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117

Influence of Urinary pH on Concentrations of Phenylbutazone and its Metabolites in Post-Race Urine Samples

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Abstract

We measured the plasma and urinary levels of phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone for more than 200 thoroughbred horses racing in Kentucky in the spring of 1983. In each case the plasma levels were well fitted by log-normal distributions and were not influenced by urinary pH values. The urinary concentrations of phenylbutazone were also well fitted by a log-normal distribution but those of oxyphenbutazone and γ-hydroxyphenylbutazone were more complex. In each case higher urinary concentrations tended to be associated with higher urinary pH values. When this data was fitted to a model of exponential increase in drug or drug metabolite concentration with increasing urinary pH, the effect of urinary pH was significant at $p < 0.0001$. This analysis showed that 33 per cent of the variability in urinary phenylbutazone concentrations, 58 per cent of the variability in urinary oxyphenbutazone concentrations, and 28 per cent of the variability in urinary γ-hydroxyphenylbutazone concentrations were due to the effects of urinary pH. This is consistent with the known effects of alkaline urinary pH on urinary concentrations and clearance of acidic drugs in other species.

Introduction

Phenylbutazone is an effective non-steroidal anti-inflammatory drug for treating musculoskeletal disorders and is widely used for racehorses.[1] It can be detected for seven days or more and is generally present in the body fluids of horses during racing.[2] However, the amounts actually found in post-race urine samples are probably influenced considerably by the medication regulations of the jurisdiction the horses race in.[1]

Objections to phenylbutazone and its metabolites in race-day urine samples come from analysts who may hold that these substances interfere with, or 'mask', the detection of other drugs.[1,3] No published scientific evidence to either support or refute this charge exists.

Before one can assess whether phenylbutazone can mask the detection of illegal medication one needs to know what concentrations of phenylbutazone are actually found in race-day plasma and urine samples. We have therefore surveyed the concentrations of phenylbutazone and its metabolites in plasma and urine from horses racing in Kentucky in the spring of 1983.

While analyzing the results it became clear that a major factor affecting these concentrations in urine was the pH of the urine sample. We therefore analyzed the data from this survey to determine the significance of pH as a factor in urinary drug concentrations. This showed that pH has a substantial effect on urinary drug concentrations, consistent with data reported for acidic drugs in other species.

Materials and Methods

Phenylbutazone and oxyphenbutazone were obtained from Ciba Pharmaceuticals (Summit, NJ) and

γ-hydroxyphenylbutazone from Ciba-Geigy (Basel, Switzerland). Liquid-chromatographic grade methanol and water were obtained from Alltech (Deerfield, IL). All other solvents and reagents were of analytical grade from Fisher Scientific Company (Louisville, KY).

The samples tested were all post-race blood and urine samples submitted to the Kentucky Equine Drug Testing Laboratory by the Kentucky State Racing Commission. Blood samples were obtained when the horses voided urine post-race. All samples were tightly sealed immediately after collection and stored at 4°C. The pH of the urine samples was taken immediately on arrival at the University of Kentucky and the drug analysis completed within 48 hours. (Phenylbutazone is a permitted medication for thoroughbred horses racing in Kentucky.)

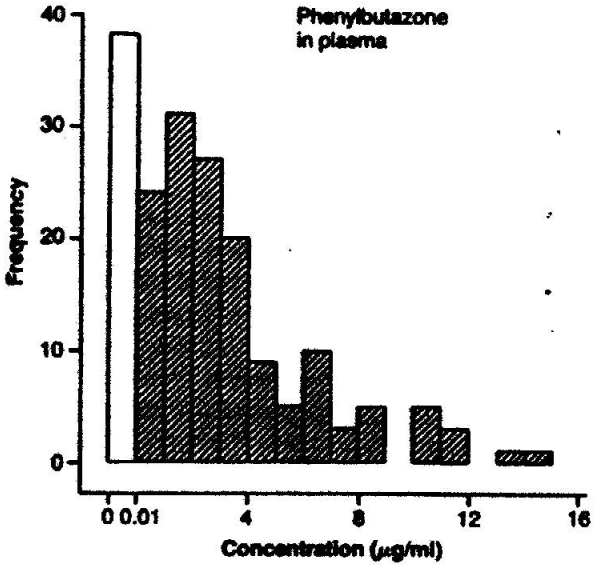
Phenylbutazone and its metabolites were detected and quantified by liquid chromatography, as described by Houston *et al.*[4] The frequency distributions of plasma and urine levels of phenylbutazone and its metabolites were analyzed for normality using the Shapiro–Wilk's statistic.[5] The best-fit transformations were determined and the means and standard deviations estimated. For those distributions that could be logarithmically transformed to normal distributions the standard deviations are presented here as the antilogs of the standard deviations of the logarithmically transformed data. Where the data did not normalize when log-transformed the arithmetic standard deviation is given.

