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Phenylbutazone and its metabolites in plasma and urine of thoroughbred horses: population distributions and effects of urinary pH

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A survey of plasma and urinary concentrations of phenylbutazone and its metabolites in thoroughbred horses racing in Kentucky was carried out. Post-race blood samples from more than 200 horses running at Latonia Racetrack and Keeneland in the Spring of 1983 were analysed. The modal plasma concentration of phenylbutazone was between 1 and 2 μg/ml, the mean concentration was 3.5 μg/ml and the range was up to 15 μg/ml. Oxyphenbutazone had a modal plasma concentration between 1 and 2 μg/ml, a mean concentration of 2.07 μg/ml and a range of up to 13 μg/ml. γOH-phenylbutazone had a modal plasma concentration of less than 1 μg/ml, a mean level of 1.39 μg/ml and a range of up to 7.32 μg/ml. All plasma concentration frequency distributions were well fitted by log normal distributions.

Urinary concentrations of phenylbutazone yielded modal concentrations of less than 1 μg/ml, a mean urinary concentration of 2.9 μg/ml, with a range of up to 30.5 μg/ml. This population fitted a log-normal distribution. For oxyphenbutazone, the modal concentration was less than 3 μg/ml, the mean concentration was 15.26 μg/ml, with a range to 81.5 μg/ml. The frequency distribution of these samples was apparently bimodal. For γOH-phenylbutazone, the modal concentration was less than 4 μg/ml, the mean concentration 21.23 μg/ml, with a range of up to 122 μg/ml. The population frequency distribution for γOH-phenylbutazone was indeterminate.

Analysis of the pH of these post-race urine samples showed a bimodal frequency distribution. The pH values observed ranged from 4.9 to 8.7, with peaks at about pH 5.25 and 7.25. This bimodal pattern of urinary pH values is consistent with observations made in England and Japan.

Urinary pH influenced the concentrations of phenylbutazone, oxyphenbutazone and γOH-phenylbutazone found in the urine samples. The concentration of these metabolites found in alkaline urines were from 32 to 225 times greater

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than those found in acidic urines. Plasma concentrations of phenylbutazone and its metabolites, however, were unaffected by urinary pH.

In interlaboratory experiments, horses running at Hollywood Park were dosed with phenylbutazone at about 2 g/1000 lbs 24 and 48 h before racing, and a mean dose of 0.6 g/1000 lbs at 72 h prior to racing. Post-race plasma samples from these horses showed phenylbutazone concentrations ranging from 0.44 to 9.97 μg/ml, with a mean concentration of 4.09 μg/ml. Plasma oxyphenbutazone concentrations in these horses varied from 0.8 μg/ml to 11.3 μg/ml, with a mean of 5.3 μg/ml.

Comparison of plasma concentrations of phenylbutazone and oxyphenbutazone in horses racing in Kentucky, with the results of dosing experiments in horses in training, suggests that most horses racing in Kentucky are being dosed with amounts of phenylbutazone broadly equivalent to, or less than, proposed 'no-race-day medication rule' dosing schedules.

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INTRODUCTION

Phenylbutazone is a non-steroidal anti-inflammatory drug (NSAID) widely used in racing horses (Tobin, 1981). Because of its effectiveness in the treatment of musculo-skeletal disorders, phenylbutazone may be used in training or racing horses (Tobin, 1979). Since phenylbutazone may take 66 days or longer to clear a horse's system, and can be detected for at least 7 days after dosing, it is very commonly detectable in the body fluid of horses during races (Tobin *et al.*, 1982). However, the levels actually found in post-race urine samples are likely to be influenced to a considerable extent by the medication regulations of the jurisdiction in which the horses race (Tobin, 1981).

Objections to the presence of phenylbutazone or its metabolites in the urine of racing horses come from analysts, who may hold that the presence of phenylbutazone or its metabolites in equine urine interferes with or 'masks' the detection of other drugs (Tobin, 1981; Takade & Vassilaros, 1982). Further, it has been suggested that horsemen may deliberately administer phenylbutazone at high doses to racing horses to 'mask' or conceal the presence of illegal medications in horse urine (Takade & Vassilaros, 1982). No published scientific evidence either to support or to refute such charges exists.

Before one can assess the ability, or otherwise, of phenylbutazone to 'mask' the detection of illegal medications, one needs to know the concentrations of phenylbutazone found in the plasma and urine of racing horses. We have, therefore, surveyed the concentrations of phenylbutazone and its metabolites in plasma and urine samples from horses racing in Kentucky in the Spring of 1983. In addition, the results of tests on plasma concentrations of phenylbutazone and oxyphenbutazone in horses racing in California are reported.

MATERIALS AND METHODS

Phenylbutazone (PB) and oxyphenbutazone (OPB) were obtained from Ciba Pharmaceuticals (Summit, NJ) and γ-hydroxyphenbutazone (γ-OHPB) from Ciba-Geigy (Basle, 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 submitted to the Kentucky Equine Drug Testing Laboratory by the Kentucky State Racing Commission. Blood samples were obtained and urine samples were collected when the horse voided urine post-race. All samples were tightly sealed immediately after drawing and stored at 4°C throughout. The pH of the urine samples were taken immediately on arrival at the University of Kentucky and the drug

analysis completed within 48 h. 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-μl loop was used on the 210 sample injector valve. An ultrasphere-ODS 5-μm column (4.6 mm × 25 cm) with a guard column (Pellicular C-18, 4.6 mm × 5 cm, Alltech) between the injector and analytical column was used for separation of the compounds.

