

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS:

(1) INHIBIT ENZYMES WHICH FORM PROSTAGLANDINS AND THEREFORE SLOWLY REDUCE TISSUE PROSTAGLANDINS

(2) REDUCE PAIN IN INFLAMED TISSUES BY REDUCING TISSUE PROSTAGLANDIN LEVELS

(3) REDUCE FEVER (ARE ANTIPYRETIC)

(4) ACCUMULATE IN STOMACH, KIDNEY AND SMALL INTESTINES AND TEND TO PRODUCE LESIONS IN THESE TISSUES.

Figure 2. Effects of nonsteroidal anti-inflammatory drugs.

increased. In 1958, Colorado approved phenylbutazone for use in racing, and the era of controlled medication was born.[8]

Phenylbutazone and the other nonsteroidal anti-inflammatory drugs produce their anti-inflammatory effects by inhibiting the production of chemicals called "prostaglandins."[14] Prostaglandins are produced in large amounts in inflamed tissues and are involved in the blood vessel changes which cause the reddening, heat, swelling, pain and loss of function that we associate with inflammation. They also have another peculiar action, in that they act to supersensitize pain receptors in the inflamed area to agents which cause pain. Anybody who has had anything as simple as a good sunburn is familiar with the great increase in sensitivity to even the lightest touch which occurs in sunburned tissue. This hypersensitivity is a typical effect of certain prostaglandins. In fact, if minute amounts of prostaglandins are injected into a normal joint, that joint soon becomes excruciatingly painful to move. This sensitizing action of the prostaglandins is represented schematically in Figure 3. It is clear from this mechanism of action that if the concentration of prostaglandins in inflamed tissues can be reduced, the signs of inflammation, swelling, and particularly the perception of pain in that area will also be reduced.

When phenylbutazone is injected intravenously it takes about 30 minutes to distribute throughout the horse and to begin blockage of formation of the prostaglandins. There are, however, unusually large amounts of prostaglandins already present in an inflamed tissue.[15] Therefore, even though the formation of new prostaglandins is blocked, phenylbutazone will not appear to take effect until these high prostaglandin levels have been reduced. This process usually takes about three to four hours, and once the excess prostaglandin has dissipated, the supersensitivity to pain reduces and the swelling and heat in the area begin to decline. Loss of these signs of inflammation will require an additional period of time, and it may take up to 12 hours or more for the full pharmacological action of phenylbutazone to become apparent.[12]

Since all the nonsteroidal anti-inflammatory agents produce their effects in more or less the same way, they all take a period of at least several hours to begin producing effects, even when they are given by intravenous (IV) injection. On the other hand, when blood levels of these drugs decline, the concentration of the prostaglandins in the inflamed tissues builds back up again, and the pain and other signs of inflammation begin to return. It is then time for another dose of phenylbutazone. It is clear from this mechanism of action that the therapeutic effects of phenylbutazone are directly related to its ability to reduce inflammation, and that any analgesic action it may have is secondary to and dependent on the anti-inflammatory effect. Phenylbutazone has no anesthetic actions and is not an anesthetic. It is simply a very effective anti-inflammatory drug, and as such, prevents the appearance of pain, which is one of the cardinal signs of inflammation.

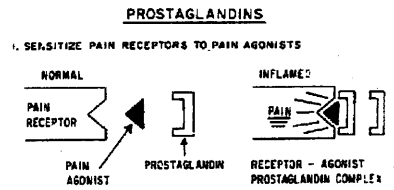

Figure 3. Sensitizing action of the prostaglandins.

In an average-sized horse (1000 lbs), a dose of 2 to 3 g/day of phenylbutazone IV, or about 4 g orally, should produce the optimal anti-inflammatory effect. Two grams daily should then maintain the effect.[11] As the dose of the drug is increased, the blood levels increase and the plasma half-life of the drug is also increased (Figure 4).[9] If the drug is given orally instead of IV it takes about five hours for peak blood levels to be attained, and the time to attain peak pharmacological effect will be further delayed (Figure 5).[11] Urinary levels of phenylbutazone are usually higher than plasma levels of the drug, and urinary levels are detectable for at least 24 hours after a 2 g/1000 lb dose IV (Figure 6). Since the anti-inflammatory effect is not good for more than about 24 hours, daily dosing with phenylbutazone is required.[11]

For a long time the only way to assess the anti-inflammatory and lameness-alleviating efficacy of phenylbutazone was simply by "eyeballing" a treated horse. This led to some confusion about how effective the drug really was, how long it took to act, and for how long a period it was effective. Recently, however, Professor Pratt and his co-workers at the Massachusetts Institute of Technology have developed an instrument called a "force plate" which can provide an objective measurement of lameness and the effects of drugs on lameness.[12] This instrument measures small variations in the load on a horse's leg. It turns out that a horse is very unsteady on a lame leg, and that this unsteadiness, in the form of constant small readjustments of weight on this leg, can be measured by means of the "force plate." After administering phenylbutazone, the horse becomes much more comfortable and thus steadier on his sore leg, which begins to be comparable with his good leg. Using this instrument, 2 g phenylbutazone orally has been shown to take several hours to act after administration of an IV dose and to be largely over by 24 hours. If the dose is given intravenously, the onset of action is a little faster, but probably not much faster, because the reduction of tissue prostaglandins by phenylbutazone and other NSAIDs takes some time to develop.

Though the pharmacological action of phenylbutazone is over within 24 hours, the time for the animal to "eliminate" phenylbutazone is indefinite, as it is with any drug given to a horse. Since phenylbutazone is relatively easy to detect, it can be detected for a very long period indeed if the analyst so desires. Figure 7 shows some of the best data on plasma levels of phenylbutazone in the horse that has come to my attention.[10] In this experiment horses were given varying doses of phenylbutazone for up to three days, and plasma and urinary levels of the drug followed. Plasma levels started at about 20 to 30 μg/ml, declined with a half-life of about nine hours, and were still detectable in plasma eight days after dosing and, in some urine samples, on the ninth day after dosing. This experiment, when taken in conjunction with the previous data, emphasizes the point that while the pharmacological actions of phenylbutazone may be over within 24 hours, detection of phenylbutazone in plasma and urine after treatment with this drug is possible for very long periods indeed.

Although the "clearance times" for phenylbutazone in plasma and urine obviously depend on the

[8] Bierhaus, G.H.: *Personal communication.* Colorado Racing Commission Veterinarian (1979).

