

## PHENYLBUTAZONE LEVELS IN POST-RACE SAMPLES

### FROM HORSES RACING IN KENTUCKY: A REVIEW

Teresa Houston, PhD; Sylvia Chay, BSc; W.E. Woods, MSc;  
Jerry W. Blake, PhD and Thomas Tobin, MVB, MRCVS

#### Introduction

When I came to Kentucky in about 1975 to start the Kentucky Equine Drug Research Program, I was told that I could work on any drug that I wished except phenylbutazone (PB). According to my authority, phenylbutazone was a drug whose characteristics were well known and was of little interest to regulators. Since then, however, phenylbutazone has become the drug of greatest interest in racing and a major part of our work is devoted to this problem. Among the more recently perceived problems with phenylbutazone is the thought that it may "mask" the detection of other drugs and the Kentucky Equine Drug Research Program is currently working on a project to determine the impact, if any, of the supposed masking ability of this drug on the efficacy of equine drug testing (Tobin, 1981, Takade, 1982, Woods, 1984a, 1984b).

Masking is thought to occur when the presence of one drug in a sample interferes with the detection of another (Woods, 1984a, 1984b). Usually this is thought of as occurring in thin layer chromatographic systems and the masking is considered to be due to simple physical obstruction of the visualization of the "masked" drug by either phenylbutazone or one of its metabolites (Woods, 1984b). For this problem to be studied in the laboratory, however, one needs to know the levels of both phenylbutazone and its metabolites actually found in the blood and urine of horses post-race. This report represents the results of such a survey done on Thoroughbred horses racing in Kentucky in the spring of 1983. In addition, we have presented data on the levels of phenylbutazone found in horses after dosing with standard schedules of phenylbutazone in order that these levels may be compared with those found in actual post race urine samples.

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Correspondence should be addressed to Dr. Tobin.

## Materials and Methods

Phenylbutazone (PB) and oxyphenbutazone (OPB) were obtained from Ciba Pharmaceuticals (Summit, NJ) and  $\gamma$ -hydroxyphenylbutazone ( $\gamma$ -OHPB) from Ciba-Geigy (Switzerland). Liquid chromatographic grade methanol and water were obtained from Burdick-Jackson (Alltech, Deerfield, IL). All other solvents and reagents used were of analytical grade from Fisher Scientific Company (Louisville, KY).

All blood and urine samples tested were post-race blood and urine samples or samples from cooperative studies submitted to the Kentucky Equine Drug Testing and Research Laboratories by the Kentucky State Racing Commission or study participants. Blood samples were drawn and urine samples collected when the horses voided urine post-race. All post-race samples were stored at 4° C and shipped to the University of Kentucky for analysis within 48 hours. All samples from interlaboratory studies were received as frozen plasma samples. Phenylbutazone is a permitted medication for Thoroughbred horses racing in Kentucky.

A Beckman 341 liquid chromatograph equipped with a 421 controller, an Altex model 100 pump and an Altex model 153 UV detector (254 nm) were used. A 20  $\mu$ l loop was used on the 210 sample injector valve. An ultrasphere-ODS 5 $\mu$  column (4.6 mm x 25 cm) with a guard column (Pellicular C-18, 4.6 mm x 5 cm, Alltech) between the injector and analytical column was used for the separation of the compounds. All interlaboratory samples were analyzed as described by Chay *et al.*, (1984).

The pH of the urine was measured on a Fisher Accumet Model 230 pH meter when the samples were received in the laboratory.

The chromatographic procedure used was based on that described by Marunaka *et al.*, (1980). The mobile phase was a linear gradient of 50% methanol-50% 0.01 M sodium acetate (pH 4) as the initial concentration and 100% methanol (at 5%/min) as the final concentration. The column was maintained at room temperature with a flow rate of 1.0 ml/min and the eluted compounds were recorded by the detector at 254 nm.

Blood samples were collected in 20 ml Vacutainer tubes containing potassium oxalate and sodium fluoride. Urine samples were collected in glass jars when the horses voided. To 1 ml of plasma was added 4 ml saturated  $\text{KH}_2\text{PO}_4$  and 6 ml dichloromethane at room temperature. The samples were rotoracked 6 min, centrifuged for 2 min at 2000 rpm and the organic layer was transferred to a clean tube and evaporated to near dryness in a water bath (65° C). The samples were completely dried under a stream of  $\text{N}_2$ . To the residue, 100  $\mu$ l of methanol were added and 20  $\mu$ l of the sample injected onto the column. The urine samples were prepared in a similar manner except 4 ml of pH 3.3 saturated  $\text{KH}_2\text{PO}_4$  was added.

Standard curves were prepared for the determination of phenylbutazone, oxyphenbutazone and  $\gamma$ -hydroxyphenylbutazone by adding known amounts of authentic standards to blank plasma or urine and assaying by the same extraction procedure. Concentration ranges of 0.5 - 50.0  $\mu$ g/ml were used. Peak heights were plotted against the concentration.

## Statistical Analysis

The frequency distributions of plasma and serum levels of phenylbutazone and its metabolites were analyzed for normalcy by using the Shapiro-Wilk's Statistic (Chay et al., 1983). The best fit transformation was determined and a distribution curve was estimated using the methods and moments based on the calculated mean and standard deviation. The probability of attaining a given plasma concentration of phenylbutazone and oxyphenbutazone after different dosing schedules was then calculated using this calculated mean and standard deviation.