Results

The plasma concentrations of phenylbutazone for 182 thoroughbred horses racing in Kentucky in the spring of 1983 are shown in Figure 1. No phenylbutazone was detected in 38 of these horses and the modal blood concentration of phenylbutazone was less than 2 μg/ml. The distribution of plasma concentrations was 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 Figure 2. No oxyphenbutazone was detected in 26 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.

Figure 1 *Frequency distribution of plasma phenylbutazone levels in 182 thoroughbreds racing in Kentucky. The open bar represents plasma samples in which no phenylbutazone was detected. The hatched bars show how often each concentration of phenylbutazone occurred. The values ranged from 0.20 to 15.0 μg/ml, with the mode between 1.0 and 2.0 μg/ml, and a mean (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.*

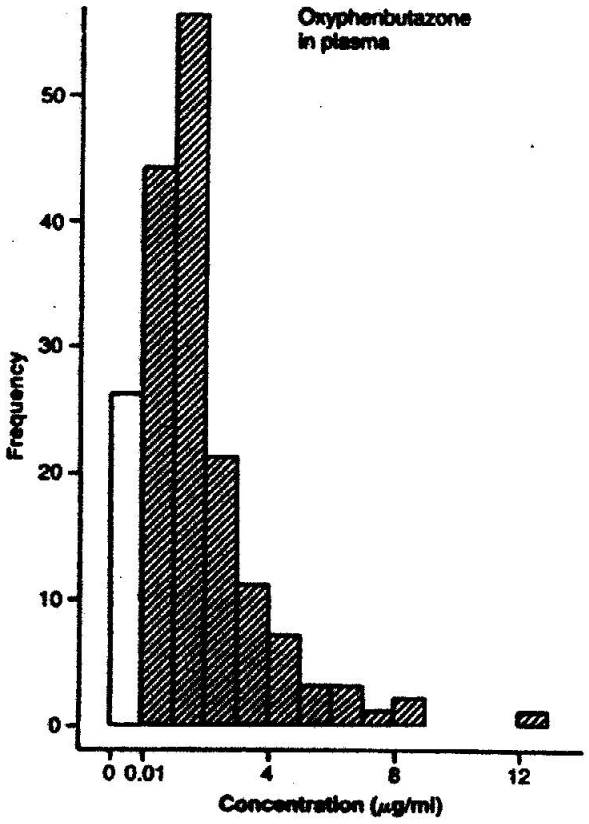


The plasma concentrations of γ-hydroxyphenylbutazone (the 'alcohol metabolite') found in 161 of these horses are presented in Figure 3. The modal concentration of γ-hydroxyphenylbutazone was less than 1 μg/ml. Again, the distribution was log-normal, with the highest plasma concentration being less than 8 μg/ml.

Urinary concentrations of phenylbutazone for 155 of these horses are presented in Figure 4. No phenylbutazone was found in 25 of the samples, and the modal concentration was less than 1 μg/ml. All but eight of the samples showed urinary concentrations of less than 10 μg/ml. Although the overall distribution was highly skewed with a long 'tail' of individual high urinary concentrations of phenylbutazone, the distribution was well fitted by a log-normal distribution.