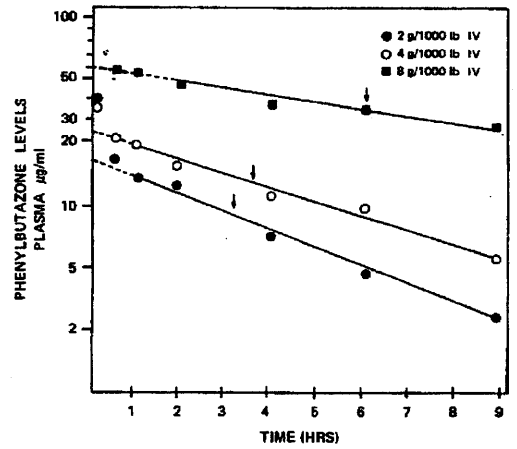

Figure 4. Plasma levels of phenylbutazone in a horse after administration of three different doses (after Piperno *et al.*).[11]

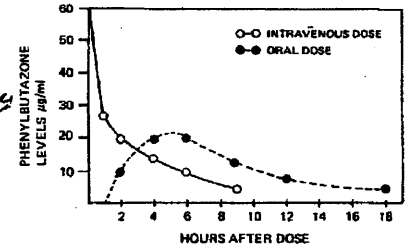

Figure 5. Plasma levels of phenylbutazone following 4 g/1000 lb (453.6 kg) administered by the oral and intravenous routes (after Piperno *et al.*).[11]

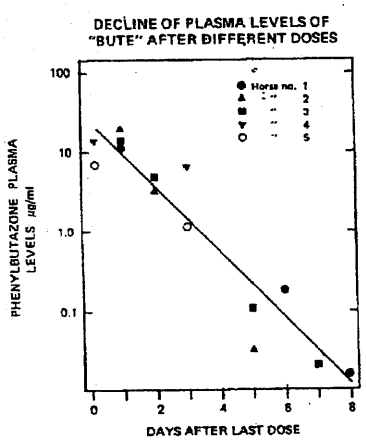

Figure 7. Symbols show plasma levels of phenylbutazone in horses which had been dosed with 2, 4, and 6 g phenylbutazone for up to three days before measurement of plasma levels. Phenylbutazone was detectable in plasma for eight days and urine for up to nine days (after Norheim *et al.*).[10]

very safe and effective drug in the horse with minimal side effects and toxicities.

This pattern of minimal toxicity due to phenylbutazone in the horse contrasts sharply with the serious toxicities seen in man. In man, some type of side effect is seen in from 10 to 40% of patients, including peptic ulcer, liver and kidney problems and, most seriously, inability to form red cells or white cells in the blood. A number of phenylbutazone-dependent deaths have been recorded, and for this reason its use is restricted in man, and it is best used only in short-term therapy.[K]

Because a dose of phenylbutazone persists a long time in the blood of man (half-life about three days) compared with its relatively short plasma half-life in the horse (six to eight hours), it has been suggested that its lack of toxicity in the horse is due to its rapid metabolism by the horse. This theory ignores observations that drug toxicities are very often due to drug metabolites rather than to the drug itself, metabolites which are of course formed at a much greater rate in the horse than in man.

The question about the effect of phenylbutazone on the performance of horses has often been raised and has not been satisfactorily answered. Reviewing the subject of phenylbutazone and furosemide[c] in racing horses, the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners concluded that phenylbutazone does not change the innate ability of a horse to race, but by relieving inflammation may enable him to race nearer to his maximum capacity.[4] Studying the effects of phenylbutazone in time trials in horses, Sanford and his colleagues found that phenylbutazone administered intramuscularly 23 hours before "time" trials improved performance in their horses.[13] These workers were apparently rather surprised by this result and concluded that phenylbutazone had acted to relieve subclinical lameness rather than to stimulate the horses.[13] This interpretation is well supported by some experiments from our laboratory, which show no effect of phenylbutazone on fentanyl-stimulated trotting in horses, minimizing suggestions that phenylbutazone either stimulates or depresses horses at the usually used clinical doses.[4]

Another important question with phenylbutazone is whether or not it interferes with the detection of other drugs, the popularly called "masking" effect. While there is no doubt that the presence of any drug must make the detection and unequivocal identification of another drug somewhat more difficult, the consensus of the Veterinary Chemists Advisory Committee was that the usual doses of phenylbutazone given on race day may or may not interfere with the detection of other medications, depending on the analytical methods used *and, perhaps just as importantly, on what other drugs are being tested for.*[4]

[c] Lasix, National Laboratories Corporation, Somerville, NJ.
[K] Italicized portion represents this author's comments.

Part two of this review on nonsteroid anti-inflammatory drugs will appear in the July issue of The Journal.

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Pharmacology Review: The Corticosteroids

Thomas Tobin, D.V.M., Ph.D.

Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40506.

The physiological importance of the adrenal cortex was recognized over 100 years ago, and by the 1930s crude adrenal extracts were being used in human medicine. Over the next 20 years, the synthesis and chemical structures of the major natural and synthetic steroids were elucidated and the principles of their pharmacology and therapeutics worked out. Today, the natural and synthetic corticosteroids can be divided into three main groups based on their pharmacological characteristics. The first and most important group for the equine practitioner is the glucocorticoids, which predominantly affect protein and carbohydrate metabolism and produce the potent anti-inflammatory effects of these drugs. The second group is the mineralocorticoids, represented in nature by aldosterone, which gives rise to sodium and water retention. The third group consists of the adrenal sex hormones.[4] Because the glucocorticoid actions of the corticosteroids are those with which the equine practitioner is primarily involved, this review will be devoted to those actions of the corticosteroids. Table 1 shows the relative anti-inflammatory effectiveness of some natural and synthetic corticosteroids and how effective the selection, for anti-inflammatory action among the synthetic glucocorticoids, has been.

TABLE 1

Relative potency of corticosteroids.

	Sodium retention	Anti-inflammatory effect
Natural Steroids:		
Cortisol	1	1
Corticosterone	15	0.3
Synthetic Steroids:		
Prednisolone	0.8	4
Dexamethasone	0	25
Flumethasone	0	700

Plasma Level and Control

Cortisol and corticosterone are the principal glucocorticoids found in equine plasma, at levels of about 70 ng/ml, and 5 ng/ml, respectively.[5] Their rate of secretion in the horse is not known, but is probably comparable with the 0.5 to 1 mg/kg/day secreted by the dog and human. Secretion of cortisol by the adrenal cortex is under direct control of the adrenocorticotropic hormone (ACTH) of the anterior pituitary, and administration of ACTH brings about a prompt increase in plasma cortisol concentration. After administration of 200 units of ACTH, equine plasma cortisol levels peak at about 150 ng/ml five hours after dosing, and then decline toward control over the next 30 hours (Figure 1). Since cortisol is not stored in the adrenal cortex, the rate of secretion is also the rate of formation of this hormone, and both are directly controlled by ACTH.[6]

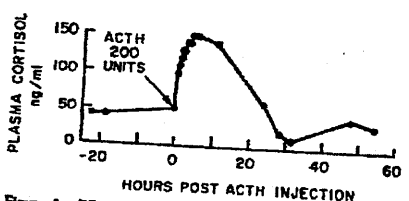

Figure 1. Effect of ACTH on plasma cortisol in the horse. The solid circles (•-•) show plasma levels of cortisol before and after intramuscular injection of 200 units of ACTH gel in a pony (adapted from James[5]).

The rate of release of ACTH from the pituitary is influenced by a number of factors: exercise, stress, surgery, cold exposure, hypoglycemia, etc. The most important control on natural ACTH release, however, is feedback inhibition by high plasma levels of cortisol or its synthetic analogues. This gives rise to the major problem with prolonged corticosteroid therapy, which is "feedback inhibition" of ACTH release, resulting in reduced adrenal cortex function and finally in atrophy of the adrenal cortex. If corticosteroid therapy is abruptly terminated, the animal is left with reduced or no natural corticosteroids which can be fatal.[4] In a milder form this may cause the "turning out" syndrome, which is seen when horses are "turned out" after a racing season during which they received large doses of corticosteroids. These horses tend to be un

this effect which leads to the use of glucocorticoids in certain types of lymphoma.