All data are described as the range and mean of the values reported. For those distributions which could be logarithmically transformed to normal distributions, the standard deviation represents the antilog of the standard deviation of the logarithmically transformed data. Where the data did not normalize when log-transformed, the arithmetic standard deviation is presented.

## Results and Discussion

The plasma levels of phenylbutazone in 182 horses racing in Kentucky are shown in Figure 1. Of these horses about 38 had no detectable phenylbutazone in their blood, the modal level was between 1-2  $\mu\text{g/ml}$  and the mean blood level was about 3.5  $\mu\text{g/ml}$ . The overall distribution was log-normal and the highest plasma levels observed in these horses were about 14  $\mu\text{g/ml}$ . These levels compare well with those observed after dosing with recommended daily dosing schedules of phenylbutazone (Figures 4, 5 and Table 1).

The plasma levels of oxyphenbutazone found in these horses are presented in Figure 2. No oxyphenbutazone was detected in the plasma of about 28 horses, the modal level was between 1 and 2  $\mu\text{g/ml}$  and the highest plasma level observed was about 13  $\mu\text{g/ml}$ . Again the distribution was well fitted by a log-normal distribution and the plasma levels were generally less than the corresponding levels of phenylbutazone observed in these samples.

The plasma levels of  $\gamma$ -hydroxyphenylbutazone found in these samples is presented in Figure 3. No  $\gamma$ -hydroxyphenylbutazone was detected in about 38 of these samples and the modal level of  $\gamma$ -hydroxyphenylbutazone was less than 1  $\mu\text{g/ml}$ . The highest level of  $\gamma$ -hydroxyphenylbutazone observed was about 8  $\mu\text{g/ml}$  and the overall distribution was well fitted by a log-normal distribution.

These plasma levels of phenylbutazone and oxyphenbutazone compared well with those observed in horses racing in California. In these horses (Figure 4) the mean plasma level of phenylbutazone was about 4.09  $\mu\text{g/ml}$ , the modal level was about 3.5  $\mu\text{g/ml}$  and the highest level observed was about 10  $\mu\text{g/ml}$ . The plasma level distribution was log-normal and compared well with the levels found in horses racing in Kentucky, as did the levels of oxyphenbutazone found in these horses (Table 1).

FREQUENCY DISTRIBUTION OF  
PLASMA PHENYLBUTAZONE  
LEVELS IN THOROUGHBRED  
HORSES RACING IN KENTUCKY

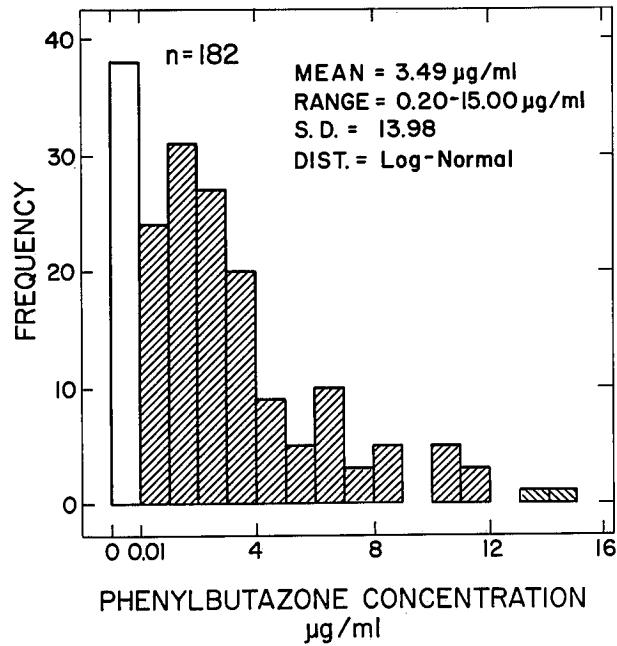


Figure 1

Frequency distribution of plasma phenylbutazone levels in 182 Thoroughbred horse racing in Kentucky.

The open bars represent those plasma samples in which no phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of phenylbutazone. The range of values observed was from 0.20 to 15.0 µg/ml, with a modal level between 1.0 and 2.0 µg/ml, and the mean level, not including those samples in which no drug was detected, of 3.49 µg/ml. The standard deviation of this distribution was 12.98 µg/ml, and the population was well fitted by a log-normal distribution, with a Shapiro-Wilk's statistic of > 0.15. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

FREQUENCY DISTRIBUTION OF  
PLASMA OXYPHENBUTAZONE  
LEVELS IN THOROUGHBRED  
HORSES RACING IN KENTUCKY

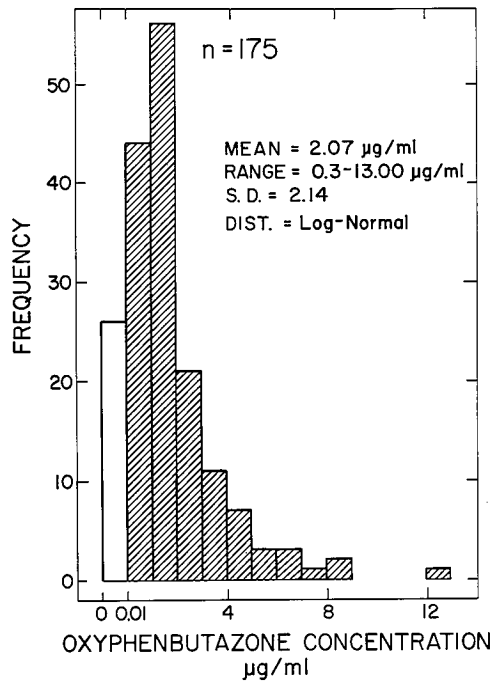


Figure 2

Frequency distribution of plasma oxyphenbutazone levels in 175 Thoroughbred horses racing in Kentucky.