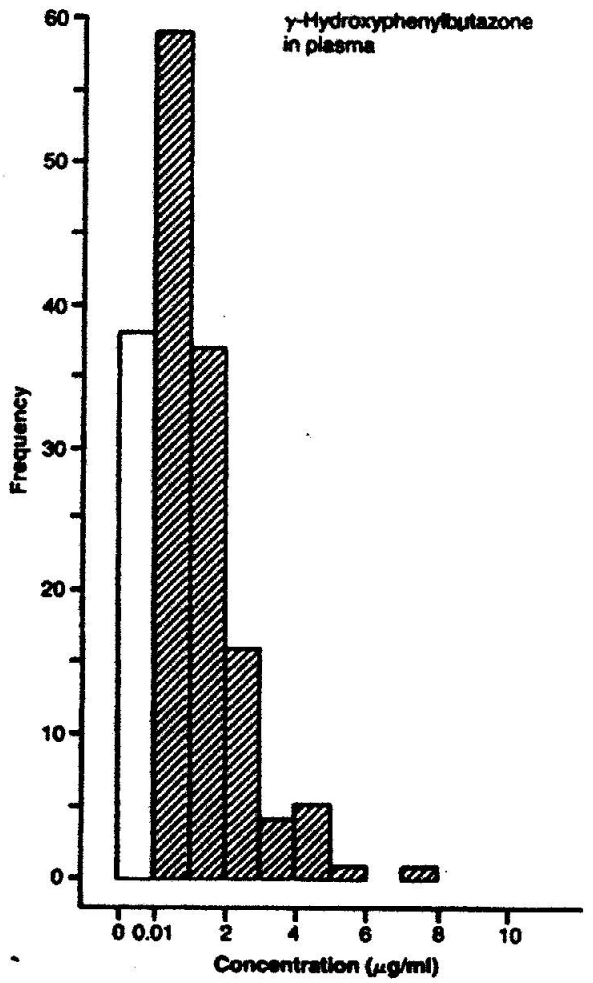
Urinary concentrations of oxyphenbutazone for

Figure 2 *Frequency distribution of plasma oxyphenbutazone levels in 175 thoroughbreds racing in Kentucky. The open bar represents plasma samples in which no oxyphenbutazone was detected. The hatched bars show how often each concentration of oxyphenbutazone occurred. The values ranged from 0.30 to 13.00 μg/ml, with the mode between 1.0 and 2.0 μg/ml, and a mean (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.*



168 of these horses are shown in Figure 5. No oxyphenbutazone was detected in 11 of these horses, and the modal concentration was less than 3 μg/ml. The distribution then fell away sharply, showing only two samples with concentrations between 15 and 18 μg/ml. Thereafter, however, urinary concentrations of oxyphenbutazone increased (with eight horses showing concentrations of 36 to 39 μg/ml), then declined (with a highest recorded concentration of 81.5 μg/ml). The population distribution might best be described as bimodal.

Figure 3 *Frequency distribution of plasma γ-hydroxyphenylbutazone levels in 161 thoroughbreds racing in Kentucky. The open bar represents plasma samples in which no γ-hydroxyphenylbutazone was detected. The hatched bars show how often each concentration of γ-hydroxyphenylbutazone occurred. The values ranged from 0.10 to 7.32 μg/ml, with the mode between 0.1 and 1.0 μg/ml, and a mean (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.*



Urinary concentrations of γ-hydroxyphenylbutazone for 152 of these horses are presented in Figure 6. No γ-hydroxyphenylbutazone was detected in 11 of the samples. The modal concentration in equine urine was less than 4 μg/ml. Concentrations

Figure 4 *Frequency distribution of urine phenylbutazone levels in 155 thoroughbreds racing in Kentucky. The open bar represents urine samples in which no phenylbutazone was detected. The hatched bars show how often each concentration of phenylbutazone occurred. The values ranged from 0.10 to 30.5 μg/ml, with the mode between 0.1 and 1.0 μg/ml, and a mean (not including those samples in which no drug was detected) of 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.*

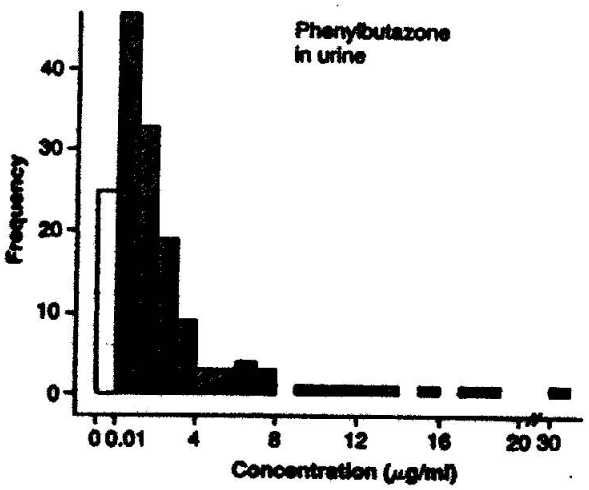


Figure 5 *Frequency distribution of urine oxyphenbutazone levels in 168 thoroughbreds racing in Kentucky. The open bar represents urine samples in which no oxyphenbutazone was detected. The hatched bars show how often each concentration of oxyphenbutazone occurred. The values ranged from 0.30 to 81.50 μg/ml, with the mode between 1.0 and 3.0 μg/ml, and a mean (not including those samples in which no drug was detected) of 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).*