Despite the fact that glucocorticoids do not affect the titer of circulating antibodies, they have marked effects on the immune response. The symptoms of certain allergic states are often dramatically reduced by the corticosteroids. The corticosteroids are particularly useful in preventing the consequences of cell-mediated (delayed hypersensitivity) immune reactions such as graft rejection.[4]

Effects on Growth and the Central Nervous System (CNS)

The glucocorticoids have marked effects on both growth and the central nervous system. Their antianabolic effect appears as a suppression of growth, which is a widespread effect of the glucocorticoids. In the fully grown animal this same effect is seen as a delay in wound healing. The glucocorticoids also increase the rate of demineralization of bone, and prolonged administration can lead to osteoporosis and compression fractures. In the CNS, the glucocorticoids produce an antipyretic effect similar to that of the nonsteroidal anti-inflammatory drugs. Unlike the nonsteroidal group, however, large doses of the glucocorticoids can cause an elevation of mood and euphoria, and this effect has led to abuse of corticosteroids by humans.[4]

Effects on Water and Electrolyte Metabolism

In general, the potent glucocorticoids have minimal effects on water and electrolyte metabolism, and even large doses do not affect these parameters. However, excessive administration of glucocorticoids with significant mineralocorticoid activity leads to sodium and water retention. Continued administration can result in edema, hypokalemia and metabolic alkalosis. Excessive administration of corticosteroids will thus result in muscle weakness from both protein catabolism and hypokalemia.

Anti-Inflammatory Effect

The glucocorticoids, and particularly the potent synthetic members of this group, have the capacity to prevent or suppress the local heat, redness, swelling, and pain by which inflammation is grossly recognizable. At the microscopic level, they inhibit both the early and late phases of the inflammatory process. In the early phases they inhibit edema formation, fibrin deposition, capillary dilation, and the migration of leukocytes and phagocytic activity. They also inhibit the later stages, such as capillary and fibroblast proliferation and cicatrization. The precise mechanisms of these effects are not well understood, but the corticosteroids produce these effects quite independent of the type of agent which elicits the response.[4] The effect is therefore a suppression of the tissue response to trauma rather than any direct antagonism of the causative agent.

Because the anti-inflammatory effect simply suppresses tissue response, treatment with corticosteroids in no way neutralizes or removes a primary cause, but merely suppresses clinical signs of the disease. The suppression of clinical signs is the most dangerous aspect of corticosteroid therapy. A patient with severe bacterial pneumonia treated with corticosteroids may appear to improve, and an inflamed joint injected with corticosteroids will appear normal, and the horse will be improved or go sound. In truth, however, these conditions progress at at least the same rate as they would have in the absence of corticosteroids, and collapse, when it does occur, is sudden and without warning. This aspect of the action of the corticosteroids has given rise to the grim comment that "a patient on corticosteroids can walk all the way to the autopsy room." Similarly, a horse can wear a joint surface right down to the bone by running on a glucocorticoid-injected joint.

Therapeutic Uses of Corticosteroids

Two main guidelines have been developed covering use of the corticosteroids which should be kept in mind when these drugs are used: (1) a single large dose of a corticosteroid, or even therapy for a few days, is unlikely to produce harmful effects; (2) prolonged corticosteroid therapy, to the extent that the dose exceeds substitution therapy, can produce potentially lethal effects, especially if therapy is abruptly withdrawn.[4]

These rules lead to the principle that long-term maintenance on corticosteroid therapy is reserved for serious or life-threatening diseases, while short-term therapy is more easily justified.[4]

Systemic Corticosteroids

Because of the ability of glucocorticoids to suppress the immune response, they should be used with great care in the presence of bacterial or viral infections. Corticosteroids are commonly given in association with broad-spectrum antibiotic treatment of infections and, undoubtedly in this situation, will act to improve an animal's appetite and appearance, and reduce fever. Use of these drugs, however, carries the risks of blocking the animal's natural response and

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Phenylbutazone in Horses: A Review

Albert A. Gabel, D.V.M., M.S. Ohio St.
Thomas Tobin, D.V.M., Ph.D. U of Ky
Richard S. Ray, D.V.M., M.S., Ph.D. Ohio St.
George A. Maylin, D.V.M., M.S., Ph.D. Cornell

From Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by: Gary P. Carlson, University of California; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; and David Snow, University of Glasgow; and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Finley, A.V. Villatico, Robert E. Vessiny, Dr. Francis Ozog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Rierhaus, and General Wayne Kester.

1. Phenylbutazone is a very effective nonsteroidal anti-inflammatory agent in horses. Through its anti-inflammatory action, it is an analgesic and an antipyretic. It is not an anesthetic.

2. Phenylbutazone does not change a horse's innate ability to race, but by relieving inflammation it may enable him to race nearer to his maximum capability.

3. Its mechanism of action is thought to be by inhibition of prostaglandins.

4. Onset of action occurs within a few hours, and optimum effect lasts for less than 48 hours after the last dose.

5. In horses phenylbutazone is safe; side effects and toxicity are rare at the usual doses.

6. In blood, phenylbutazone can be detected for 24 hours and occasionally up to 48 hours.

7. Although the quantity of phenylbutazone in the urine is not reliable for the determination of the time that the last dose of phenylbutazone was administered, reasonable estimates can be made by a comparison of the ratios of phenylbutazone to its major metabolites.

8. Trace amounts of phenylbutazone and its metabolites can be detected in urine for up to 96 hours by utilizing electron capture derivative formation and detection by gas chromatography.

9. The usual doses of phenylbutazone given on race day may interfere with the detection of other medications, depending upon analytical methods used.

"Still a Work"

Introduction

Phenylbutazone is a safe, effective nonsteroidal anti-inflammatory drug* (NSAID) with antipyretic and analgesic activity. In the recommended doses, there is no evidence that it changes a horse's innate ability to race, except to make him perform more nearly normal if he has pain due to inflammation of part of his musculoskeletal system.

Mechanism of Action

Phenylbutazone is the (NSAID) most widely used in horses. Its mechanism(s) of action is(are) not clearly understood. Studies involving the mechanism(s) of action of phenylbutazone in the horse have not been reported. Most evidence indicates that (NSAID)'s exert their action by inhibiting the biosynthesis of prostaglandins (at the last step of biosynthesis) which directly and/or indirectly mediate the inflammatory and pain response.[1,2] This inhibition probably reduces the concentration of prostaglandins in inflamed tissues.[1,22] Prostaglandins increase permeability of blood vessels and result in swelling and inflammation of tissue.[22] Prostaglandins also sensitize pain receptors in tissues to inflammatory and pain mediators; this blockade of prostaglandin formation results in reduced pain.[22] Because accumulation of phenylbutazone in the tissue is slow and the drug only prevents formation of new prostaglandins, the anti-inflammatory action of the drug develops slowly as preformed tissue prostaglandin concentrations must first decline.[4] Blockade of prostaglandin synthesis thus slowly returns the inflamed area toward normal. The analgesic action of phenylbutazone occurs directly in the inflamed tissue (not in the brain) and is directly related to its anti-inflammatory effects.