The open bars represent those plasma samples in which no oxyphenbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration oxyphenbutazone. The range of values observed was from 0.30 to 13.0 µg/ml, with the modal level between 1.0 and 2.0 µg/ml, and the mean level, not including those samples in which no drug was detected, of 2.07 µg/ml. The standard deviation of this distribution was 2.14 µg/ml, and the population was well fitted by a log-normal distribution, with a Shapiro-Wilk's statistic of > 0.023. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

FREQUENCY DISTRIBUTION OF PLASMA  
 $\gamma$ -OH PHENYLBUTAZONE LEVELS IN  
 THOROUGHBRED HORSES RACING IN  
 KENTUCKY.

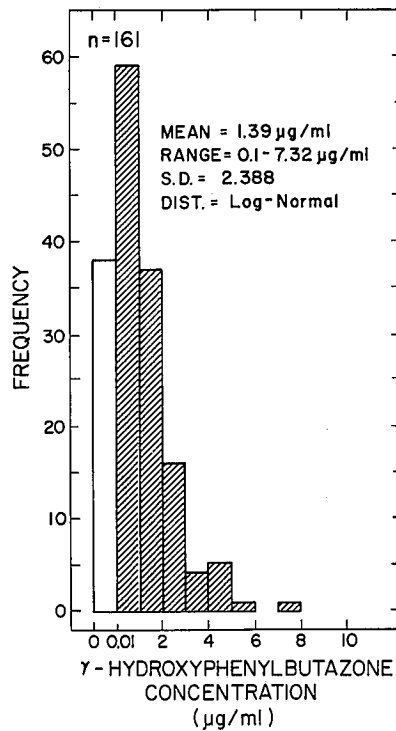


Figure 3

Frequency distribution of plasma  $\gamma$ OH-phenylbutazone levels in Thoroughbred horses racing in Kentucky.

The open bars represent those plasma samples in which no  $\gamma$ OH-phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of  $\gamma$ OH-phenylbutazone. The range of values observed was from 0.10 to 7.32  $\mu\text{g/ml}$ , with the modal level between 0.1 and 1.0  $\mu\text{g/ml}$ , and the mean level, not including those samples in which no drug was detected, of 1.39  $\mu\text{g/ml}$ . The standard deviation of this distribution was 2.39  $\mu\text{g/ml}$ , and the population was well fitted by a log-normal distribution, with a Shapiro-Wilk's statistic of  $> 0.124$ . (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

PLASMA LEVELS OF PHENYLBUTAZONE AND  
 OXYPHENBUTAZONE IN HORSES RACING  
 IN CALIFORNIA

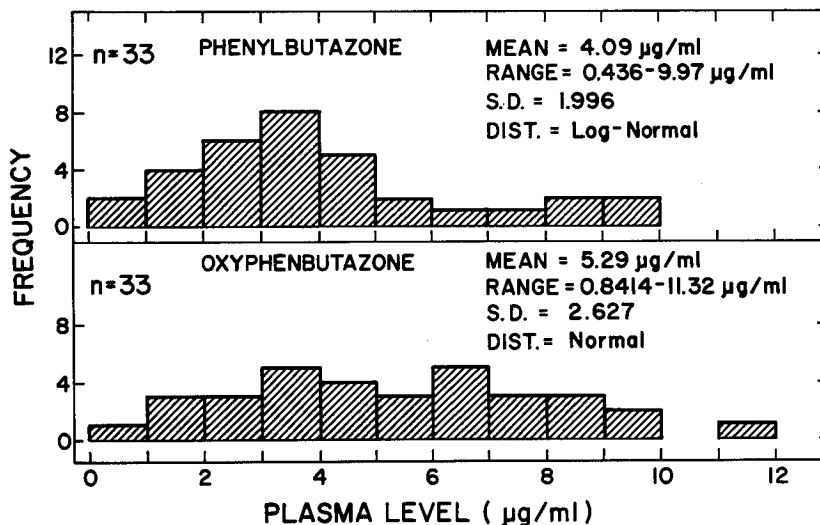


Figure 4

Plasma levels of phenylbutazone and oxyphenbutazone in horses running at Hollywood Park, California, 1983.

Thirty-four horses racing in Hollywood Park were dosed with phenylbutazone either orally or intravenously (IV) at 72, 48 and 24 hour prior to post. At 24 hours prior to post, 26 of these horses received 2 gm/horse, either orally or IV, 7 received 3 gm orally, and one received 4 gm orally. At 48 hours prior to post, 2 horses received no phenylbutazone, 26 received 2 gm either orally or IV, and 6 horses received no dose of phenylbutazone, 8 received 2 gm orally and one horse received 3 gm orally. One horse received 2 gm orally at 96 hours prior to post. The mean dosage rates, therefore, were 2.3 gm/1000 lbs at 24 hours, 2.0 gm/1000 lbs at 48 hours. The variable pattern of dosing at 72 hours is difficult to evaluate, but amounted to a mean dose of about 0.6 gm/1000 lbs. All levels were measured as plasma levels. The Shapiro-Wilk's statistic was 0.333 for the phenylbutazone distribution and 0.757 for the oxyphenbutazone distribution. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

Table 1

Comparison and Statistical Projections From Data on Horses Running in  
Kentucky and California and "No Race Day Medication Rule" Studies

