

II. PHARMACOLOGICAL STUDIES OF DRUGS IN HORSES PHENYLBUTAZONE AND OTHER DRUGS IN HORSES RACING UNDER A CONTROLLED MEDICATION PROGRAM

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ABSTRACT

A study of blood and urinary concentrations of phenylbutazone in horses racing under controlled medication rules was carried out. Under these rules free administration of phenylbutazone was permitted, while administration of furosemide was stipulated as being limited to I.V. administration four hours or more pre-race. Plasma and urinary levels of phenylbutazone in 58 post-race samples were monitored. Plasma levels were determined by EC detection, while urinary levels of "phenylbutazone and its metabolites" were estimated by measuring the U.V. absorbance of an acidic extract of the urine sample at 258nm. Plasma levels of phenylbutazone average about 9.0 ug/ml, with an approximately symmetric distribution about the mean, and an upper range of about 24 ug/ml. Urinary concentration of phenylbutazone and its metabolites were highly variable, ranging from 0 to an apparent 350 ug/ml of "phenylbutazone and its metabolites" in urine. There was, however, no correlation ($r = .14$) between the actual plasma level of phenylbutazone and the urinary levels of "phenylbutazone and its metabolites" in these horses.

Because 21 of the horses in this study had been treated pre-race with furosemide, it was possible to determine the magnitude of the diluting effect of furosemide on the concentrations of "phenylbutazone and its metabolites" in equine urine. The results showed that the mean concentration of phenylbutazone in the urine of furosemide-treated horses was reduced by less than 50%. Further, the "phenylbutazone" concentrations in the urines of furosemide-treated horses formed a subpopulation bracketed by the range of "phenylbutazone" concentrations in the untreated horses.

These results suggest that the free use of phenylbutazone in horses does not lead to overdosing with this drug. They show that the urinary levels of "phenylbutazone and its metabolites" as traditionally measured in U.S. racing laboratories bears little relationship to plasma levels of this drug. Use of furosemide led to less than a 50% reduction in the concentration of "phenylbutazone and its metabolites" in urine, despite the fact that the time or route of its administration was not monitored.

In the 1970's, in the United States of America, many horse racing jurisdictions elected to allow the use of certain medications, most commonly phenylbutazone and furosemide, under controlled conditions^{1,2,3}. While the approval of these drugs for use has generated considerable debate, few of the jurisdictions involved have reported on the blood levels of drugs found in horses racing under such medication programs. In this report we present some data gathered on post-race blood and urine samples from horses racing under a controlled medication program in the United States.

In these experiments, about 58 post-race blood and urine samples were collected and analyzed. All samples were routine post-race samples¹ drawn within about 35 ± 20 minutes after post time. Blood samples were drawn into VACUTAINER (R) tubes, while urine samples were collected by spontaneous voiding. Once obtained, they were cooled at 0°C and shipped to the drug testing laboratory at the University of Kentucky where urinary concentrations of "phenylbutazone and its metabolites" were measured by UV analysis¹. Plasma levels of phenylbutazone were measured by gas chromatography in the Kentucky Equine Drug Research Program laboratory¹.

Fig. 1 shows plasma levels of phenylbutazone in these post-race blood samples. The median plasma level in these animals was about 9 ug/ml, with a relatively symmetrical distribution about the mean. The highest blood levels found in 2 horses were above 20 ug/ml.

In order to estimate the therapeutic significance of a mean blood level of 9.0 ug/ml, we compared these blood levels with phenylbutazone blood level data from the work of a number of authors.^{4,5,6,7,8} At about 12 hours after dosing in the experiments, the blood levels of most horses were in range of about 9.0 ug/ml (Fig. 2). Since the optimal pharmacological effect of phenylbutazone is obtained at about 12 hours after dosing¹, these data suggest that phenylbutazone use in Kentucky racing is consistent with its use for optimal therapeutic effect.

Furosemide is the drug of choice in the treatment of epistaxis or exercise-induced pulmonary haemorrhage (EIPH) in racing horses⁹. As such, it is approved for use in a number of racing jurisdictions.¹ The principal problem with this approval is that furosemide may act to dilute out certain drugs or drug metabolites in equine urine.^{10,11} In an attempt to estimate the practical significance of this problem, we

analyzed the effect of furosemide pre-treatment on the concentrations of phenylbutazone and its metabolites in equine urine.