Phenylbutazone (and other NSAID) are all highly protein bound acidic drugs which accumulate in inflamed tissues (which is important for their action), but they also accumulate in the stomach, small intestine, and kidney.[4] Thus, all drugs in this group have the potential to produce lesions of the stomach, small intestine, and kidney tissue.[1] Since prostaglandins are involved in the cause of fevers, members of this group of drugs are also antipyretic. Even large doses do not reduce liver glycogen content in experimental animals, as do the salicylates.[22] Phenylbutazone interferes with several enzymes of amino acid and amino sugar metabolism.[23] It also inhibits mucopolysaccharide biosynthesis and collagen formation and uncouples oxidative phosphorylation. The other (NSAID)'s share these inhibitory properties.[22]

*Other members of the nonsteroidal anti-inflammatory group of drugs are flunixin meglumine, aspirin, ibuprofen, naproxen, flufenamic acid, flumanic acid, mefenamic acid, indomethacin, niflumic acid, and meclofenamic acid. All these drugs share the same basic mechanism of action and general pharmacology.

Clinical Efficacy

Currently, phenylbutazone is the most effective and dependable anti-inflammatory agent on the market for the equine. It is especially effective in cases of bone and joint inflammation and laminitis[5,6,7] as well as being useful in soft tissue inflammation.

At low doses, the effect of the drug appears to be dose related.[22] In response to a single administration intravenously or orally, a dose of 4 g has an optimal effect for a 1,000-pound horse. Two grams are required daily to maintain optimal effect. First response to the drug can be seen a few hours after administration. Optimum effect is variable but occurs approximately 12 hours after administration. Intravenous injection appears to speed onset of action, on the average by about 2 to 4 hours compared to oral use.[7] Onset of action is variable after oral administration, depending on whether the horse eats before or after the drug is given.[24] Optimal clinical effect appears to continue for a variable time, usually for somewhat less than 48 hours after the last dose of the drug, depending on the dose and length of the course of treatment.[12,24] In other species, several drugs not commonly used in horses can be altered in their degradation when phenylbutazone is used with them.[25] Therefore, their duration and intensity of action change. This action has not been demonstrated in horses. Conversely, several drugs known to inhibit drug metabolism in other species have been of little or no effect on the half-life of phenylbutazone in the horse.[21]

Metabolism

Phenylbutazone is almost completely metabolized in the horse. In separate studies, only 3.7% of a 2 g/1000 lb dose was recovered in the first 24 hours,[22] and by dosing 2 g/1000 lbs at consecutive 24-hour periods, between 1 and 2% of the parent drug was recovered in the urine.[19] The principal metabolites reported in the equine are oxyphenbutazone and γ-hydroxyphenylbutazone, the "alcohol metabolite." Together, these account for about 25% of the drug administered.[19] A second minor alcoholic metabolite has been found, whose structure has tentatively been determined by nuclear magnetic resonance to be a primary alcohol.[19] Other metabolites and conjugated forms have been reported in the rat,[2] but to date 75% of the metabolites have not been accounted for in the horse.

Because the pharmacologically inactive γ-hydroxyphenylbutazone is less tightly protein bound than either phenylbutazone or oxyphenbutazone,[2] it is readily excreted in urine and is the principal metabolite found in urine (14%) after a single dose of the drug.[26] However, if dosing is continued, the proportion of the alcohol metabolite excreted drops to 6% of the dose.[19] This change in the proportion of different metabolites in the urine as dosing

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Equine Communications

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Pharmacology Review: A Review of Recent Research on Furosemide in the Horse—Thomas Tobin, Kentucky Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, KY

Furosemide* is a member of the "high ceiling" group of diuretics and is a safe and very effective diuretic in the horse.[4] Outside of the usual medical indications for diuretics, furosemide is also useful in racehorses because it is believed to be effective in the control of epistaxis (nosebleed). Epistaxis, however, only occurs in about 2% of horses, while up to 80% of the horses running in some meets may be on furosemide.[3] This article will review recently acquired knowledge concerning furosemide in the horse and may suggest some reasons for the widespread use of furosemide in racing horses.

Furosemide was introduced into the equine medical literature by Dr. Marvin Beeman and his colleagues in Colorado.[9] It is useful in edema from all causes. It is particularly useful in cases of pulmonary edema because it acts to rapidly remove this fluid, even before it begins to move fluid out through the kidneys.[8] Its ability to increase the volume of urine may also be used to dilute out substances in urine and prevent renal damage. Thus, when a horse "ties up," the released myoglobin may deposit in and damage the kidneys. In situations such as this, the increased urine volume due to furosemide greatly reduces the possibility of renal damage.[4]

The diuretic action of furosemide is due to its inhibition of active reabsorption of chloride in the renal tubules. Sodium and water reabsorption normally follow chloride reabsorption, so administration of furosemide leads to greatly increased losses of sodium, chloride, and water.[8]

In a 1,000-pound horse, 4 ml of furosemide (50 mg/ml) produces about 4 liters of urine in about 40 minutes. This is the dose recommended for use in epistaxis (nosebleed) in the horse. A 10-ml dose, which is a common clinical dose, produces about 9 liters of urine, mostly within 1 hour. Forty milliliters of furosemide will produce about 21 liters of urine within about 2 hours, and this is close to the maximum response in a normal horse.[8] 2 mg/kg

The diuretic action of furosemide is therefore relatively short lived, especially after small intravenous (IV) doses. This short lived action of furosemide is not due to any inability of the horse to respond, as a second dose of furosemide will usually produce a very good response. Rather, the action of furosemide in the horse is brief because furosemide is rapidly pumped out of the blood into the renal tubules.[8] This pumping accounts for the short plasma half-life ($T_{\frac{1}{2}}$ = about 35 minutes) of the drug, its very rapid onset of action in the kidneys, and the fact that up to 60% of the drug is found unchanged in the urine. This is a very high proportion of a drug to be excreted unchanged in the horse.[8]

Because furosemide is secreted directly into the urine, it is found there in very high concentrations. A good analyst can easily detect furosemide in equine urine for 12 hours after a 10-ml dose, and the drug can be detected for up to 52 hours. Because the pharmacological actions of furosemide are largely over within 2 hours after an intravenous dose, there appears to be no need for detection of the drug for more than 12 hours after its administration. Racing chemists should bear this in mind when setting the level of sensitivity of their test systems.[8]

If furosemide is given intramuscularly (IM), its plasma half-life is greatly increased ($T_{\frac{1}{2}}$ = 86 minutes), and the diuretic response also lasts longer. After IM injection of 1 mg/kg of furosemide, about a 50% greater urinary response is observed than after its in-

*All the work reported here refers to injectable Lasix®, 50 mg/ml from National Laboratories Corp. subsidiary of American Hoechst Corp., Somerville, NJ

Continued on p. 320.