Phenylbutazone*	Range ( $\mu\text{g/ml}$ )	Median ( $\mu\text{g/ml}$ )	Mode ( $\mu\text{g/ml}$ )	Mean ( $\mu\text{g/ml}$ )	5% ( $\mu\text{g/ml}$ )	1% ( $\mu\text{g/ml}$ )	0.1% ( $\mu\text{g/ml}$ )
Kentucky (Post-Race)	0.20- 15.00	2.52	1-2	3.49	11.07	15.8	35.8
Keystone (I.V.)	1.5- 9.88	4.00	3-4	4.75	8.8	11.8	16.2
Keeneland (Oral-I.V.)	1-14	5.16	2-3	5.32	10.3	18.6	23.5
California	0.44- 9.97	3.65	3-4	4.09	10.45	16.78	23.18
Florida (Serum)	0.27- 1.80	0.76	1-2	0.94	1.62	1.90	2.21

\*All data reported here refer to drug or drug metabolite concentrations from these studies as assayed by the Kentucky Equine Drug Testing and Research Programs.

In an interlaboratory study (Soma *et al.*, 1984), horses in training at Keystone Racetrack were dosed with 2 gm of PB/1000 lbs for four days intravenously. On day five plasma was drawn for analysis. The distribution of plasma levels of phenylbutazone in these animals is shown in Table 1. The plasma levels observed ranged from about 1 to 10  $\mu\text{g/ml}$ , with a modal level of about 3.5  $\mu\text{g/ml}$  and a mean level of about 4.75  $\mu\text{g/ml}$ . A statistical projection of these data show that about one in 1000 horses dosed with this schedule may be expected to yield blood levels above 16.2  $\mu\text{g/ml}$ . This blood level is comparable with those observed in these horses racing in Kentucky under "no race day medication" restrictions.

In another study (Chay *et al.*, 1984), we dosed horses with 4 gm/1000 lbs of phenylbutazone orally, either by bolus, stomach tube or as a paste preparation for three days, then with 2 gm/1000 lbs I.V. on day four. The plasma levels observed on day five, 24 hours after the last dose, are presented in Figure 5. The plasma levels varied from about 1 to 14  $\mu\text{g/ml}$ , with a modal level of about 2.5  $\mu\text{g/ml}$  and a mean blood level of about 5.3  $\mu\text{g/ml}$ . Based on a statistical projection of these data, one can predict that one horse in about 1000 will have a blood level above 23.5  $\mu\text{g/ml}$ . Again, these data are in good agreement with the blood levels of phenylbutazone and its metabolites found in the plasmas of horses post-race in Kentucky. These comparisons of the blood levels and the statistical projections developed from are presented in tabular form in Table 1.

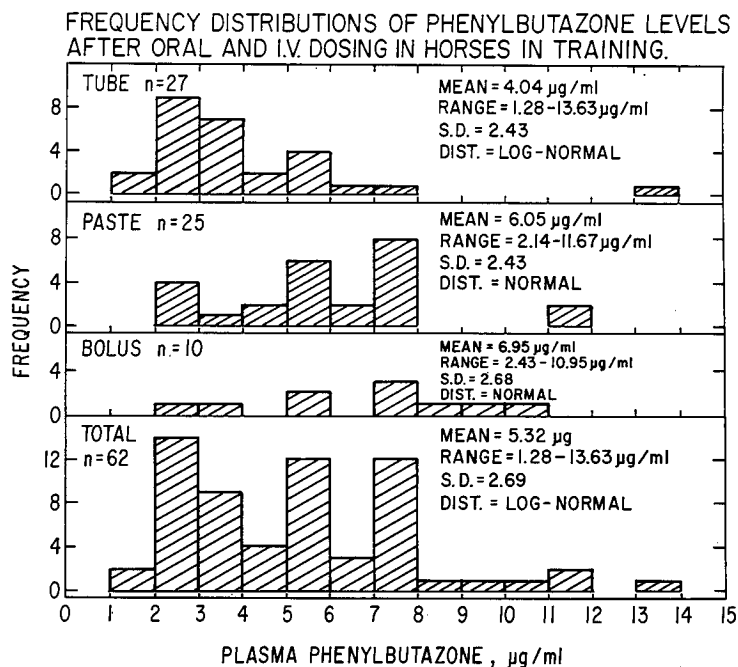


Figure 5

Frequency distribution of phenylbutazone levels after oral and intravenous dosing.

The vertical bars show plasma levels of phenylbutazone in horses after dosing with 8.8 mg/Kg of phenylbutazone orally and 4.4 mg/kg intravenously to Thoroughbred horses in training. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

The urinary concentrations of phenylbutazone found in the urines of horses racing in Kentucky are presented in Figure 6. No phenylbutazone was found in the urines of about 24 horses, and the modal level of phenylbutazone in these horses was less than 1 µg/ml. Thereafter the urinary concentrations of phenylbutazone fell rapidly, with very few urinary concentrations above 8 µg/ml. The greatest urinary concentrations observed in these horses was about 30 µg/ml, although this appeared to be an exceptionally high concentration. The urinary concentrations of phenylbutazone were well fitted by a log-normal distribution.

FREQUENCY DISTRIBUTION OF URINE PHENYL-BUTAZONE LEVELS IN THOROUGHBRED HORSES RACING IN KENTUCKY.