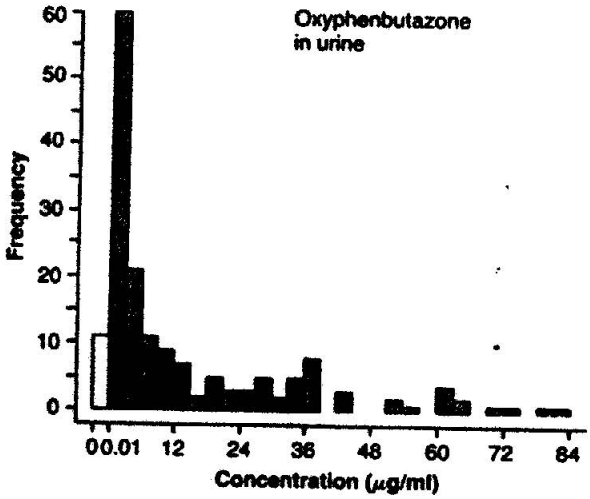


Figure 6 *Frequency distribution of urine γ-hydroxyphenylbutazone levels in 152 thoroughbreds racing in Kentucky. The open bar represents urine samples in which no γ-hydroxyphenylbutazone was detected. The hatched bars show how often each concentration of γ-hydroxyphenylbutazone occurred. The values ranged from 0.05 to 122.0 μg/ml, with the mode between 1.0 and 4.0 μg/ml, and a mean (not including those samples in which no drug was detected) of 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).*

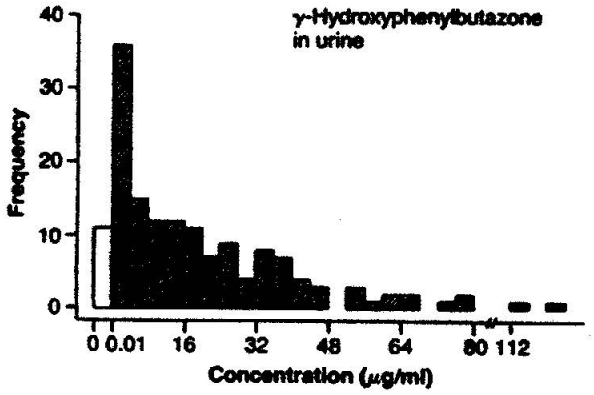
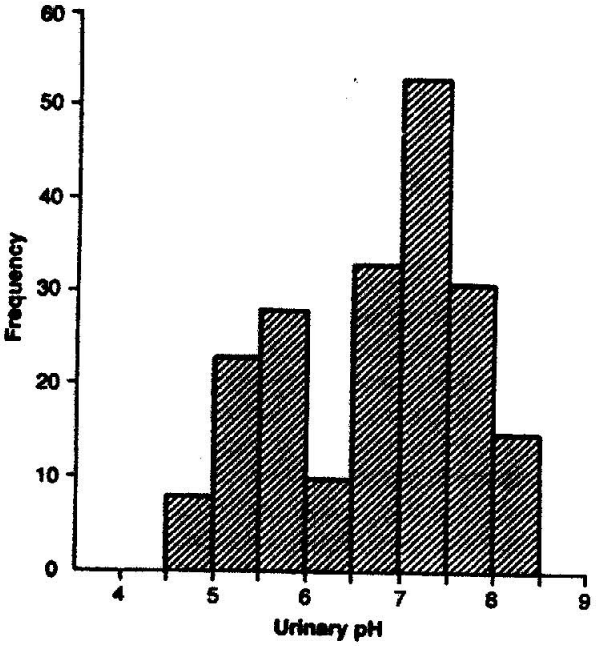


Figure 7 *Population distribution of urinary pH values. The hatched bars show the frequency of pH values in 202 post-race urine samples of horses racing in Kentucky. The mean pH was 6.8.*



ranged up to 80 μg/ml except for two horses which had urine concentrations of γ-hydroxyphenylbutazone above 112 μg/ml. Although clearly skewed to the left, the population distribution was not well fitted by a log-normal distribution and might best be described as indeterminate.

This data shows clearly that urinary concentrations of phenylbutazone and its metabolites follow more complex distributions in urine than in plasma. Although the plasma concentrations of phenylbutazone and its metabolites followed apparently log-normal distributions the concentrations of phenylbutazone metabolites in urine tended to follow more complex patterns.