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travenous injection. Onset of the diuretic effect is, however, almost as fast as after IV injection, so IM injection would appear to be the route of choice if a prolonged or greater diuretic effect is required.[6]

Like the diuretic effects of furosemide, the cardiovascular effects of furosemide are also transient. After intravenous injection, furosemide reduces pressure in the pulmonary circulation within minutes. This effect occurs independently of the diuretic effect and may be the basis of the action of furosemide against epistaxis. In the systemic circulation, furosemide produces a decrease in cardiac output with a small increase in heart rate. Furosemide does not alter blood pressure in the systemic circulation. After 1 mg/kg (about 10 ml) intravenously, these effects are usually over within 2 hours.[5]

After intravenous injection, 10 ml furosemide increases total plasma solids about 10%, hematocrit by about 5%, and rapidly reduces plasma K^+ levels. These effects peak between 10 and 30 minutes after administration of the drug and decline back to control levels by 2 hours postdosing.[5,6]

In studies in a number of laboratories, furosemide was found not to reduce the plasma levels of any drugs tested to date.[4] Furosemide does not, therefore, act to "flush" drugs out of the blood or plasma of horses and should not interfere with blood testing for drugs.[7] Similarly, it did not significantly affect the urinary concentrations of a number of drugs tested, such as procaine or methylphenidate, and a closely related drug did not affect amphetamine.[4] These drugs are all basic, relatively lipid soluble drugs, and it appears likely that furosemide has little or no effect on urinary concentrations of this group of drugs.[7]

On the other hand, furosemide treatment caused up to 50-fold reductions in the urinary concentrations of both phenylbutazone and the major metabolite of pentazocine, which is a water soluble pentazocine glucuronide conjugate. In the case of phenylbutazone, this reduction in concentration was sufficient to render the drug difficult to detect in our routine screening tests on racetrack samples at the University of Kentucky. Since both furosemide and phenylbutazone are permitted medications in Kentucky, we now routinely take both blood and urine samples for testing. In our opinion, blood samples are necessary to accurately test for medication with phenylbutazone if the use of furosemide is also permitted.[7]

Some racing jurisdictions, such as Illinois or California, have rules which set upper limits on the concentration of phenylbutazone permitted in horse urine on race day. These rules make little sense if furosemide is also a permitted medication. This is because simple administration of furosemide either IV or IM as close to race time as possible will easily reduce the concentration of phenylbutazone in the postrace urine to below the critical level.[7]

By and large, however, the actions of furosemide on phenylbutazone concentrations in urine are not a serious problem for the racing chemist, in contrast with the likely actions of furosemide on some other groups of drugs. The 50-fold dilution of the major water soluble metabolite of pentazocine also probably occurs with all other drugs detected as water soluble metabolites, such as apomorphine, morphine, and related narcotics, and the phenothiazine tranquilizers. Since these drugs are effective at very low plasma concentrations and are difficult or impossible to find in equine plasma, their dilution by furosemide in equine urine is a serious problem for the analyst. The potential for furosemide to interfere with detection of these drugs in urine is probably the most serious problem associated with the approval of furosmide for use in racing horses.[7]

The performance effects of furosemide have been studied in some detail at both the University of Kentucky and the Ohio State University.[8] As pointed out earlier, furosemide has a very rapid action against pulmonary edema and is thought by track veterinarians to greatly assist the breathing of horses with respiratory problems, such as follicular pharyngitis. However, double blind trials on healthy Standardbred horses at both the University of Kentucky and the Ohio State University showed no statistically significant effects of furosemide on the performance of Standardbred horses.[4,8]

Because the numbers of horses involved in these trials were small and the horses were all clinically normal, we elected to perform a further study on the effects of furosemide on racing performance under track conditions. To this end, the Kentucky Harness Commission made available to us the "Lasix list" for the Louisville Downs Summer 1977 Harness Racing Meet. 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 furosemide. From the "Lasix list," 58 horses were selected, and for these horses 160 times prefurosemide and 232 times postfurosemide were obtained. It turned out that the horses were actually 0.14 seconds slower on furosemide than before they went on furosemide, though this difference was not statistically significant. The only conclusion from this experiment seems to be that the horsemen at Louisville Downs or their veterinary advisors were, on the whole, not able to improve the performance of their horses with furosemide.[8]

Furosemide appears to be a very safe drug in the horse. The usual dose administered in the treatment of epistaxis is a relatively small dose, and when administered IV, its action is brief. Given access to water and salt, any drug-induced losses of fluid or ions should be rapidly replenished. In another series of experiments 10-ml (1 mg/kg) doses of furosemide were given daily to horses for 4 consecutive days, and a series of hematological parameters followed. No cumulative changes in any of these parameters over the 4-day test period were observed. However, if these same 4 doses were given at hourly intervals, a 40% drop in serum K^+ was observed. Thus, the only likely acute toxicity with furosemide would appear to be the possibility of inducing hypokalemia with rapidly (hourly) repeated doses.[8]

In conclusion, furosemide is a very effective, safe, and rapidly acting diuretic in the horse. It clearly acts to dilute out and interfere with testing for certain drugs in equine urine. It does not appear to affect the performance of Standardbred horses. Clinical experience suggests that furosemide is effective in the treatment of epistaxis, and it may be useful in horses with respiratory problems, but no hard evidence to support these clinical impressions is currently available.

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Piperazine Toxicity in Horses

—P. H. McNeil, B.V.Sc. and G. B. Smyth, B.V.Sc., M.A.C.V.Sc., Dept. of Veterinary Clinical Sciences, University of Melbourne, Veterinary Clinical Centre, Werribee, 3030, Victoria, Australia

Clinical signs of horses involved in an epidemic of iatrogenic piperazine citrate poisoning are described. The disease was reproduced by similarly overdosing an experimental horse. No treatment was necessary for a complete recovery.

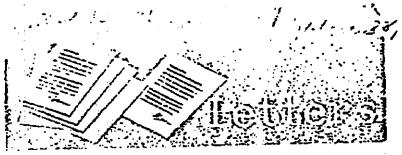
Introduction

Toxic reactions subsequent to oral administration of piperazine compounds for the treatment of helminth infestations of man and domestic animals are uncommon. Vomiting, urticaria, blurred vision, weakness, nystagmus, and convulsions may occur with normal or excessive dosing in humans.[1,3,10,12] Occasional emesis, diarrhea, and transient neurotoxic symptoms are seen with excessive dosing in small animals.[4,5,15,19,20] Mild gastrointestinal signs are reported after administering high doses to calves.[18] Therapeutic activity is attributed to the neuromuscular blocking activity of these compounds in the parasite, which allows normal intestinal peristalsis of the host to expel the intact parasite from the gut. The effects of the drug on neuromuscular activity within the host are minimal.[10,18]

Reports of toxicity in horses are rare. Toxic signs were not produced in foals treated with 6 times the normal dosage levels of piperazine adipate.[17] However, in a more recent study, involving trials with both levamisole and piperazine, neurotoxic signs were produced in a horse dosed with piperazine monohydrochloride at 6 times the normal dosage levels.[7] This paper reports the clinical signs associated with an outbreak of iatrogenic piperazine citrate poisoning on a Standardbred stud and the experimental reproduction of the disease.

Case History

A group of 10 weanling Standardbred colts were running in a 50-acre paddock grassed with improved pasture. All had suffered a mild upper respiratory tract infection during the 3 weeks prior to anthelmintic treatment. There was no other history of illness in any of the horses. During a 2-day period, every horse on the property had been treated via nasogastric intubation with piperazine citrate and thiabendazole pow-



Use of Bumetanide in Horses

December 9, 1976

Dear Sir:

In the article "Use of Bumetanide, a Potent Diuretic, to Obtain Urinary Samples for Dope Testing in Horses," (*AJVR*, Nov, 1976), Frey et al, report on the use of a diuretic, bumetanide, to obtain postrace urine samples for equine dope testing. These workers concluded that use of bumetanide "did not interfere with the detection of doping drugs" and that "by enhancing the clearance of drugs used for doping, bumetanide even provides favorable conditions for the detection of such drugs." It is our belief that these statements should be treated with caution.