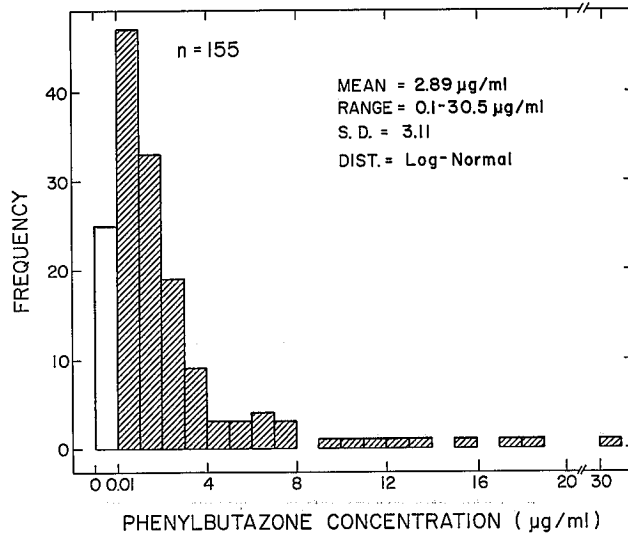


Figure 6

Frequency distribution of urine phenylbutazone levels in Thoroughbred horses racing in Kentucky.

The open bars represent the urine samples in which no phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of phenylbutazone. The range of values observed was from 0.20 to 30.5 µg/ml, with the modal level between 0.1 and 1.0 µg/ml. The mean level, not including those samples in which no drug was detected, was 2.89 µg/ml. The standard deviation of this distribution was 3.11 µg/ml and the population was well fitted by a log-normal distribution with a Shapiro-Wilk's statistic of > 0.15. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

The urinary concentrations of oxyphenbutazone observed in these horses are presented in Figure 7. No oxyphenbutazone was detected in the urines of 11 horses, with the modal concentration of oxyphenbutazone being less than 3 µg/ml and the range of concentrations observed (going up to) about 8 µg/ml. This distribution was not well fitted by a normal distribution, but rather appeared to be a bi-modal, with a secondary peak occurring at about 40 µg/ml.

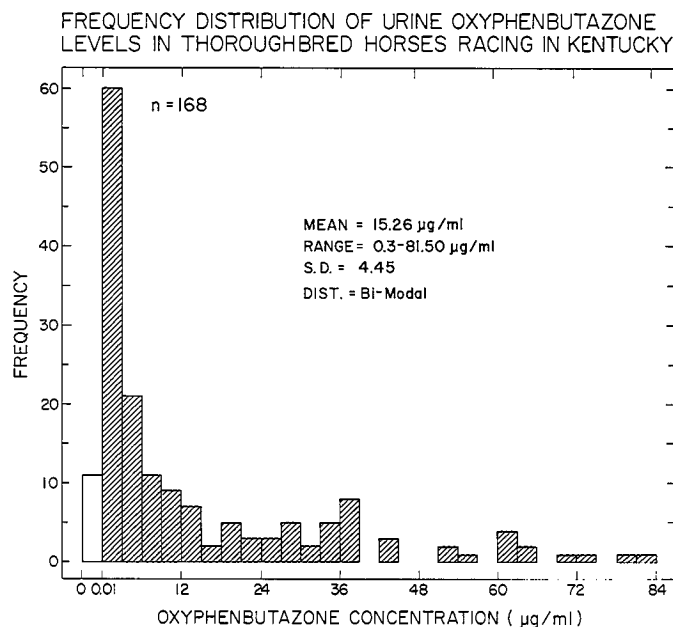


Figure 7

Frequency distribution of urine oxyphenbutazone levels in Thoroughbred horses racing in Kentucky.

The open bars represent those urine samples in which no oxyphenbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of oxyphenbutazone. The range of values observed was from 0.30 to 81.50 µg/ml, with the modal level between 1.0 and 3.0 µg/ml. The mean level, not including those samples in which no drug was detected, was 15.26 µg/ml. The standard deviation of this distribution was 4.45 µg/ml and the population was not fitted by a log-normal distribution (Shapiro-Wilk's statistic, < 0.01). (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

The urinary concentrations of  $\gamma$ -hydroxyphenylbutazone found in these samples is presented in Figure 8. No  $\gamma$ -hydroxyphenylbutazone was found in the plasma of about 11 horses and the modal level of the metabolite was less than 4 µg/ml. The mean level was about 21 µg/ml and the highest concentration was about 120 µg/ml. Again, the distribution was not well fitted by a normal or log-normal distribution and was described as indeterminate.

FREQUENCY DISTRIBUTION OF URINE  $\gamma$ -OH PHENYL-BUTAZONE LEVELS IN THOROUGHBRED HORSES RACING IN KENTUCKY.

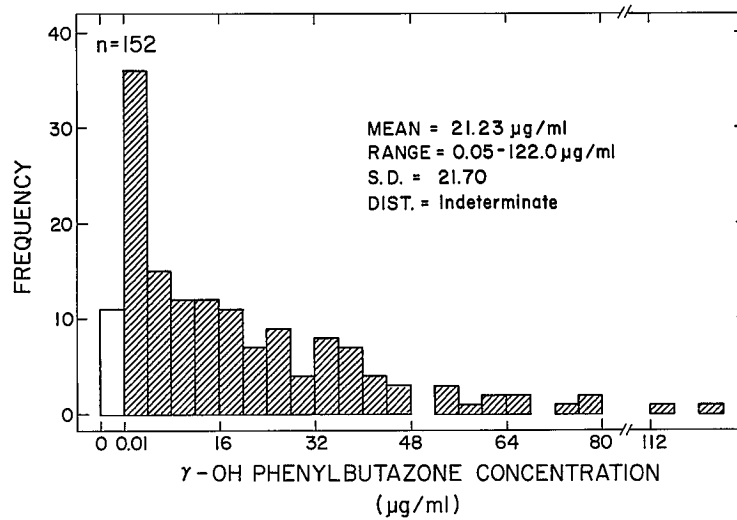


Figure 8

Frequency distribution of urine  $\gamma$ OH-phenylbutazone levels in Thoroughbred horses racing in Kentucky.