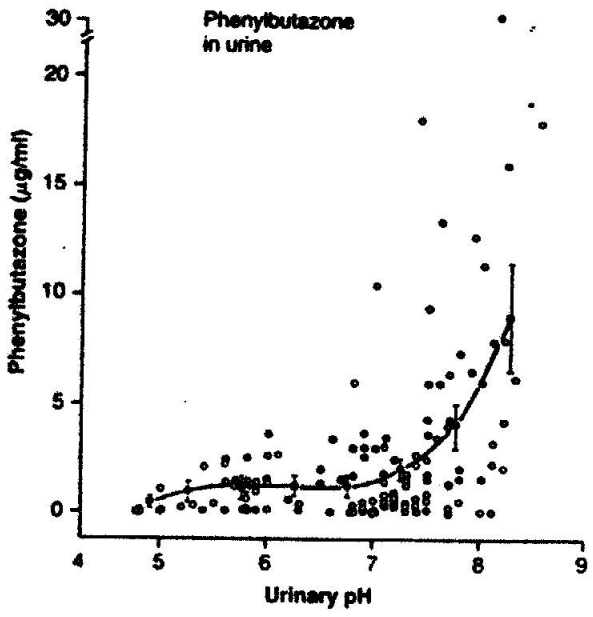
In a preliminary analysis of some of this data, Houston *et al.*[4] have shown that urinary concentrations of oxyphenbutazone were strongly influenced by urinary pH. Because this observation might explain the skewed populations we studied the effects of urinary pH on plasma and urinary concentrations of phenylbutazone and its metabolites.

Figure 7 shows the population distribution of pH values in the 202 post-race urine samples available to us. The population distribution was apparently bimodal, with a population peak at approximately 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.[1]

Figure 8 shows the relationship between urinary pH and urinary concentrations of phenylbutazone. For urine samples with pH values between 4.5 and 5.0 the mean concentration of phenylbutazone was 0.04 μg/ml. This increased rapidly at first, more slowly between pH 6.0 and 7.0, then sharply between 7.5 and 8.5. Overall, the increase of urinary pH appeared to result in an approximately 225-fold increase in the urinary concentrations of phenylbutazone in these post-race horse urines. However plasma concentrations of phenylbutazone were essentially unaffected by urinary pH [Table 1].

Figure 9 shows the relationship between urinary concentrations of oxyphenbutazone and urinary pH. For urinary pH values of 4.5 to 5.0 the average concentration of oxyphenbutazone was 0.6 μg/ml. As urinary pH increased the mean urinary concentrations of oxyphenbutazone increased approximately 66-fold to 40.1 μg/ml at pH 8.0 to 8.5. As with phenylbutazone, oxyphenbutazone concentrations in equine plasma were unaffected by urinary pH [Table 1].

Figure 8 *Effect of urinary pH on urinary concentrations of phenylbutazone. The open circles show urinary concentrations of phenylbutazone (from Figure 4) plotted against urinary pH. The solid circles (with bars) show the mean urinary concentration of phenylbutazone (± the standard error of the mean) for each half pH unit. (Between pH 4.5 and 5.0 this was 0.04 ± 0.04 μg/ml and between pH 8.0 and 8.5, 9.0 ± 2.7 μg/ml.) The line connecting the solid circles was fitted by eye.*



A broadly similar pattern was observed with γ-hydroxyphenylbutazone [Figure 10]. At a urinary pH of 4.5 to 5.0 the mean urinary concentration was 1.4 μg/ml. This concentration increased rapidly until, at pH 6.0 to 6.5, the mean concentration was 28.3 μg/ml. Thereafter the urinary concentration dropped, then rose again rapidly to a mean of 44.2 μg/ml above pH 8. Urinary concentrations of γ-hydroxyphenylbutazone thus increased approximately 32-fold with increasing urinary pH over the range. However, the plasma levels of γ-hydroxyphenylbutazone either were not affected or appeared to decrease with increasing urinary pH [Table 1].