Phenylbutazone was chosen for this test both because it is commonly present in urine samples and because it is apparently fully susceptible to the diluting effects of furosemide.^{4, 14, 15} On the principle that any "metabolites" of phenylbutazone are likely to be more susceptible to dilution than phenylbutazone itself, we measured the UV absorbance of urines from phenylbutazone-treated horses at 258 nm. The absorbances measured in each urine sample were compared with a phenylbutazone standard curve and the results expressed as a concentration of phenylbutazone. This is a standard method used in many North American drug-testing laboratories to quantitate "phenylbutazone and its metabolites" in equine urine.

As shown in Fig. 3, in the absence of furosemide there was no clear-cut distribution of the urine levels of phenylbutazone and its metabolites. Urine levels ran from 40 to 360 ug/ml. with apparently equivalent numbers in each grouping. The mean level of phenylbutazone and its metabolites in the non-furosemide treated horses was 181 ug/ml.

In the furosemide-treated horses, the distribution of urinary concentrations was compressed, with the largest numbers occurring in the 40 to 50 ug/ml range, and extending up to about 180 ug/ml. The mean urinary level of phenylbutazone in these horses was about 100 ug/ml, more than half of that in the untreated horses. The data suggest that use of furosemide in racing horses is associated with about a 50% reduction in the urinary concentrations of phenylbutazone and its metabolites.

The data of Fig. 3 were obtained from horses racing under the Kentucky Rules of Racing which specify that furosemide should be administered at least 4 hours prior to post-time. To determine the duration of the diluting effect of anti-epistaxis doses of furosemide, we studied the effects of

its administration on urinary levels of fentanyl, morphine and phenylbutazone.

For these studies, the dose of furosemide selected was about 0.4 mg/kg, which is the dose commonly used in the treatment of epistaxis. The horses were pre-treated with either phenylbutazone, fentanyl or morphine and then challenged with furosemide IV. In each case (Fig. 4, 5, 6), urinary concentrations of the drug rose toward control values, to become statistically indistinguishable from control values within 2-3 hours after dosing with furosemide. The experiments suggest that by 3 hours after furosemide treatment the diluting effects of an anti-epistaxis dose of furosemide are minimal.¹¹

An important category of drug metabolites whose concentration in urine is diluted out by furosemide are the glucuronide metabolites. Because these glucuronides are poorly lipid soluble, they are concentrated in urine. Prior to analysis these drugs must be released from their glucuronide conjugates.

Recent work from this laboratory^{12, 13} indicates that B-glucuronidase from *Patella vulgata* provides a high yield of free glucuronide. Since this enzyme preparation can withstand 65°C, its use provides rapid hydrolysis of glucuronide complexes.

In conclusion, these experiments have shown that horses racing under a controlled medication program had median blood levels of about 9.5 ug/ml of phenylbutazone. This blood level appears to correspond with clinical doses of this drug about 12 hours prior to post-time, which is the time period over which one may expect an optimal pharmacological effect to develop. Studying the action of furosemide on urinary levels of phenylbutazone and its metabolites in these horses suggests that the drug diluting effects in post-race urines amounted to about a 50% dilution effect. If the dose of furosemide is the dose used in the treatment of epistaxis, the diluting effects are over within less than 3 hours.

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FOOTNOTE:

Published as Kentucky Agricultural Experiment Station Article 81-4-169 with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication 69 from the Kentucky Equine Drug Research Program and the Graduate Toxicology Program, University of Kentucky, Lexington, KY 40546-0076.

Fig. 1: Phenylbutazone concentrations in the blood of 58 racing horses.

In a study in a midwestern racing state, blood levels of phenylbutazone in 58 racing horses were measured. The vertical bars show the numbers of horses with blood levels of phenylbutazone in the indicated ranges. The dotted line represents an approximate distribution curve drawn by eye. The data shows that the median blood level of phenylbutazone in the blood of these horses was about 9 ug/ml, with no blood levels above 24 ug/ml. The blood levels of phenylbutazone in these horses, therefore, cluster around the level to be expected in the blood of horses after therapeutic doses of this drug. (Reproduced with permission from "Drugs and the Performance Horse", Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981).