The open bars represent those urines in which no  $\gamma$ OH-phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of  $\gamma$ OH-phenylbutazone. The range of values observed was from 0.05 to 122.0  $\mu\text{g/ml}$ , with the modal level between 1.0 and 4.0  $\mu\text{g/ml}$ . The mean level, not including those samples in which no drug was detected, was 21.23  $\mu\text{g/ml}$ . The standard deviation of this distribution was 21.70  $\mu\text{g/ml}$  and the population was not fitted by a log-normal distribution (Shapiro-Wilk's statistic  $< 0.01$ ). (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

It appears from review of the urinary concentrations of phenylbutazone and its metabolites that they are much more variable and skewed than the corresponding plasma distributions of drugs, which were all well fitted by log-normal distributions. One likely source of this variation may be the effects of urinary pH on the amounts of phenylbutazone or metabolite appearing in the urine of these horses. We therefore plotted the concentrations of phenylbutazone and its metabolites found in these urine samples against urinary pH to determine the effects of pH on the concentrations of phenylbutazone or its metabolites found in equine urine.

The range of urinary pH values found in these horses is shown in Figure 9. This distribution is similar to those observed in other jurisdictions (Tobin, 1981). It is bi-modal, ranging from a pH value of about 4.5 to 9.5, with the bi-modal peaks at about pHs 5.8 and 7.2. This 10,000-fold range of urinary pH values is theoretically more than sufficient to substantially affect the urinary concentrations of either phenylbutazone or its metabolites.

FREQUENCY DISTRIBUTION OF URINE pH  
VALUES IN HORSES RACING IN KENTUCKY

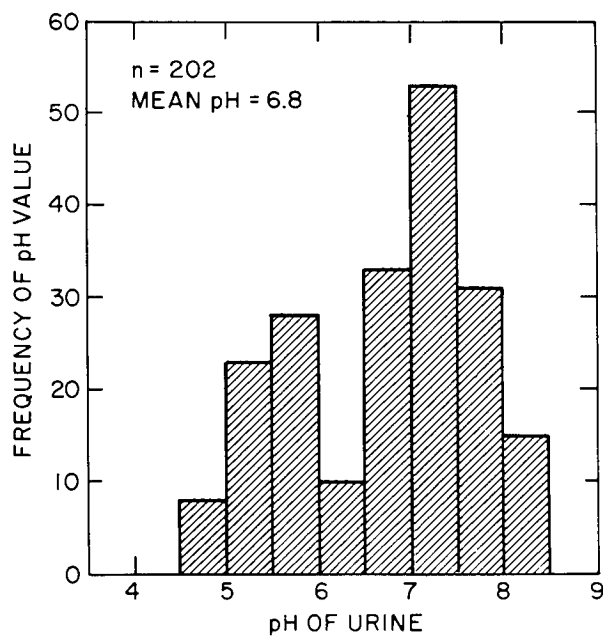


Figure 9

Population distribution of urinary pH values.

The hatched bars show the frequency of observed urinary pH values in 202 post race urine samples of horses racing in Kentucky. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

Figure 10 shows the effects of the pH of the urine sample on the concentration of phenylbutazone found in the sample. Because the relationship appeared to be complex and curvilinear, we chose to pool the urinary concentrations of phenylbutazone observed in each one half pH unit and plot the mean concentration observed for each epoch. As shown in Figure 10, there is little effect of pH on the urinary concentration of phenylbutazone until the pH of the urine sample rises above about 7.0. However, as the pH value rises above 7.0 there is a marked effect of pH on the concentration of phenylbutazone found in the sample, with the concentration increasing to about ten times that observed in the samples of less than pH 7.0. Overall there is an approximately 200-fold increase in the concentration of phenylbutazone in these urine samples as the pH of the sample increases from pH 4.5 to pH 8.5.

### EFFECT OF URINARY pH ON URINARY CONCENTRATIONS OF PHENYLBUTAZONE

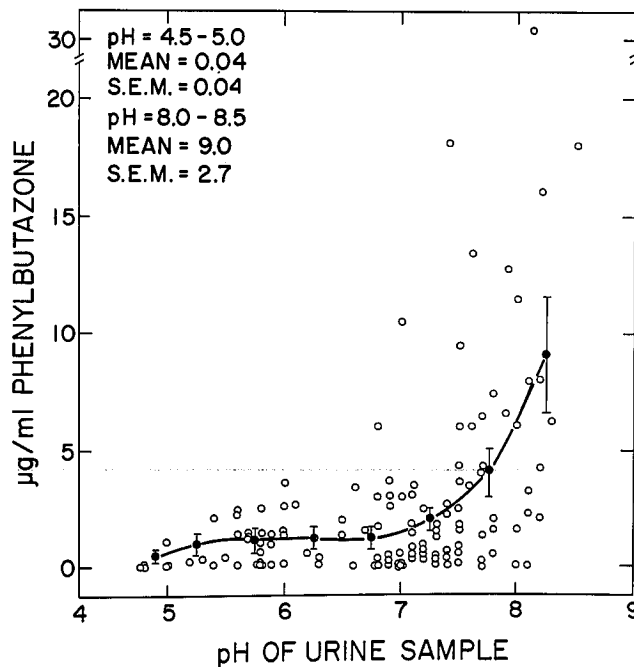


Figure 10

Effect of urinary pH on urinary concentration of phenylbutazone.