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 detected 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 of saturated KH_2PO_4 and 6 ml of dichloromethane at room temperature. The samples were rotoracked for 6 min, centrifuged for 2 min at 2000 r.p.m. 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 N_2. One hundred microliters of methanol was added to the residue, and 20 μl of the sample was injected onto the column. The urine samples were prepared in a similar manner, except 4 ml of pH 3.3 saturated KH_2PO_4 was added.

Standard curves were prepared for the determination of phenylbutazone, oxyphenbutazone and γ-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 μg/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 analysed for normality 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 of moments based on the calculated mean and standard deviation. The probability of attaining a given plasma or serum 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 deviations presented represent 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

The plasma concentrations of phenylbutazone observed in 182 thoroughbred horses racing in Kentucky in the Spring of 1983 are shown in Fig. 1. No phenylbutazone was detected in thirty-eight of these horses, and the modal blood concentration of phenylbutazone was less than 2 μg/ml. The distribution of plasma concentrations is log-normal, with ten horses showing plasma concentrations above 10 μg/ml.

The plasma concentrations of oxyphenbutazone found in 175 of these horses are shown in Fig. 2. No oxyphenbutazone was detected in twenty-six of these samples. The modal plasma concentration of oxyphenbutazone was less than 2 μg/ml. The distribution of plasma concentrations was log-normal, and one horse showed a plasma concentration of oxyphenbutazone above 12 μg/ml.

The plasma concentrations of γOH-phenylbutazone or the alcohol metabolite found in 161 of these horses are presented in Fig. 3. The modal concentration of γOH-phenylbutazone found in these horses was less

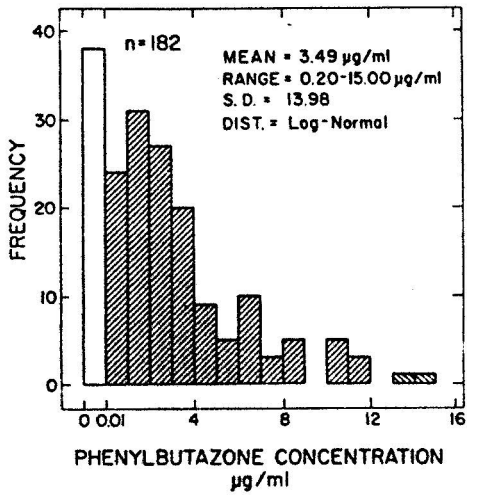


FIG. 1. Frequency distribution of plasma phenylbutazone levels in 182 thoroughbred horses racing in Kentucky. The open bar represents those plasma samples in which no phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of phenylbutazone. The range of values observed was from 0.20 to 15.0 µg/ml, with the mode between 1.0 and 2.0 µg/ml, and a mean level, not including those samples in which no drug was detected, of 3.49 µg/ml. The standard deviation of this distribution was 13.98 µg/ml, and the population was well fitted by a log-normal distribution, with a Shapiro-Wilk's statistic of > 0.15.

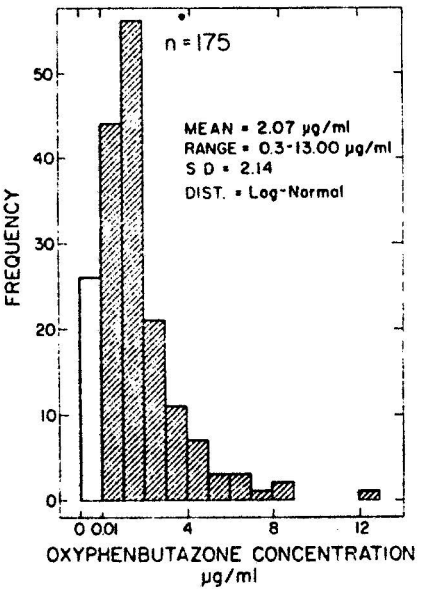


FIG. 2. Frequency distribution of plasma oxyphenbutazone levels in 175 thoroughbred horses racing in Kentucky. The open bar represents those plasma samples in which no oxyphenbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of oxyphenbutazone. The range of values observed was from 0.30 to 13.00 µg/ml, with the mode 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.

than 1 µg/ml. Again, the distribution was log-normal, with the highest plasma concentration being less than 8 µg/ml.

The concentrations of phenylbutazone found in urine samples from 155 of these horses are presented in Fig. 4. No detectable phenylbutazone was found in twenty-five of these urine samples, and the modal phenylbutazone concentration was less than 1 µg/ml. All but eight of the samples showed urinary concentrations of less than 20 µg/ml. However, the overall distribution was clearly skewed, with a long 'tail' of individual high urinary concentrations of phenylbutazone.

The concentrations of oxyphenbutazone in the urine of 168 of these horses are shown in Fig. 5. No oxyphenbutazone was detected in eleven of these horses, and the modal concentration was less than 3 µg/ml. The distribution then fell away sharply, showing only two samples with urine concentrations between 13 and 14 µg/ml. Thereafter, however, urinary concentrations of oxyphenbutazone increased, with eight horses showing concentrations of about 36 µg/ml, and then declined, with the highest recorded concentration about 83 µg/ml. The population distribution appears to be best described as bimodal.

The concentrations of γOH-phenylbutazone found in 152 of these urine samples are presented in Fig. 6. No γOH-phenylbutazone was detected in eleven of these samples. The modal concentration in equine urine was less than 4 µg/ml, followed by a frequency of concentrations which declined slowly to about 80 µg/ml. Two horses, however, showed urine concentrations of γOH-phenylbutazone above 112 µg/ml. Although clearly skewed to the left, the population distribution was not well fitted by a log-normal curve and was described as indeterminate.