Inspection of the data provided by Frey et al does indeed show that the concentration of amphetamine in the urine of the 2 horses tested was probably not affected by bumetanide treatment. This is in good agreement with results obtained in our laboratory, which have shown no substantial effects of furosemide treatment on urinary concentrations of procaine or methylphenidate. It thus seems that furosemide or bumetanide, both acidic high-ceiling diuretics, may produce little change in the concentration of basic, highly lipid soluble drugs in equine urine.

The situation with phenobarbital is, however, strikingly different. Frey et al show that bumetanide reduced urinary concentrations of phenobarbital eightfold in one experiment, threefold in another. These limited data suggest a substantial reduction in the urinary concentration of phenobarbital by bumetanide, which will make its routine detection in a sample of limited size much more difficult.

In our hands, furosemide treatment reduced urinary concentrations of phenylbutazone up to 15-fold and rendered it difficult to detect in routine screening tests.[1] These observations, similar to those observed with phenobarbital by the authors, strongly suggest that use of diuretics such as bumetanide and furosemide can substantially reduce the concentration of some drugs in equine urine. This reduction in concentration is very important in routine testing, where the size of the urine sample available to the laboratory is limited.

A number of important doping drugs such as apomorphine are detected in equine urine primarily as highly water-soluble glucuronides, which enter the urine by glomerular filtration and are then concentrated by renal concentrating mechanisms. It is probable that the urinary concentrations of these metabolites will be greatly (15- to 18-fold) reduced by the diuretic effect of drugs such as bumetanide or furosemide and their detection in routine dope testing rendered that much more difficult.

In summary, we believe that the conclusions of the authors concerning the use of bumetanide in dope testing may be misleading when applied to certain drugs in the routine dope testing situation, since both bumetanide and the closely related furosemide can be shown to greatly reduce the urinary concentration of important doping drugs. While these concentration changes may not be critical for work performed in a research laboratory, they can mean the difference between success and failure to detect dope in routine drug testing.

Tobin, DVM, PhD
J. W. Blake, MD
Lexington, Ky 40506

George A. Maylin, DVM, PhD
Ithaca, NY 14850

Reference

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ELECTRON CAPTURE DETECTION OF AN APOMORPHINE HEPTAFLUOROBUTYRATE DERIVATIVE AT LOW PICOGRAM LEVELS.

J. R. Miller, J. W. Blake and T. Tobin

Kentucky Equine Drug Research and Testing Programs
and the Graduate Toxicology Program

Department of Veterinary Science
University of Kentucky
Lexington, KY 40506

SUMMARY

An electron capturing derivative of apomorphine was prepared by incubating the drug with heptafluorobutyric anhydride (HFBA), triethylamine and heat. Mass spectral analysis suggests that HFBA reacts with both phenolic hydroxyl groups on apomorphine to give a derivative detectable at low picogram levels. This method is sufficiently sensitive for pharmacokinetic studies in the horse and is likely applicable to other dopaminergic analogues of apomorphine.

INTRODUCTION

Relatively small (0.02 mg/kg or about 12 mg) doses of apomorphine intravenously produce marked signs of central excitation in the horse. Apomorphine is thus not infrequently used as a stimulant "dope" in horse racing. However, because of the small amounts of drug which are effective, pharmacokinetic studies and correlations between plasma levels of apomorphine and behavioral and performance effects are difficult to perform. Thus in our hands, the fluorimetric methods of Butterworth & Barbeau (1975) and VanTyle & Burkham (1971) were not sufficiently sensitive for pharmacokinetic work in horses. Further, recent studies showing that apomorphine is a direct dopamine receptor agonist (Seeman et al, 1975; Moore, 1974) and studies on the treatment of Parkinson's disease with apomorphine and its congeners (Cotzias et al, 1976) suggest a need for a highly sensitive and specific assay for apomorphine analogues. In this communication we report a heptafluorobutyric anhydride (HFBA) derivatization method for determining apomorphine in equine plasma

UNIVERSITY OF KENTUCKY
LEXINGTON, KENTUCKY 40506

COLLEGE OF AGRICULTURE
RESIDENT INSTRUCTION
AGRICULTURAL EXPERIMENT STATION
COOPERATIVE EXTENSION SERVICE

DEPARTMENT OF
VETERINARY SCIENCE

In Haste, March 3, 78

Dear Committee Member:

Enclosed is the final pre-Miami draft of the "Blood and urine and pre- and post-race testing" report. This draft goes out to all members of the Committee and all who took part in the Meadowlands Conference. A draft very close to this one has been seen by most of those who took part in the Meadowlands conference and has met with varying degrees of acceptance.

The draft has been extensively re-written. The major change is the increased estimate of the efficacy of pre-race testing, up to 75%, when compared with post-race testing, and which is credited to Ray, Maylin, Woodward and Saffgastein.

I have done my best to get this report re-written and in the mail to all of you before you leave for Miami. Looking forward to seeing you there, I remain,

Yours sincerely,

Tom Tobin
Associate Professor

TT/vnr

Enclosure

in saliva is important. A number of basic drugs (pentazocine, morphine, methadone) are also highly protein bound in plasma and therefore may not be found in high concentrations in saliva.[10, 26]

Neutral drugs, such as caffeine, theophylline and ethanol, are found in the same concentrations in plasma and saliva.[9]

The volume of saliva which can be obtained is small, being not less than 20 ml and often as low as 2-3 ml.[9] Because of the reduced concentration of many drugs in equine saliva, this small volume can be a serious problem. Saliva is not, to our knowledge, routinely used as a biological fluid in most drug testing programs in the United States.

(c) BLOOD

(i) Collection of sample: Blood sampling, using prepackaged vacuum vials and needles, is rapid and cheap. The sample size is small, so transportation costs are low. For sampling alone no facilities are required. Since a veterinarian does the blood sampling the sampling is professionally done and ancillary information about the clinical condition of the horse is obtained. Blood is, *per se*, the simplest, cheapest, fastest and most reliable biological sample to take in the horse. It is the only sample suitable for pre-race testing under North American conditions. There is no evidence that venipuncture as utilized in pre- and post-race testing harms horses.

(ii) Advantages of blood testing: Outside of the speed and ease with which the blood sample can be obtained, the principal advantage of blood testing is that almost all drugs found in blood are found in an unchanged form.[2] Some drugs are found only in blood, such as reserpine,[12, 14] methadone, doxapram[14] and pseudo-ephedrine.[27] In addition, unaltered valium and acepromazine can be found only in blood, which is important if the parent drug must be identified.[12] Because the drugs are actually found in blood at or about the time of racing, the pharmacological and forensic significance of the finding is usually relatively

3/3/78

clear-cut.[19] The problems encountered in urine sampling with the recovery, identification and interpretation of the significance of the presence of drug metabolites are avoided.[19] Blood levels of drugs are essentially not affected by other drugs such as diuretics,[19] in contrast with the marked effects observed on some urinary drug concentrations.[25] Because the "clearance" of most drugs from blood occurs relatively rapidly in the horse, the possibility of an analyst finding traces of a drug in blood days after administration of the drug is in most cases remote.[2]

(iii) Disadvantages of blood testing: A perceived problem with blood testing is the small size of the sample, which is usually not greater than 20 ml. Compounding this problem is the fact that some drug or drug metabolite concentrations in blood are a good deal lower than in urine.[19] The upshot of this is that the analytical techniques required in blood sampling are somewhat more exacting than those required for urine and there is much less room for error in blood testing when compared with urine testing. Classical analytical techniques used in urine testing are not readily applicable to blood testing. Given good analytical techniques[12] the small size of blood samples is not a problem.