When the logarithms of the urinary concentrations of phenylbutazone and its metabolites were plotted against the pH of the urine samples, an *F*-test showed that the effect of pH was highly significant at $p < 0.0001$. In addition, an analysis of variance showed that 33 per cent of the variation in phenylbutazone concentration, 58 per cent of the variation

Figure 9 *Effect of urinary pH on urinary concentrations of oxyphenbutazone. The open circles show urinary concentrations of oxyphenbutazone (from Figure 5) plotted against urinary pH. The solid circles (with bars) show the mean urinary concentration of oxyphenbutazone (± the standard error of the mean) for each half pH unit. (Between pH 4.5 and 5.0, this was 0.6 ± 0.2 μg/ml and between pH 8.0 and 8.5, 40.1 ± 5.5 μg/ml.) The line connecting the solid circles was fitted by eye.*

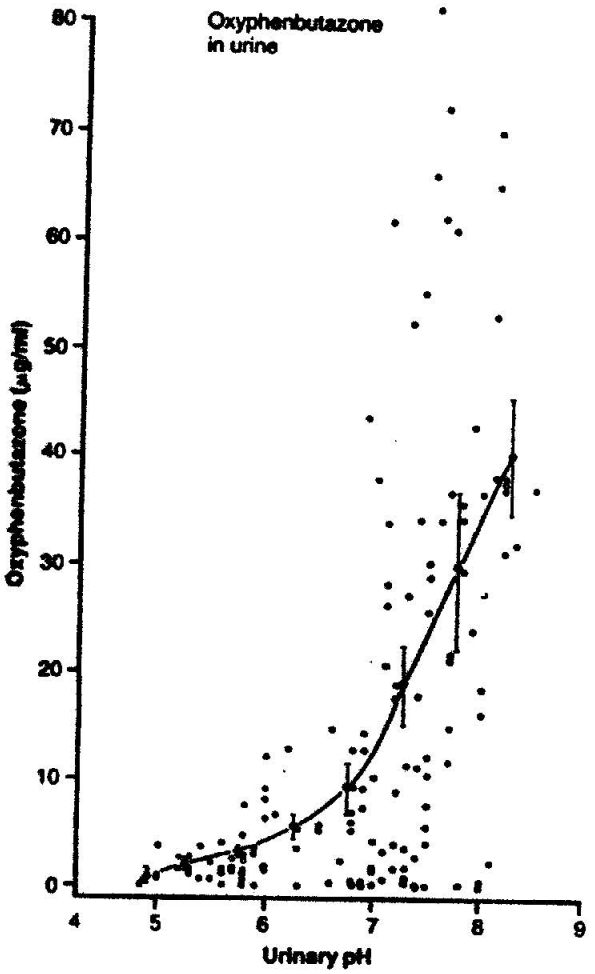
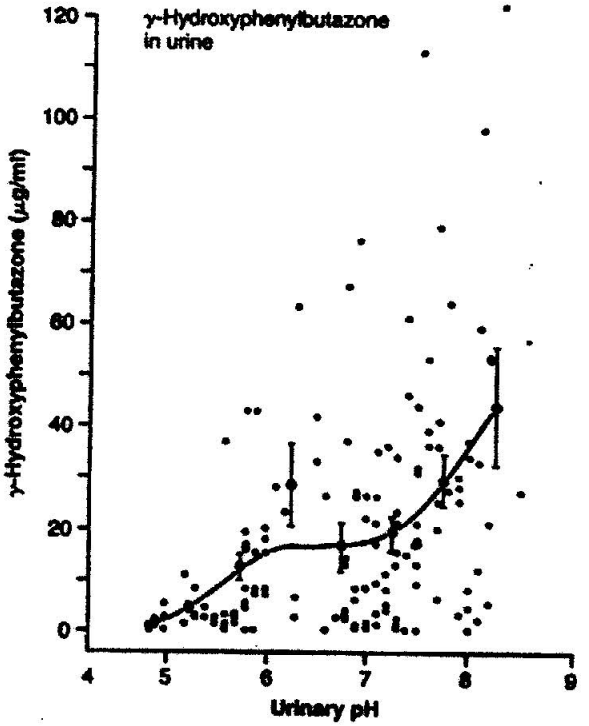


Figure 10 *Effect of urinary pH on urinary concentrations of γ-hydroxyphenylbutazone. The open circles show urinary concentrations of γ-hydroxyphenylbutazone (from Figure 6) plotted against urinary pH. The solid circles (with bars) show the mean urinary concentration of γ-hydroxyphenylbutazone (± the standard error of the mean) for each half pH unit. (Between pH 4.5 and 5.0, this was 1.4 ± 0.9 μg/ml and between pH 8.0 and 8.5, 44.2 ± 11.5 μg/ml.) The line connecting the solid circles was fitted by eye.*



in oxyphenbutazone concentration, and 28 per cent of the variation in γ-hydroxyphenylbutazone concentration in these samples were due to the differences in urinary pH.