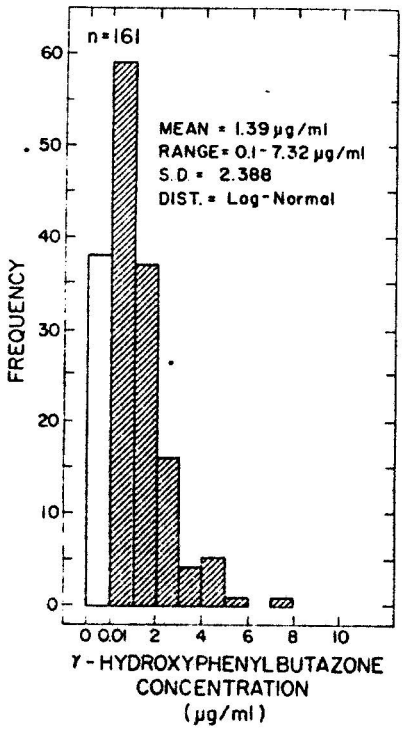


FIG. 3. Frequency distribution of plasma γOH-phenylbutazone levels in thoroughbred horses racing in Kentucky. The open bar represents those plasma samples in which no γOH-phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of γOH-phenylbutazone. The range of values observed was from 0.10 to 7.32 µg/ml, with the mode between 0.1 and 1.0 µg/ml, and the mean level, not including those samples in which no drug was detected, of 1.39 µg/ml. The standard deviation of this distribution was 2.39 µg/ml and the population was well fitted by a log-normal distribution, with a Shapiro-Wilk's statistic of 0.124.

In a preliminary analysis of some of these data, Houston *et al.* (1983) showed that urinary concentrations of oxyphenbutazone were strongly influenced by urinary pH. Because this observation might explain the skewed populations of urinary drug concentrations, we studied the effects of urinary pH on plasma and urinary concentrations of phenylbutazone and its metabolites.

Figure 7 shows the population distributions of urinary pH values in the 202 post-race urine samples available to us. The lowest value observed was pH 4.9, and the highest pH 8.7. The population distribution was apparently bimodal, with a population peak at about pH 5.75, a trough at pH 6.25 and a major peak at pH 7.25. This bimodal distribution is similar to the distributions reported from England and Japan (Tobin, 1981).

Figure 8 shows the relationship between urinary pH and urinary concentrations of phenylbutazone. For urine samples with pH values between pH 4.5 and 5.0, the mean concentration of phenylbutazone was 0.04 µg/ml. This increased rapidly at first, then more slowly between pH 6.0 and 7.0, and then sharply between 7.5 and 8.5. Overall, the effect of urinary pH on urinary concentrations of phenylbutazone in these post-race horse urines appeared to result in about a 225-fold increase from the lowest to the highest urinary pH value. On the other hand, however, plasma concentrations of phenylbutazone were essentially independent of urinary pH, as shown in Table I.

Figure 9 shows the relationship between urinary concentrations of oxyphenbutazone and urinary pH. For urinary pH values of 4.5–5.0, the average concentration of oxyphenbutazone in equine urine was 0.6 µg/ml. As urinary pH increased, the concentrations of oxphenbutazone in the urine sample increased about ten-fold to 6.5 µg/ml and about seven-fold by pH 8.5, at which the mean urinary concentration of oxyphenbutazone was 40.1 µg/ml. Overall therefore, the urinary concentrations of oxyphenbutazone increased about 66-fold, again apparently depending on urinary pH. As with phenylbutazone, the concentrations of oxyphenbutazone in equine plasma were not affected by urinary pH (Table I).

A broadly similar pattern was observed with γOH-phenylbutazone (Fig. 10). At a urinary pH of 4.5–5.0, the mean urinary concentration observed was 1.4 µg/ml. This concentration increased rapidly until, at pH 6.5, the mean concentration was 28.3 µg/ml. Thereafter, the urinary concentration dropped, but then rose again rapidly to a mean level of 44.2 µg/ml above pH 8. Urinary concentrations of γOH-phenylbutazone, therefore, showed about a 32-fold increase over the range of urinary pH values, with the concentration increasing as pH increased. On the other hand, the plasma levels of γOH-phenylbutazone appeared to decrease with

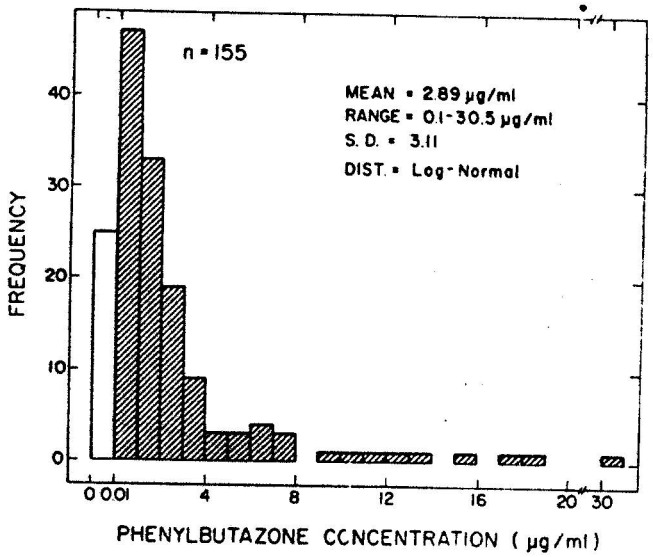


FIG. 4. Frequency distribution of urine phenylbutazone levels in thoroughbred horses racing in Kentucky. The open bar represents the urine samples in which no phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of phenylbutazone. The range of values observed was from 0.20 to 30.5 µg/ml, with the mode 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.