Because blood samples contain various enzymes which may contribute to drug breakdown, the splitting of drugs in blood samples can be a problem,[22, 23] particularly if the blood sample is to be used in post-race testing. For this reason blood sampling tubes should contain enzyme poisons and be cooled during transport and storage.[23]

The classic example of a drug which cannot be detected in blood but which is readily detectable as a metabolite in urine is apomorphine. The concentrations of apomorphine found in equine plasma during the pharmacological action of the drug are too low to be readily detectable by currently available analytical techniques.[20] However, the glucuronide metabolite of apomorphine is highly concentrated in equine

Chapter 14

CALMING HORSES FOR SHORT PERIODS: ACEPROMAZINE AND ITS COUSINS

UNTIL THE EARLY 1950s, the only drugs available to calm nervous horses were depressant drugs like chloral hydrate and the barbiturates. While these drugs would calm nervous and fractious animals, they only did so at doses that clearly rendered them drowsy and affected their motor ability and coordination. With the introduction of reserpine and the phenothiazine tranquilizers in the early 1950s, a class of drugs became available that would render animals less reactive to environmental stimuli with minimal effects on their consciousness, motor ability, and coordination. Technically, these drugs were found to block learned (conditioned) responses and not affect innate or unlearned responses, and the term "tranquilizer" was coined to describe this new group of drugs. It subsequently developed, however, that the major action and use of this group of drugs in humans were for the treatment of psychotic individuals. They are therefore now known in human medicine as the antipsychotic or neuroleptic drugs, and "tranquilizer" is considered obsolete. The term tranquilizer, however, is still widely and rather loosely used in equine circles to describe any drug that allows a horse to remain conscious and standing but enables a veterinarian to take substantial liberties with it. It is not, however, in this equine usage, in any way a precise term.

Perhaps the subtlest use of this rather heterogenous group of depressant or tranquilizing drugs is to "take the edge" off a horse. This is primarily a horseman's use of these drugs. What the horseman requires in this circumstance is a drug that will allow a somewhat nervous horse to perform in a relaxed manner in an unusual or challenging environment. Thus, a "washy" horse, which gets excited and "runs his race" in the paddock before the actual race, might benefit from being "touched" with (i.e. administered a small dose of) a tranquilizer pre-race. The idea in this case is to give a horse a dose that will keep him from being hyperexcitable in the paddock but will allow him to run "up to form" in the actual race. Other circumstances where a small dose of a tranquilizer might help is for a horse that does not take readily to starting gates, for horses that tense up and "ease over jumps" better on a small dose of tranquilizer, and for show horses. Similarly, trainers will sometimes use a small dose of a tranquilizer when first saddling a horse or teaching him to load or use a starting gate. All of these uses require very subtle drug effects and are outside the usual "clinical" uses of "tranquilizers" as drugs. Another area where "tranquilizers" have been used is, of course, in the "nobbling" or stopping of horses. In this case, a slightly larger dose of "tranquilizer" is used, the objective being to reduce the speed of the horse to the point at which he must lose the race, without affecting him to the point that he is clearly tranquilized and scratched from the race.

A somewhat more substantial "tranquilizing" effect is that which is required for

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The Pharmacology of Furosemide in the Horse
I. Effects on the Disposition of Procaine, Methylphenidate, Phenylbutazone, and Pentazocine

Thomas Tobin, D.V.M., Ph.D.
Brian L. Roberts, B.Sc.
J. Richard Miller, B.Sc.

From the Kentucky Equine Drug Research Program and The Graduate Program in Toxicology, Department of Veterinary Science, University of Kentucky, Lexington, KY 40506.

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The effects of furosemide (1 mg/kg IV) on the disposition of procaine, methylphenidate, phenylbutazone, and pentazocine in Thoroughbred horses were investigated. Furosemide did not reduce plasma levels of procaine, methylphenidate, or phenylbutazone and had little effect on urinary concentrations of procaine and methylphenidate. Furosemide reduced urinary concentrations of phenylbutazone and the major glucuronide metabolite of pentazocine about 40 to 50-fold.

These results and experience in our laboratory show that furosemide may substantially reduce the probability of detection of phenylbutazone and pentazocine in urine. For phenylbutazone, this problem may be approached either by determining plasma levels of the drug or by using more sensitive detection methods in urine. For pentazocine and other drugs excreted as glucuronides, the solution is not clear-cut. Because of limitations in current analytical technology, it appears likely that furosemide treatment may substantially reduce the probability of detection of many drugs excreted as water soluble metabolites.

Introduction

The extensive use of furosemide* in horses racing in some jurisdictions raises questions as to its effect on other drugs with which it may be administered. Thus furosemide might affect the pharmacological response to, disposition of, or urinary elimination of other drugs.[1] Further, and of particular interest to horsemen and racing authorities, furosemide treatment may affect routine forensic screening for other drugs.[1] This study was designed to provide answers to some of these questions.

*"Lasix", Hoechst-Roussel Pharmaceuticals, Somerville, N.J.

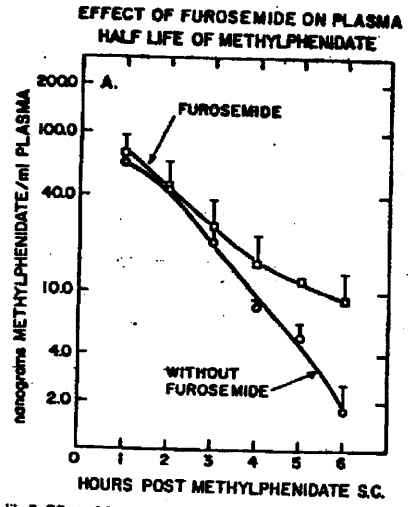

Fig 7—Effect of furosemide on plasma levels of methylphenidate. Methylphenidate, 0.33 mg/kg, was administered subcutaneously to 4 Thoroughbred mares at indicated zero time. The open circles (○ - ○) show plasma levels after methylphenidate alone, the open squares (□ - □) plasma levels after administration of 1 mg/kg of furosemide at 2 hours. The slopes of a least squares fit to each set of points are significantly different (*t* test, $P < 0.01$). All points are the means ± standard error of the mean of experiments of 4 different horses.

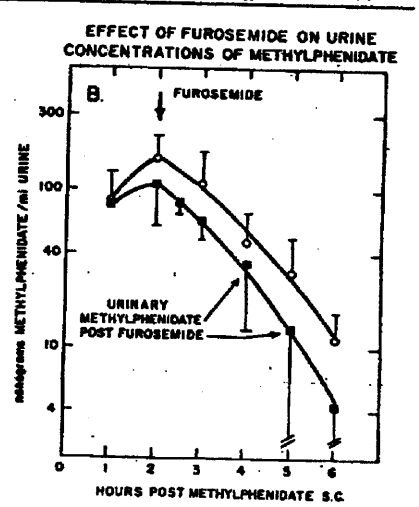

Fig 8—Effect of furosemide on urinary concentrations of methylphenidate. The open circles (○ - ○) show urinary concentrations of methylphenidate after 0.33 mg/kg of methylphenidate subcutaneously. The solid squares (■ - ■) show urinary concentrations when 1 mg/kg furosemide was given IV 2 hours after the methylphenidate. All experimental points are the means of determinations on at least 4 horses ± the standard error of the mean.

drawn to this phenomenon when routine screening of racetrack samples from furosemide-treated horses showed phenylbutazone consistently present in plasma samples but only occasionally in urine.[1] As shown in the experiments of Figs 2, 3, and 4, phenylbutazone concentrations are reduced to close to the limit of routine UV screening and flame ionization detection, while plasma levels are unaffected. Furosemide treatment, therefore, can cause a potentially important reduction in urinary concentrations of phenylbutazone for a period of hours after its administration.