Discussion

This data shows that these horses had plasma levels of phenylbutazone and its metabolites that were well fitted by log-normal distributions. However, though the urinary concentrations of phenylbutazone were well fitted by a log-normal distribution, those of oxyphenbutazone and γ-hydroxyphenylbutazone were more complex. Although the mathematical nature of the distributions of oxyphenbutazone and γ-hydroxy-

Table 1 *Effect of urinary pH on plasma levels of phenylbutazone and its metabolites*

	Urinary pH							
	4.5 to 5.0	5.0 to 5.5	5.5 to 6.0	6.0 to 6.5	6.5 to 7.0	7.0 to 7.5	7.5 to 8.0	8.0 to 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
γ-Hydroxyphenyl-butazone (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
Total (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

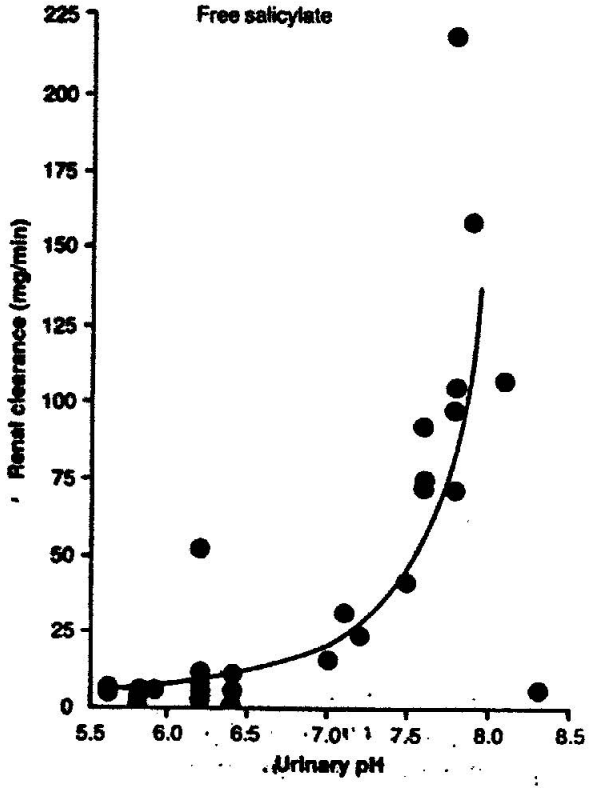
phenylbutazone in urine was not clear, analysis of the data showed that urinary pH had a major influence on concentrations of phenylbutazone and its metabolites in the urines of these horses.

It has long been known that the renal clearance of certain drugs is greatly affected by urinary pH. In 1946 Smith and his co-workers[6] showed that the renal clearance of free salicylate increased more than ten-fold as the urinary pH approached 8.0. At that time the principle of pH trapping of ionized drug molecules had not been developed but the experimental data is clear [Figure 11] and in good accord with the results reported in this paper. The 1946 data shows that the urinary clearance of free salicylate increased exponentially as the pH of the urine increased above 7.0. A broadly similar effect may be expected with phenylbutazone and its metabolites since they have similar pK_a values to salicylate.

Since Smith's initial work with salicylates was reported in 1946 the theoretical basis of this effect has been worked out. The drugs affected are weak acids or weak bases and their pK_a values must be within a certain range: The pK_a value must lie between 3.0 and 7.5 for acidic drugs and between 7.5 and 10.5 for basic drugs. The concentrations and clearance of salicylate, phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone (with pK_a values of 4.6, 4.5, 4.7, and 4.0 respectively) can therefore theoretically be affected by urinary pH.

In general, the effect of pH on equine urinary concentrations of phenylbutazone and its metabolites follows the pattern observed with salicylate. Little effect

Figure 11 *Relationship between urinary pH and urinary clearance of free salicylate for man.[6] The solid circles represent the free salicylate secreted per minute, plotted against urinary pH.*



of pH was seen below pH 7.0 for phenylbutazone and the curve does not appear to climb steeply until pH values of approximately 8.0. However, the concentration differential for phenylbutazone is large, with a 225-fold range between the acidic and basic post-race urines. Over the same pH range the data for oxyphenbutazone shows a 66-fold increase while the data for γ-hydroxyphenylbutazone shows a 32-fold increase. Overall, the variability in urinary concentrations of phenylbutazone and its metabolites turns out to be approximately 33 per cent due to pH for phenylbutazone, 58 per cent for oxyphenbutazone, and 28 per cent for γ-hydroxyphenylbutazone.