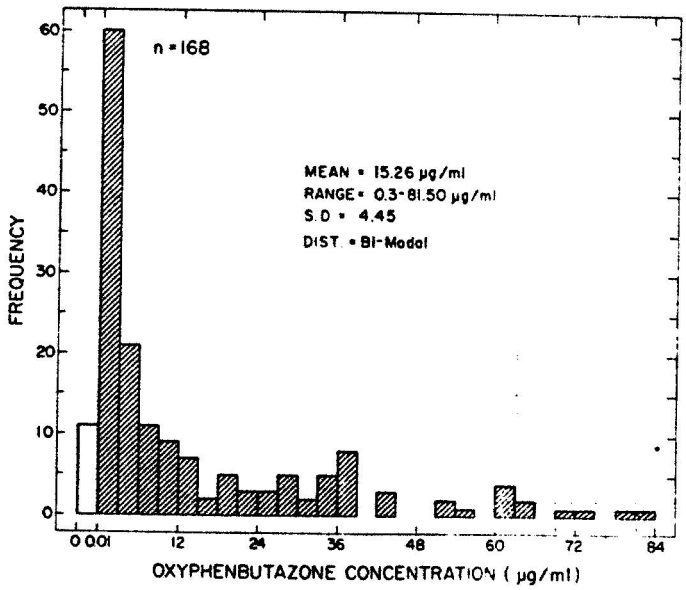


FIG. 5. Frequency distribution of urine oxyphenbutazone levels in thoroughbred horses racing in Kentucky. The open bar represents those urine samples in which no oxyphenbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of oxyphenbutazone. The range of values observed was from 0.30 to 81.50 µg/ml, with the mode 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).

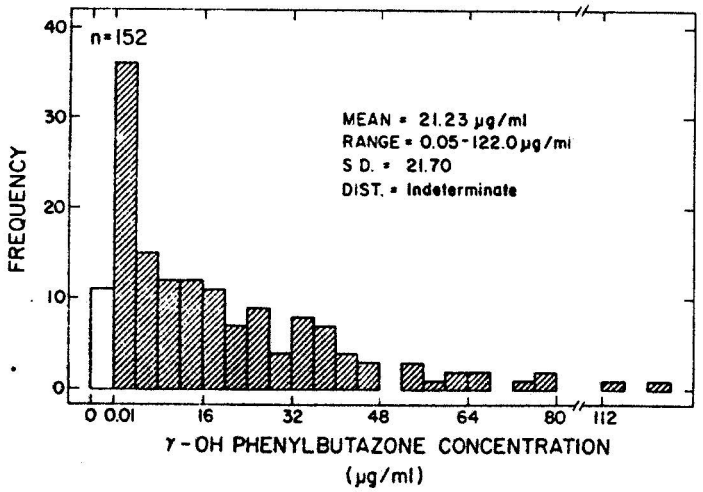


FIG. 6. Frequency distribution of urine γOH-phenylbutazone levels in thoroughbred horses racing in Kentucky. The open bar represents those urine samples in which no γOH-phenylbutazone was detectable. The hatched bars represent the frequency of occurrence of each concentration of γOH-phenylbutazone. The range of values observed was from 0.05 to 122.0 µg/ml, with the mode between 1.0 and 4.0 µg/ml. The mean level, not including those samples in which no drug was detected, was 21.23 µg/ml. The standard deviation of this distribution was 21.70 µg/ml and the population was not fitted by a log-normal distribution (Shapiro-Wilk's statistic < 0.01).

increasing urinary pH, as shown in Table 1.

Because a number of racing jurisdictions have regulations stating that certain levels of 'phenylbutazone and its metabolites' in equine urine shall not be exceeded (Tobin, 1981), we summed the concentrations of phenylbutazone, oxyphenbutazone and γOH-phenylbutazone for each half pH unit (Fig. 11). As might be expected, urinary concentrations of phenylbutazone and its metabolites increased from 2.8 µg/ml at pH 4.5 to 87.9 µg/ml at pH 8.85, an approximately 32-fold increase in urinary concentrations. As with the individual parent and metabolites, the plasma concentrations of phenylbutazone and its metabolites showed no such increase in association with increasing urinary pH (Table 1).

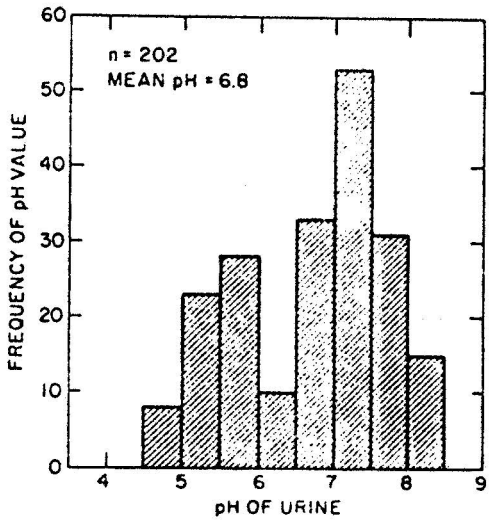


FIG. 7. Population distribution of urinary pH values. The hatched bars show the frequency of observed urinary pH values in 202 post-race urine samples of horses racing in Kentucky.

In a parallel study, horses racing in California were treated with a regimen of phenylbutazone approximately equivalent to 2 g/day for 2 days, with no medication for 24 h prior to post time. Figure 12 shows the plasma levels of phenylbutazone and oxyphenbutazone in these samples. The modal level of phenylbutazone in these samples was between 3 and 4 µg/ml, the mean 4.09 µg/ml, with a range of up to 9.97 µg/ml. These data were well fitted by a log-normal distribution. The oxyphenbutazone levels found in these samples were greater than the phenylbutazone levels, with a mean of 5.29 and a range of up to 11.32. The plasma levels of oxyphenbutazone in these horses were well fitted by a normal distribution.