The open circles (o-o) show urinary concentrations of phenylbutazone (from Figure 4) plotted against urinary pH. The solid circles (●-●) show the mean urinary concentrations of phenylbutazone for each half-pH unit epoch  $\pm$  S.E.M. The line connecting the solid circles was fitted by eye. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

A broadly similar effect was observed on the urinary concentrations of oxyphenbutazone as shown in Figure 11. The concentration of oxyphenbutazone in the urine sample increased from about 0.6  $\mu\text{g/ml}$  at a pH of 4.5 to about 40.0  $\mu\text{g/ml}$  at pH 8.5. This is about a 60-fold increase in the concentration of this metabolite in the urine sample, apparently dependent principally on the effects of urinary pH.

EFFECT OF URINARY pH ON URINARY CONCENTRATIONS OF OXYPHENBUTAZONE

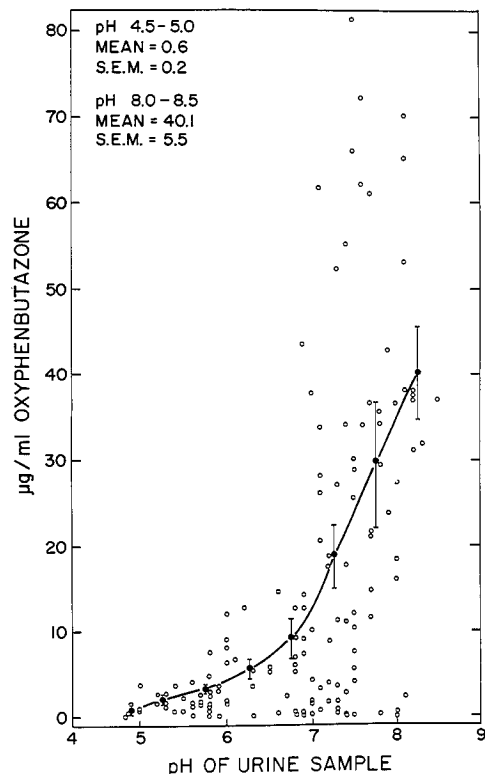


Figure 11

Effect of urinary pH on urinary concentration of oxyphenbutazone.

The open circles (o-o) show urinary concentrations of oxyphenbutazone (from Figure 4) plotted against urinary pH. The solid circles (●-●) show the mean urinary concentrations of oxyphenbutazone for each half-pH unit epoch  $\pm$  S.E.M. The line connecting the solid circles was fitted by eye. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

For  $\gamma$ -hydroxyphenylbutazone, the effect was qualitatively similar, with the urinary concentration of this metabolite increasing from 1.4  $\mu$ g/ml at pH 4.5 to about 44.2  $\mu$ g/ml at a pH of above 8.0. Overall this was more than a 30-fold increase in the urinary concentration of  $\gamma$ -hydroxyphenylbutazone, again apparently dependent on the effects of urinary pH (Figure 12).

EFFECT OF URINARY pH ON URINARY  
CONCENTRATIONS OF  $\gamma$ -OH-PHENYLBUTAZONE

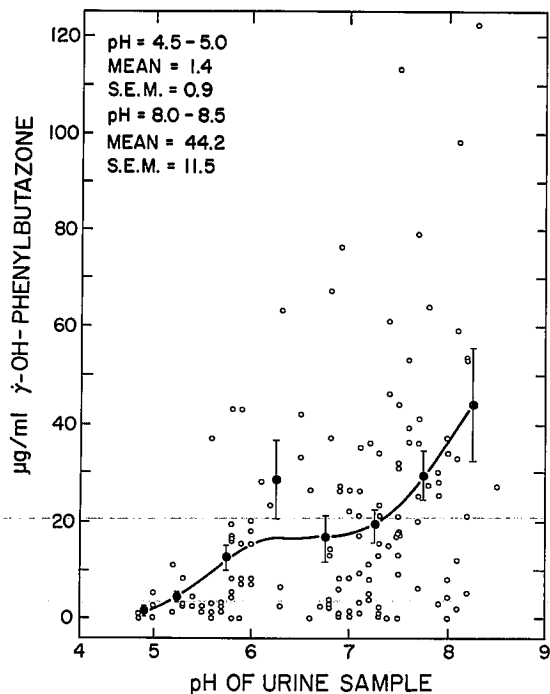


Figure 12

Effect of urinary pH on urinary concentration of  $\gamma$  OH-phenylbutazone.

The open circles (o-o) show urinary concentrations of  $\gamma$  OH-phenylbutazone (from Figure 4) plotted against urinary pH. The solid circles (●-●) show the mean urinary concentrations of  $\gamma$  OH-phenylbutazone for each half-pH unit epoch + S.E.M. The line connecting the solid circles was fitted by eye. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

Because a number of racing jurisdictions have rules which state that the levels of phenylbutazone and its metabolites in the urine of racing horses cannot exceed certain levels, most commonly about 165  $\mu$ g/ml of phenylbutazone and its metabolites (Tobin, 1981), we investigated the effects of pH on the sum of the urinary concentration of phenylbutazone and its metabolites. The data of Figure 13 shows that pH has a marked effect on the sum of the urinary concentrations of phenylbutazone and its metabolites, apparently increasing them from a level of about 2.8  $\mu$ g/ml at pH 4.5 to about 88  $\mu$ g/ml at about pH 8.5. This is an approximately 32-fold increase in the urinary concentration of these agents, apparently largely dependent on urinary pH.