The pharmacological basis for this effect on urinary concentrations of phenylbutazone is unclear. It appears to be a combination of (a) a dilution effect and (b) a reduction in tubular transport of phenylbutazone. These conclusions are based on the following observations.

While plasma and urinary concentrations of phenylbutazone are usually quite similar (i.e., about 40 μg/ml in plasma and urine after 6.6 mg/kg to a horse),[1] this equivalence of blood and urine levels is deceptive. Because phenylbutazone in plasma is 95% plasma protein bound (Fig 1), only about 2 μg/ml or less of phenylbutazone is free to be excreted by glomerular filtration. This filtered 2 μg/ml must then be increased to 40 μg/ml by (a) the renal concentrating mechanism, or (b) active tubular secretion.

Simple interference with the renal concentrating mechanism would be sufficient to account for most of the observed reduction in phenylbutazone concentration in urine. However, simply changing urine volume would not affect the total rate of excretion of phenylbutazone, while the data of Fig 4 shows a reduction in total urinary output of phenylbutazone. This means less net entry of phenylbutazone into the increased volume of urine in the presence of furosemide. Possible mechanisms for this effect include reduced glomerular filtration, less diffusion of phenylbutazone into urine, and/or less tubular transport of phenylbutazone. Because of the acidic nature of both phenylbutazone and furosemide, an action at the level of the tubular transport appears likely to account for the data of Fig 4.[5]

Many drugs used in the illegal medication of racing horses are detected in urine primarily as their glucuronide metabolites.[1] Glucuronide metabolites enter urine either

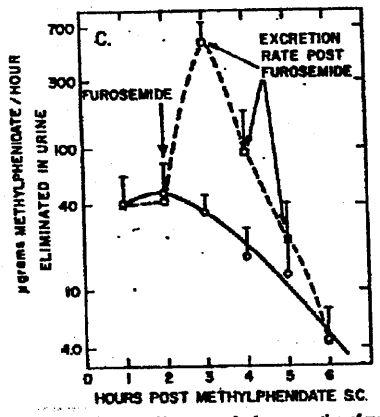

Fig 9—Effect of furosemide on rate of urinary excretion of methylphenidate. The open circles (O - O) show the rate of urinary excretion of methylphenidate (0.33 mg/kg) alone, or after 1 mg/kg furosemide IV (open squares, □ - □) 2 hours after administration of furosemide.

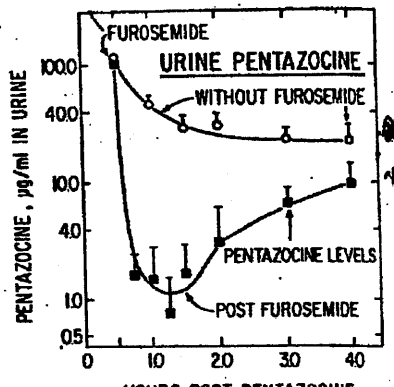

Fig 10—Effect of furosemide on urinary concentrations of a glucuronide metabolite of pentazocine. Horses were injected IV with 0.33 mg/kg pentazocine at indicated zero time. The open circles (O - O) show urinary concentrations of a glucuronide metabolite of pentazocine in control horses. The solid squares (■ - ■) show urinary concentrations of this metabolite in horses treated with 1 mg/kg of furosemide IV 30 minutes postpentazocine. All data points are means ± standard error of mean of experiments on at least 4 different horses.

by glomerular filtration or by the organic acid transport system. Since these metabolites are poorly lipid soluble, they are concentrated in urine by the renal concentrating mechanism. Urinary concentrations of these drugs may thus be many times greater than plasma levels of these drugs. Further, because sensitive analytical methods are not available for many drugs excreted as glucuronides, this concentrating effect is important in that it enables analysts to identify many of these drugs during routine forensic screening.

Pentazocine is rapidly and apparently completely metabolized to a glucuronide metabolite in the horse. Figure 10 shows the effect of furosemide treatment on urinary levels of this metabolite. Urinary concentrations are markedly reduced by about 50-fold, at peak effect in drug treated animals. As with phenylbutazone, drug levels recovered slowly and were about 50% of control levels at 4 hours postdosing. The experiment shows marked effects of furosemide treatment on urinary levels of a glucuronide metabolite in the horse.

It appears likely that this diluting effect observed for the glucuronide metabolite of pentazocine will apply to other drugs excreted as glucuronides, such as apomorphine, the phenothiazines, fentanyl, and other narcotic drugs. In good agreement with this probability are some recent experiments by Ozog[10] who reports substantial reductions in concentrations of urinary apomorphine in furosemide-treated horses, supporting the hypothesis that glucuronide metabolites are particularly susceptible to diuretic dilution.[11] Further, because many of these drugs are active at extremely low dose levels (phenothiazines) and because analytical methodology for their detection is either marginal or nonexistent (fentanyl), this effect of furosemide on glucuronide metabolites is likely to be highly significant for routine analytical work. Abolition of the renal concentrating effect by furosemide renders detection of these drugs in urine as technically challenging as is their detection at the low levels observed in plasma.

In some recent experiments Frey *et al.*[4] concluded that the diuretic bumetanide "did not interfere with the detection of doping drugs." The results reported here and experience in our Equine Drug Testing Laboratory suggests that this conclusion is in error.[13] Because sample size is limited in routine dope testing, changes in urinary concentrations of the magnitude reported in this report may be critical for drug testing and can mean the difference between success and failure.

As a practical matter, these results show that administration of furosemide can reduce the concentrations of some drugs or their metabolites in urine from 40 to 50-fold. The practical significance of this effect depends entirely on the techniques and skill of the analyst. In our testing laboratory, more sophisticated gas chromatographic methods had to be substituted for our UV and thin layer screening for phenylbutazone. Given adequate equipment, it appears unlikely that detection of phenylbutazone and other acidic drugs should be seriously compromised by furosemide. Further, plasma levels of these drugs are not likely to be affected, and detection of plasma levels of acidic drugs is readily possible with current technology.[11]

The situation for drugs such as morphine, acepromazine, and apomorphine, which are detected as glucuronide metabolites, is far less clear-cut. Since these drugs are effective at very low concentrations, the analyst is operating close to the detection limits of available methodology, and the urinary concentrating mechanism assists in their detection. It seems highly likely that use of furosemide in combination with these drugs substantially reduces the probability of their detection, given the current state of the art of drug screening. Blood testing could recommended where the use of furosemide within 4 hours of racing is permitted,[2] is not likely to be helpful with this group of drugs, since satisfactory blood testing methods are not available for many members of this group.

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