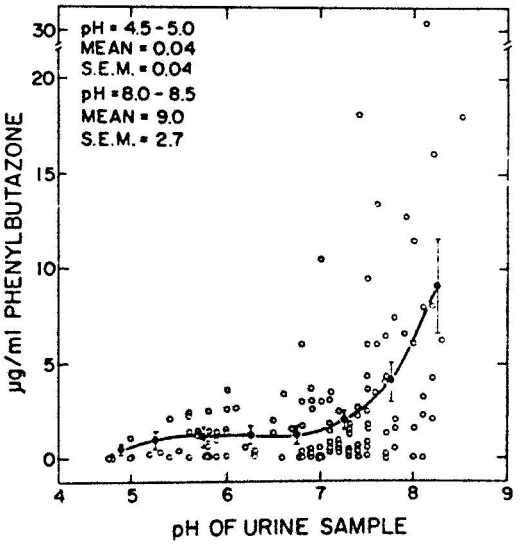


FIG. 8. Effect of urinary pH on urinary concentrations of phenylbutazone. The open circles (○) show urinary concentrations of phenylbutazone (from Fig. 4) plotted against urinary pH. The solid circles (●—●) show the mean urinary concentrations of phenylbutazone for each half pH ± SEM. The line connecting the solid circles was fitted by eye.

DISCUSSION

Inspection of the data reported in this paper reveals a number of clear patterns. Firstly, the mean and modal concentrations of phenylbutazone and its metabolites in both plasma and urine were surprisingly low. No modal plasma concentration greater than 2 µg/ml was seen for phenylbutazone or any of its metabolites. Further, the modal urinary concentrations of phenylbutazone were less than 1 µg/ml. These modal concentrations are lower than those which might be expected in horses racing on no-race-day medication schedules (Chay *et al.*, 1984). They are also lower than might be expected because of the fact that Thoroughbred racing in Kentucky has no restrictions on the use of phenylbutazone.

Despite the fact that the use of phenylbutazone in Kentucky is unrestricted, the plasma concentrations of phenylbutazone found in these horses compare very favorably with those observed in 'no-race-day medication rule' experiments. For example, the American Association of Equine Practitioners has suggested that 2 g injected i.v. is a 'non-abusive' dose of phenylbutazone for a racing horse (Harvey, 1983). Based on these considerations, Dr Larry Soma of the University of Pennsylvania, in co-operation with the Pennsylvania Horseman's Benevolent and Protective Association (HBPA), dosed horses in training at Keystone Racetrack with 2 g/1000 lbs of phenylbutazone i.v. for 4 days and measured plasma level residues of the drug on the fifth day (Soma, 1983; L. Soma, personal communication).

Analysing plasma samples from these horses in our laboratory, we found that the mean plasma concentration of phenylbutazone in these forty-three horses was 4.75 µg/ml, with a range of from 1.5 to 9.8 µg/ml (Table 11). Both the mean and modal concentrations of phenylbutazone in the horses running in Kentucky were less than those in these samples from the Keystone study. Statistical projections of the concentrations from the Keystone distribution suggests that about one horse in 1000 dosed with the Keystone schedule would reach 16.2 µg/ml. Of the 182 Kentucky samples analysed for phenylbutazone, the highest plasma concentration observed was about 15 µg/ml. Overall, the data show that the actual concentrations of phenylbutazone in horses running in Kentucky were close to those which might be found under the no-race-day medication rule recommended by the American Association of Equine Practitioners.

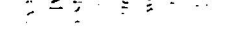
The plasma concentrations of oxyphenbutazone found in horses racing in Kentucky showed modal concentrations less than 2 µg/ml and a mean concentration of 2.07 µg/ml. The range was up to 13.00 µg/ml. These concentrations compared well with those obtained in our analysis of the Keystone study, where the mean and modal concentrations were about 3.5 µg/ml and the range up to 8 µg/ml. Inspection of Fig. 8 shows that two of the 176 Kentucky samples were 9 µg/ml, with only one above this level. Again, the concentrations of oxyphenbutazone in horses racing in Kentucky under no medication restrictions are comparable to concentrations likely to be found under a no-race-day medication rule.

The plasma concentrations of γOH-phenylbutazone found in horses racing in Kentucky had a modal concentration of less

TABLE I. Effect of urinary pH on plasma levels of phenylbutazone and its metabolites

Urinary pH	4.5–5.0	5.0–5.5	5.5–6.0	6.0–6.5	6.5–7.0	7.0–7.5	7.5–8.0	8.0–8.5
Phenylbutazone (μg/ml plasma)	2.6 ± 1.5	4.5 ± 0.9	2.8 ± 0.5	2.3 ± 0.9	2.0 ± 0.7	2.3 ± 0.4	3.5 ± 0.8	2.7 ± 0.7
Oxyphenbutazone (μg/ml plasma)	1.7 ± 0.7	3.2 ± 0.8	2.0 ± 0.4	1.2 ± 0.3	1.1 ± 0.2	1.1 ± 0.2	1.8 ± 0.4	1.7 ± 0.4
γOH-phenylbutazone (μg/ml plasma)	2.7 ± 1.0	2.3 ± 0.4	1.2 ± 0.2	0.8 ± 0.3	0.9 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2
Phenylbutazone and its metabolites (μg/ml plasma)	6.9 ± 2.5	10.1 ± 1.8	6.0 ± 1.2	4.3 ± 1.5	3.9 ± 1.1	4.0 ± 0.6	5.9 ± 1.2	5.0 ± 1.2

For each half pH unit, the plasma concentrations of phenylbutazone or its metabolites, or the sum of phenylbutazone and its metabolites were averaged.