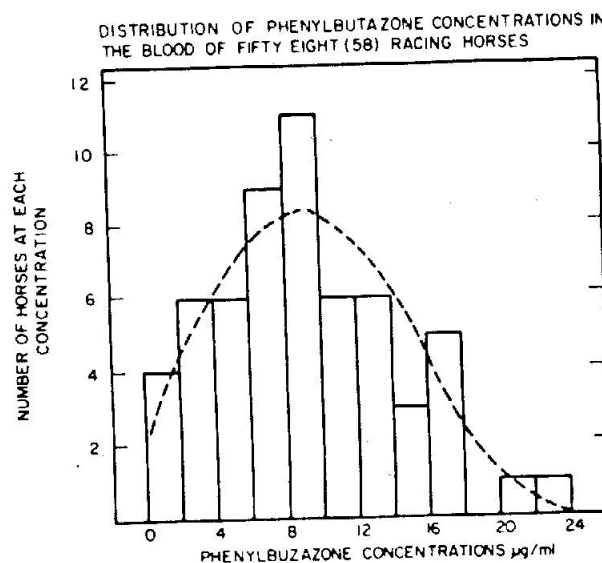


Fig. 2: Plasma levels of phenylbutazone after different dosage schedules.

The symbols show the plasma levels of phenylbutazone observed after administration of different dosage schedules of this drug to horses by different workers, as indicated below. The data show that therapeutic dosage schedules of phenylbutazone are associated with blood levels of this drug of about 10 $\mu\text{g}/\text{ml}$ at the time of optimal therapeutic effect, which occurs at about twelve hours after dosing. The key presented below gives the data source, number of horses, dose of phenylbutazone, and method of dosing.

- o Tobin, unpublished data (1/80); $n = 2$. 4 mg/kg IV, GC method.
- Tobin, Am J Vet Res. **38**: 123, 1977; $n = 4$. 6.6 mg/kg IV, spectrophoto. method.
- Tobin, Am J Vet Res. **38**: 123, 1977; $n = 4$. 6.6 mg/kg IV, GC method.
- Maylin, Proc 20th Meeting AAEP, 1974, pp 243-48; $n = 3$. 4.4 mg/kg IV, GC method.
- △ Maylin, Proc 20th Meeting AAEP, 1974; same experiment, multiple doses (0 hr, 24 hr, 48 hr, 72 hr); data from 72 hr injection.
- ▲ Piperno *et al.* J Am Vet Med Assoc. **153**: 195, 1968; $n = 6$. 4.4 mg/kg IV, spectrophoto. method.
- ▽ Piperno *et al.* J Am Vet Med Assoc. **153**: 195, 1968; $n = 1$. 8.8 mg/kg IV, spectrophoto. method.
- ▼ Piperno *et al.* J Am Vet Med Assoc. **153**: 195, 1968; $n = 1$. 17.6 mg/kg IV, spectrophoto. method.
- ◇ Piperno *et al.* J Am Vet Med Assoc **153**: 195, 1968; $n = 1$. 4.4 mg/kg IV, spectrophoto. method.
- ◆ Tobin *et al.* J Equine Med Surg **1**: 402, 1977. $n = 7$. 6.6 mg/kg, GC method.

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PLASMA PHENYLBUTAZONE LEVELS AFTER IV AND ORAL ADMINISTRATION

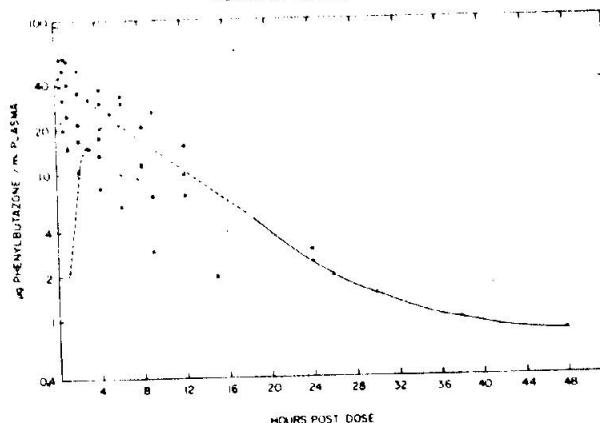


Fig. 3: Urinary concentrations of phenylbutazone and its metabolites in furosemide-treated and untreated horses.