In assessing the significance of these estimates of variability it must be remembered that there are other large sources of variability. First, an inspection suggests the data is more complex than simple exponential increases in urinary concentrations with increasing pH. An approximate mathematical model can only underestimate the variability that is due to pH.

Second, these samples were taken from horses racing in Kentucky in the spring of 1983 when there were no restrictions whatsoever on phenylbutazone use. Thus these horses represent the complete range of dosing with this drug—from zero phenylbutazone to medication right up to race day, including possible race-day medication. This is probably by far the most important source of variability overall and it is a tribute to the power of urinary pH to affect the concentrations of drugs that for one metabolite (oxyphenbutazone) pH was in fact the major contributor.

Acknowledgments

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Discussion

GALL Tom, is it not true that there are no restrictions, either, on the use of furosemide in the state of Kentucky?

TOBIN That is correct.

GALL Do you not give any significance to the fact that the horsemen are using furosemide within a few hours of the race and that that affects the urinary levels of phenylbutazone?

TOBIN The regulation in Kentucky for furosemide is four hours prior to post. I'm not familiar with its use outside that: I have no comment on that.

GALL There are no means of enforcing the four hours in Kentucky. Is that not correct?

TOBIN There are two means of enforcing it: There is the detention-barn system and there is a plasma tolerance. Those are the two means that can be used, but neither of these are used in Kentucky currently.

GALL You're using the honour system?

TOBIN That's correct.

SMITH (R L) Tom, looking at the phenylbutazone values of plasma, the variability's huge. I think the figure you gave suggested it ranged from 0.20 to 15.00 micrograms per ml within the population you looked at. Have you any grasp as to the source of this variability—how far it's genetic or environmental in origin? And from the genetic point of view, one would have thought one has a handle in terms of pedigree relationships to look for sibling correlations. Have you looked at this at all?

TOBIN The range in blood levels is not huge; it's infinite, because it goes from zero (or nondetectable) to about fifteen micrograms per ml. I do not know what the factors that contribute to this range are. If you asked me to speculate, I would suggest the first one is the dosage schedule determined by the veterinarian. The second would be the time prior to post that administration of the drug ceased. And that would be about as far as I could go. I wouldn't care to go beyond that except to point out that the range is not that dissimilar to ranges that you get if you dose horses with the clinically accepted doses of phenylbutazone for about three to four days. Does that answer your question, Bob?

SMITH (R L) Yes.

LAMBERT Just two questions. Do any of the American authorities use urine levels in their regulations?

TOBIN That was the general pattern until the early eighties. I'm not sure whether any juristdictions still do or not. I would suspect that some do.

LAMBERT The second question is, are these all winners or is it random sampling?

TOBIN These are just the post-race samples that come to us in the drug-testing laboratory. They're determined in the usual way: winners; beaten favourites; whatever the stewards recommend. If there's exotic betting, the horses finishing second and third will be sampled.

LAMBERT Why I asked that is, is it possible that the fitter horses would have a more acidic urine and be less likely to be on phenylbutazone, whereas the horses that romp in near the end of the field might have a more basic pH and could be on higher doses of phenylbutazone? I'm not disagreeing with your conclusion because it's very logical, but would that be another possibility?

TOBIN That's quite reasonable, because we have no control of the samples presented to us. It's simply an analysis of what went through the laboratory in this period and I can't determine the place of finish of a horse or, indeed, its fitness.

IRVINE Tom, since you've shown so clearly that pH has a considerable influence on urinary concentration, accentuating the importance of pH, I wonder if you could tell us a little about the reasons for the bimodal distribution of urinary pH instead of what one would have expected—a normal distribution.

TOBIN I really can't speculate as to that, Cliff. Perhaps David Snow might like to tackle that one if David is here. Could I throw that one to David?

SNOW From an analysis of urine pHs in pre- and post-race samples, a much higher frequency of

basic urines was seen in the pre-race samples. Details of this and possible explanations for more acidic urines occurring after racing are presented in the *Proceedings* of the Toronto meeting.

IRVINE Well, why isn't it a normal distribution? Why are there two peaks in it?

TOBIN It would be intriguing with the small, consistent population of horses in Hong Kong here to see whether some of them yield acid urines repeatedly post-race and whether there're actually two populations there (one which excretes acid in their urines more readily and consistently) which would be one hypothesis. And the other hypothesis would be David's—that it's simply a random event depending on the volume of urine in the bladder prior to post. However, this would be the place to answer this experimental question.

SNOW You've given David Crone some good work to do.