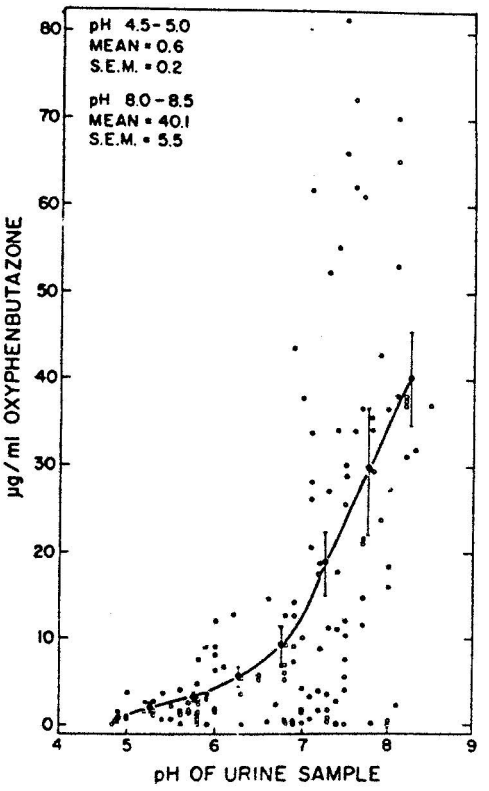


FIG. 9. Effect of urinary pH on urinary concentrations of oxyphenbutazone. The open circles (O) show urinary concentrations of oxyphenbutazone (from Fig. 4) plotted against urinary pH. The solid circles (●—●) show the mean urinary concentrations of oxyphenbutazone for each half pH unit ± SEM. The line connecting the solid circles was fitted by eye.

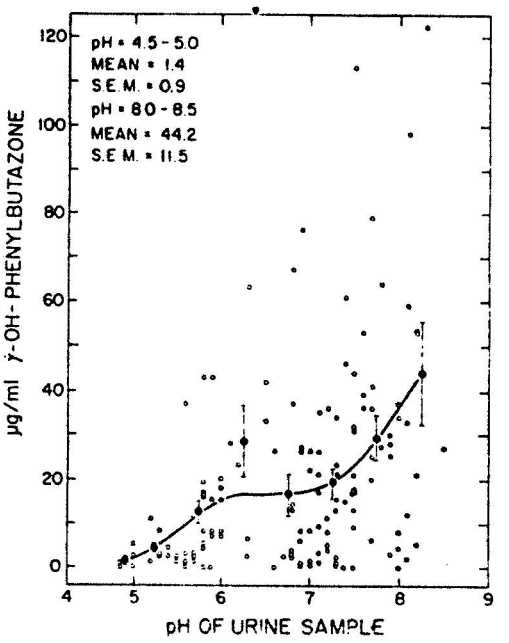


FIG. 10. Effect of urinary pH on urinary concentrations of γOH-phenylbutazone. The open circles (O) show urinary concentrations of γOH-phenylbutazone (from Fig. 4) plotted against urinary pH. The solid circles (● – ●) show the mean urinary concentrations of γOH-phenylbutazone for each half pH unit ± SEM. The line connecting the solid circles was fitted by eye.

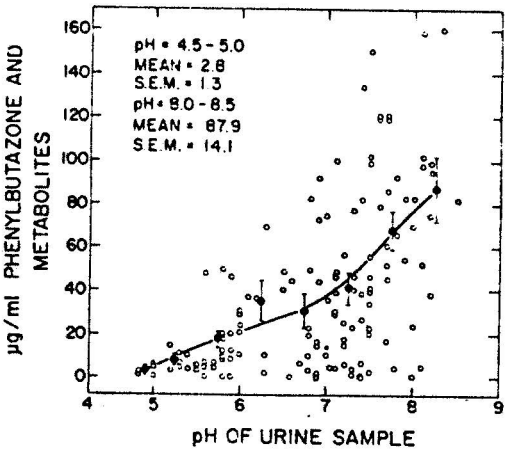


FIG. 11. Effect of urinary pH on urinary concentrations of phenylbutazone and its metabolites. The open circles (O) show urinary concentrations of phenylbutazone (from Fig. 4) plotted against urinary pH. The solid circles (●—●) show the mean urinary concentrations of phenylbutazone and its metabolites for each half pH unit ± SEM. The line connecting the solid circles was fitted by eye.

than 1 µg/ml and a mean concentration of 1.39 µg/ml. These plasma concentrations are much less than those of phenylbutazone and oxyphenbutazone, consistent with the lesser protein binding and more rapid elimination of γOH-phenylbutazone from plasma. Plasma concentrations of γOH-phenylbutazone were not measured in the Keystone or other interlaboratory studies, so no comparative data are available.