Urine samples from horses racing in a midwestern state were analyzed for their content of phenylbutazone or phenylbutazone metabolites by measuring the absorbance of acidic extracts of these urines at 258nm. The upper panel shows urinary concentrations of "phenylbutazone" or its metabolites in furosemide-treated horses, while the lower panel shows the urinary concentrations in horses not treated with furosemide. The mean "phenylbutazone" concentration in the furosemide-treated horses was more than 50% of that in the control horses, and a number of untreated horses had phenylbutazone concentrations below those of the control horses. (Reproduced with permission from "Drugs and the Performance Horse", Charles C. Thomas, Publishers, Springfield, Illinois 62717, 1981.)

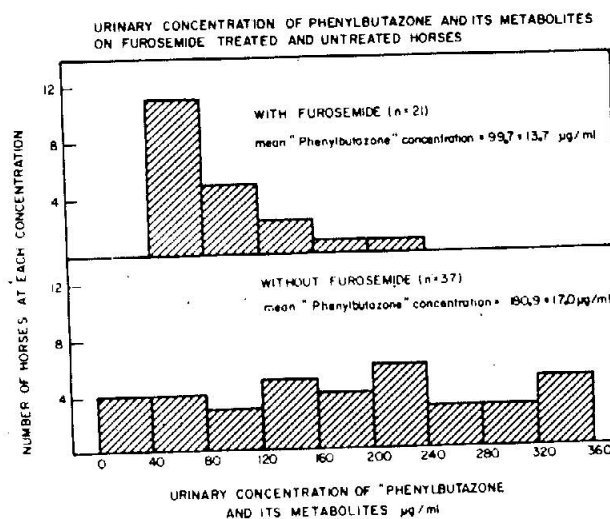


Fig. 4: Effect of furosemide treatment on urinary phenylbutazone concentration.

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 (● — ●).

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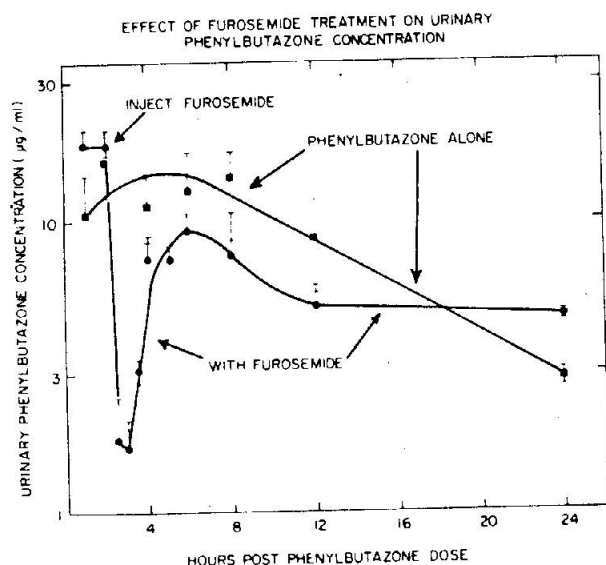


Fig. 5: Furosemide effect on urine fentanyl levels.

Urinary fentanyl levels were determined by RIA in horses given fentanyl alone (shown by the solid circles, ● — ●) and given fentanyl and furosemide (shown by the open circles, ○ — ○).