EFFECT OF URINARY pH ON URINARY CONCENTRATIONS OF "PHENYLBUTAZONE AND METABOLITES"

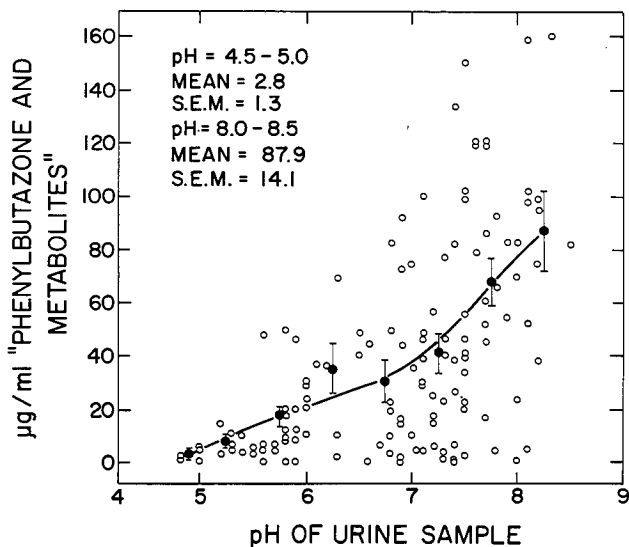


Figure 13

Effect of urinary pH on urinary concentration of phenylbutazone and its metabolites.

The open circles (o-o) show urinary concentrations of phenylbutazone (from Figure 4) plotted against urinary pH. The solid circles (●-●) show the mean urinary concentrations of phenylbutazone and its metabolites for each half-pH unit epoch + S.E.M. The line connecting the solid circles was fitted by eye. (Reproduced with permission from the J. Vet. Pharm. Ther., 1985.)

These observations are in good agreement with classical pharmacological principles. According to Melmon and Morrelli (1978), when acidic drugs have a pKa of between 3.0 and 7.5 their reabsorption by the renal tubule is likely to be sensitive to changes in urinary pH. Phenylbutazone, oxyphenbutazone and YOH-phenylbutazone, with pKa values of 4.5, 4.7 and 4.0, respectively (Soma, 1983), are therefore candidates for effects of urinary pH on their concentration and excretion rates in urine.

As acidic drugs, these agents will be largely in their ionized forms at alkaline urinary pH values and will therefore tend to "trap" in alkaline urine. The pH-partition theory predicts that the concentrations of these agents in equine urine will increase with increasing urinary pH. This is consistent with what was observed, as the urinary concentrations of these agents increased between 32- and 225-fold as the pH of the urine samples increased.

While pH-partition theory will predict the direction of a pH-dependent effect and can predict the theoretical limits of the effect, it alone cannot predict the actual size of the effect likely to be observed in practice. This is because attainment of the full theoretical distribution in a system such as the kidney depends on the amount of time available for the ion trapping phenomenon to operate and the actual rate at which the unionized drug moves across biological membranes. The rate at which drug transfer occurs depends, in turn, on the lipid solubility of the unionized species of the drug. Therefore, although the theoretical limits for the ion trapping phenomenon are on the order of about 10,000-fold, concentration changes (Tobin, 1981), the effects observed will in most cases be less. For example, if the drug or drug metabolite is poorly lipid soluble, then the unionized species will move relatively slowly in response to pH gradients and the effects of pH gradients on the final urinary concentrations of this drug or drug metabolite will be small. On the other hand, if the unionized form of the drug is highly lipid soluble, then the effect may be large and for some agents might approach the theoretical limit for that agent under the obtaining pH range.

The finding that urinary concentrations of phenylbutazone was most sensitive to changes in urinary pH is also consistent with what is known about the ion trapping phenomenon. Of the three agents whose concentrations in urine were measured, phenylbutazone is the most lipid soluble, with a saline/peanut oil partition coefficient of about 2.2, compared with a partition coefficient of about 0.6 for the other two agents (Soma et al., 1983). This means that phenylbutazone is the agent most readily able to follow the gradients caused by pH differences, with the other less lipid soluble agents being less able to follow the changes dictated by pH-partition theory. In general, therefore, the effects of pH on urinary concentrations of phenylbutazone and its metabolites are consistent with those which are predicted by pH-dependent ion trapping and the known greater lipid solubility of phenylbutazone.

In conclusion, these data show that the plasma levels of phenylbutazone and its metabolites in horses racing in Kentucky are broadly similar to those found in horses dosed with recommended schedules of phenylbutazone, with the last dose of phenylbutazone given 24 hours before post time. The plasma levels of phenylbutazone and its metabolites found were all well fitted by log-normal distributions, and the plasma levels of the metabolites found were generally less than those of the parent drug. The urinary concentrations of phenylbutazone and its metabolites showed greater variability than the plasma levels of the corresponding agent, with the modal concentrations being lower and the range being considerably higher than the levels observed in plasma.

Urinary pH appeared to play a major role in determining the levels of phenylbutazone and its metabolites found in the urine samples, with the concentration of this agent or its metabolites increasing from 30- to 200-fold in basic urine samples. These data suggest that rules which seek to restrict the levels of phenylbutazone in racing horses by limiting its concentration in urine are most likely without scientific basis.

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