The plasma concentrations of phenylbutazone and its metabolites found in horses racing in Kentucky also compare favorably with those found after a no-race-day medication rule based on oral dosing schedules. Recently, Chay *et al.* (1984) dosed horses in training at Keeneland with 4 g/1000 lbs of phenylbutazone orally for 3 days, followed by

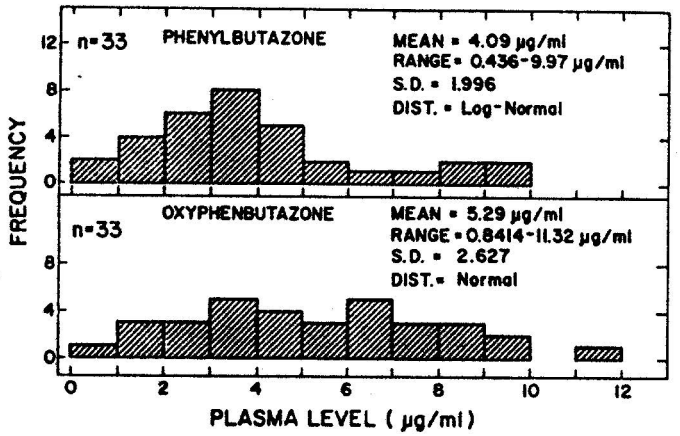


FIG. 12. Plasma levels of phenylbutazone and oxyphenbutazone in horses running at Hollywood Park, California, 1983. Thirty-four horses racing in Hollywood Park were dosed with phenylbutazone either orally or intravenously (i.v.) at 72, 48 and 24 h prior to post. At 24 h prior to post, 26 of these horses received 2 g/horse, either orally or i.v., 7 received 3 g orally, and one received 4 g orally. At 48 h prior to post, 2 horses received no phenylbutazone, 26 received 2 g either orally or i.v., and 6 horses received no dose of phenylbutazone, 8 received 2 g orally and one horse received 3 g orally. One horse received 2 g orally at 96 h to post. The mean dosage rates, therefore, were 2.3 g/1000 lbs at 24 h, and 2.0 g/1000 lbs at 48 h. The variable pattern of dosing at 72 h is difficult to evaluate, but amounted to a mean dose of about 0.6 g/1000 lbs. The Shapiro-Wilk's statistic was 0.333 for the phenylbutazone distribution and 0.757 for the oxyphenbutazone distribution.

2 g i.v. on Day 4. Plasma levels of phenylbutazone and oxyphenbutazone were assayed on Day 5 to determine the blood concentrations of phenylbutazone and oxyphenbutazone consistent with a 24-h rule after oral dosing with this schedule of phenylbutazone.

The modal plasma concentration observed in this study was about 2.5 µg/ml. The mean plasma concentration was 5.32 µg/ml, with a range of up to 13.63 µg/ml. Statistical projection of these data showed that one horse in 1000 may be expected to show blood concentrations of phenylbutazone of 23.5 µg/ml (Table 1).

These data are broadly similar to the blood concentration data of Fig. 1, where the mean and median are less than those observed in the oral dosing experiments, and the range is remarkably similar.

The oxyphenbutazone concentrations observed in this survey are also similar to those observed in the oral/i.v. study. The median plasma concentration of oxyphenbutazone in the oral i.v. study was 2.5 µg/ml, with a range of up to 13.72 µg/ml. The plasma samples from the horses running in Kentucky showed a modal concentration between 1 and 2 µg/ml, a mean concentration of 2.07 µg/ml, and a range of up to 13 µg/ml. These data clearly suggest that thoroughbred horses in Kentucky are running on the approximate equivalent of an 'oral dosing' no-race-day medication rule.

In parallel experiments in California, thirty-four horses racing at Hollywood Park were dosed with approximately 2 g of phenylbutazone orally at 24 and 48 h prior to post time. Post-race plasma samples from these horses were analysed in our laboratory and these data are presented in Fig. 12. These samples showed mean blood plasma levels of 4.09 µg/ml, with a range of 0.4 µg/ml to 9.97 µg/ml. This range is very close to that of the Keystone study (Table II), and again suggests that these horses have been dosed with dosage schedules close to those of a 'no-race-day medication' schedule. However, it must be borne in mind that the California study represents a limited group of horses, and does not constitute a survey of phenylbutazone use over a period.

The second important finding from these experiments was the observation that plasma concentrations of phenylbutazone and its metabolites in horses racing in Kentucky follow log-normal frequency distributions.

TABLE II. Comparison and statistical projections from data on horses running in Kentucky and California and 'no-race-day medication rule' studies

	Number of horses	Range (μg/ml)	Mode (μg/ml)	Median (μg/ml)	Mean (μg/ml)	5% (μg/ml)	1% (μg/ml)	0.1% (μg/ml)
*Phenylbutazone**								
Kentucky (post-race)	182	0.20–15.00	1–2	2.52	3.49	11.07	15.8	35.8
Keystone (i.v.)	43	1.50–9.88	3–4	4.00	4.75	8.8	11.8	16.2
Keeneland (oral — i.v.)	62	1.28–13.63	2–3	5.16	5.32	10.3	18.6	23.5
California	33	0.44–9.97	3–4	3.65	4.09	10.45	16.78	23.18
Florida (serum)	57	0.27–1.80	1–2	0.76	0.94	1.62	1.90	2.21
*Oxyphenbutazone**								
Kentucky (post-race)	175	0.30–13.00	1–2	1.40	2.07	5.4	9.12	16.2
Keystone (i.v.)	43	1.04–7.70	3–4	3.55	3.52	6.6	8.9	12.4
Keeneland (oral — i.v.)	62	0.88–10.26	1–3	3.22	3.20	8.9	13.6	21.7
California	33	0.84–11.32	6–7	5.03	5.29	9.61	11.41	13.38
Florida (serum)	57	0.30–2.52	1–2	1.04	1.09	2.04	2.43	2.87

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

This is despite the fact that there were no restrictions on dosing. These findings contrast with the fact that the frequency distribution patterns in urine for the metabolites of phenylbutazone were more complex, and in some cases could not be readily described mathematically.