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FUROSEMIDE EFFECT ON URINE FENTANYL LEVELS

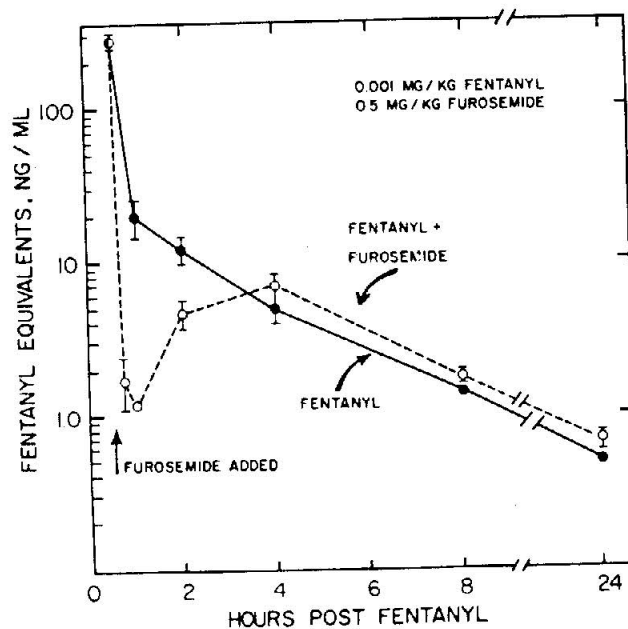
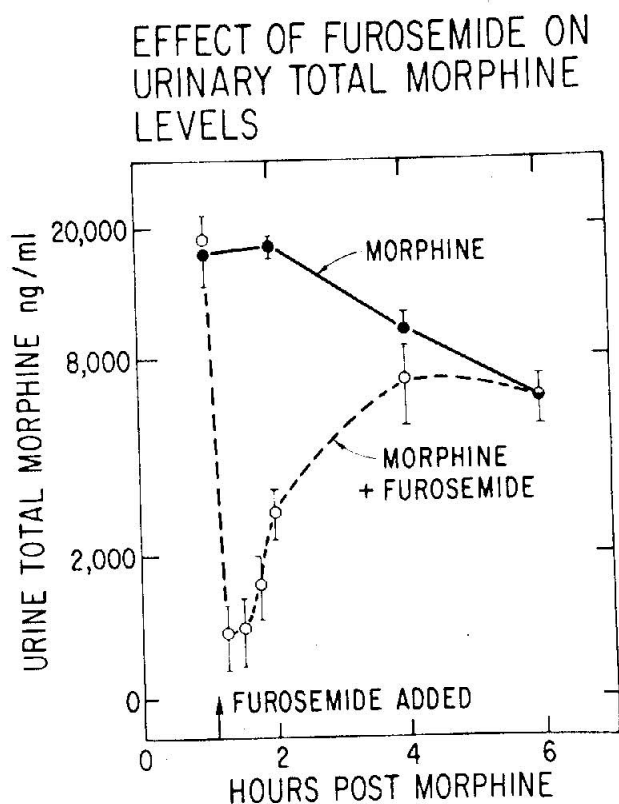


Fig. 6: Effect of furosemide on urinary total morphine levels.

Horses were dosed with 0.1 mg morphine per kg body weight and urine levels of morphine were analyzed following hydrolysis with *B*-glucuronidase (shown by the solid circles, • — •). 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, ○ — ○).

(Reproduced by permission from Combie *et al.*, *Journal of Equine Veterinary Science*, 1, 1981).



DISCUSSION

MOSS: Is there a correlation between the non-furosemide graph and the furosemide graph? Is there a correlation of the different urine flow rates?

TOBIN: We have not done a rigorous analysis. However, it would appear for the glucuronides that there is more than simple dilution.

REILLY: How did you acquire the *Patella vulgata*?

TOBIN: I believe it came from Sigma, but I am not certain.

SMITH: On the *B*-glucuronidase hydrolysis, it was interesting to "steam" it up by raising the temperature. In working with other species, you sometimes find that urine samples are refractory to the effect of *B*-glucuronidase because of the presence of natural inhibitors. Therefore, it is not always reliable unless you include some sort of control. Did you encounter this with horse urine samples?

TOBIN: We concluded quite some time ago that there were very many natural inhibitors present, and heat gave us an increased reaction rate. This is why we also chose to increase the concentration of enzymes. I am not precisely sure of the type of experiment of which you are thinking.

SMITH: What we normally do, for example, is to include phenolphthalein glucuronide just to show that it is working. Sometimes it does not.

TOBIN: When this work was completed, the researcher went ahead and worked on glucuronides of some other drugs, and the *Patella vulgata* remained superior, so that would cover that question to some extent. However, we did not have a standard glucuronide in the system.