This finding is of regulatory importance because it means that blood levels of drugs are mathematically much more predictable than urinary levels of drugs. This is an important point for regulatory scientists, who may be requested to develop tolerances or residual levels for certain drugs or drug metabolites in body fluids. Because of the mathematical predictability of plasma levels of drugs in comparison with urinary levels of drugs, plasma is a much more satisfactory medium for regulatory and forensic work, especially if quantitation or time rules are required by regulators.

As well as being less predictable, urinary concentrations of phenylbutazone were, by and large, lower than plasma concentrations of the drug. The modal concentration of phenylbutazone in these urine samples was less than 1.0 μg/ml, and the mean concentration was just under 3.0 μg/ml. This was in contrast with plasma concentrations of the drug, where the modal concentration was between about 1 and 2 μg/ml and the mean was 3.5 μg/ml.

While the bulk of the urinary concentrations of phenylbutazone were, thus, lower than plasma concentrations of this drug, there

was a clear-cut 'tail' of individual urinary concentrations above 10 μg/ml. These high urinary concentrations of PB were apparently related to the alkaline pH of these urine samples. However, despite this effect of pH on the distribution of phenylbutazone into alkaline urines, the phenylbutazone values presented in Fig. 4 were well fitted by a log-normal distribution.

In contrast, the urinary concentrations of oxyphenbutazone and γOH-phenylbutazone were not well fitted by log-normal distributions. Both of these population distributions were more complex, and we were unable to fit these populations to simple mathematical models.

Inspection of the frequency distribution of oxyphenbutazone in equine urine shows a modal concentration of oxyphenbutazone in equine urine of less than 3 μg/ml, with the frequency of higher concentrations dropping away rapidly to yield only two samples with urinary concentrations of oxyphenbutazone between 13 and 14 μg/ml. Thereafter, the frequency of higher oxyphenbutazone concentrations increased, with a secondary peak in the frequency distribution of oxyphenbutazone concentrations at about 36 μg/ml. From 36 μg/ml upwards, the frequency of higher oxyphenbutazone concentrations decreased, with a single concentration of 8.15 μg/ml being observed in these samples.

We were also unable to describe mathematically the frequency distribution of concentrations of γOH-phenylbutazone in these urine samples. Inspection of the curve of Fig. 6 showed that the distribution is markedly skewed to the left, but the distribution was not fitted by a log-normal distribution (Shapiro-Wilk's Statistic < 0.01). We therefore described the distribution as indeterminate. However, since it again appeared likely that urinary pH was a major cause of the high concentrations of γOH-phenylbutazone in the equine urine samples, we determined the effect of pH on the concentration of phenylbutazone, oxyphenbutazone and γOH-phenylbutazone and the sum of these metabolites in all the urine samples tested.

A possible explanation for these skewed distributions of urinary oxyphenbutazone and γOH-phenylbutazone concentrations may be the pH of the urine samples. As acidic metabolites, they are likely to concentrate in alkaline urines. Examining this hypothesis, Houston *et al.* (1983) have shown that urinary concentrations of oxyphenbutazone were positively correlated with pH, and that high urinary values were associated with high urinary concentrations of oxyphenbutazone. These data prompted us to examine the effects of urinary pH on urinary concentrations of phenylbutazone and its metabolites.

As shown in Fig. 7, the frequency distribution of the urine pH values observed in these samples is similar to those reported from other jurisdictions (Tobin, 1981). Work from England and Japan has shown a broad range of pH values, with values starting at a pH of about 4.0, a first peak at about pH 5.0, a trough at about 6.0, rising to a secondary peak at about 8.0, and then declining, with no value greater than a pH of about 9.0. These distributions suggest that a broad and apparently bimodal pattern of pH distribution in post-race equine urine is a consistent phenomenon on post-race urines from thoroughbred horses. While the pH range observed in these post-race urine samples is likely to underestimate the range of pH values actually found in the renal tubules, the range observed is consistent with reports from other laboratories and large enough to be an important factor in drug distribution.

When one plots the concentrations of phenylbutazone and its metabolites against urinary pH for each of these agents, a pattern of increasing urinary concentrations of these agents as pH increases is observed. For phenylbutazone (Fig. 7), its urinary levels increase at least 225-fold as urinary pH increases. For oxyphenbutazone (Fig. 8), urinary concentrations increase about 66-fold, and for γOH-phenylbutazone (Fig. 9), its concentrations increase about 32-fold. These increases are well within the range of those which may be expected from ion-trapping theory, and they are more than sufficient to render the quantitation of urinary concentrations of phenylbutazone and its metabolites meaningless for regulatory purposes.

These observations are in good agreement with classical pharmacological principles. According to Melmon & Morrelli (1972), 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 γOH-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. pH-partition theory, therefore, 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.

In summary, therefore, these data show the plasma and urinary levels of phenylbutazone and its metabolites in horses racing in Kentucky in 1983. They show that the modal levels and the mean levels of phenylbutazone and its metabolites in horses running in Kentucky were less than those found in the plasma of horses dosed with amounts of phenylbutazone consistent with 'no-race-day medication' rules. The distributions of plasma levels of phenylbutazone and its metabolites in horses racing in Kentucky followed log-normal distributions. While the levels of phenylbutazone in urine were readily described by log-normal distributions, the urinary concentrations of oxyphenbutazone and γOH-phenylbutazone did not follow this distribution. The urinary concentrations of oxyphenbutazone were apparently bimodally distributed. Urinary concentrations of γOH-phenylbutazone were markedly skewed to the left, and also could not be readily described mathematically. In each case, it appeared that a major factor in determining the urinary concentration of phenylbutazone or its metabolite is the pH of the urine sample. Because of this, the regulatory significance of urinary quantitation of phenylbutazone or its metabolites is, at best, tenuous.

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