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EFFECT OF URINE pH ON URINE LEVELS OF PHENYLBUTAZONE, OXYPHENBUTAZONE AND γ-HYDROXYPHENYLBUTAZONE IN RACING HORSES

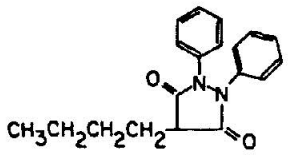
TERESA HOUSTON, THOMAS TOBIN and J.W. BLAKE

ABSTRACT

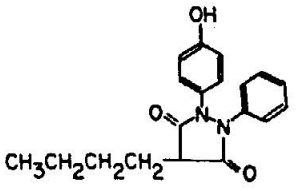
Quantitation of oxyphenbutazone levels in post-race equine urine samples is of forensic importance in regulating the usage of phenylbutazone. Urine pH in racing horses is unusual in that it may range from pH 4.5 to pH 10.0. HPLC was used to measure blood and urine levels of oxyphenbutazone in post-race samples from Thoroughbred horses racing in Kentucky and correlated with urinary pH. Observed pH values ranged from 4.8 to 8.5 and was consistent with the bimodal distribution seen for urine pH in several thousand horses. Data showed that plasma levels of oxyphenbutazone do not vary with pH since similar levels were found independent of the horses urine pH. However, urine concentration of oxyphenbutazone was very variable and highly dependent on urinary pH. Oxyphenbutazone concentration in urine varied nearly 1000-fold depending on whether the urine is acidic or basic.

INTRODUCTION

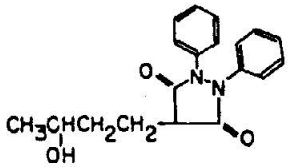
Phenylbutazone (PB) is a non-steroidal anti-inflammatory with effective analgesic and anti-pyretic effects. It is the most widely used anti-inflamatory in equine medicine and its use in racing horses is often regulated by quantitating urine concentrations of phenylbutazone and its metabolites. Phenylbutazone is metabolized in the liver by hydroxylation into two primary metabolites: oxyphenylbutazone (OPB) the active component and an essentially inactive γ-hydroxyphenylbutazone (γ-OHPB) (Figure I).



Phenylbutazone



Oxyphenbutazone



Gamma-hydroxyphenylbutzone

Fig. I

Use of phenylbutazone on the racetrack is regulated by each individual racing jurisdiction. Each racing jurisdiction sets its own rules on phenylbutazone usage - some ban all medication, others have a "24-hour rule", and others have an upper limit on the concentration of PB and OPB that is detectable in the urine usually from 120-165 μg/ml. Kentucky Thoroughbred racing has no upper limit on phenylbutazone in either blood or urine

These differing rules on phenylbutazone usage reflect different perceptions of the effects of phenylbutazone usage in racing horses. For example, some racing chemists hold that phenylbutazone can mask or interfere with the detection of illegal drugs. Others, however, hold that masking by phenylbutazone is not a significant problem in equine forensic testing.

Central to the masking dispute is the concentrations of phenylbutazone and metabolites found in equine urine. Some analysts hold that horsemen, by "over-dosing" with phenylbutazone, can raise urinary concentrations of phenylbutazone and its metabolites to the point at which they make the detection of other drugs impossible. We, therefore, elected to carry out a survey to determine the plasma and urinary levels of phenylbutazone and its metabolites in horses racing in Kentucky. Information as to the concentrations of phenylbutazone and its metabolites found under racing conditions are necessary to allow scientific studies on the masking problem to be designed and interpreted.

MATERIAL AND METHODS

The urine and plasma samples were received from the tracks within 24-48 hours and held at 4 C. The analysis of the urine and plasma was made by HPLC. Following the procedure of Marunaka and co-workers[1] a gradient of methanol and 0.01 M Na acetate starting at 50-50% to 100% methanol over 10 mins. was used. The column was a Ultrasphere ODS 5 μ 25 cm length with a precolumn. The detector was a fixed wavelength UV at 254 nm. The HPLC system was a Beckman-Altex, utilizing a program controller. Standard curves were prepared by spiking urine or plasma with a known amount of PB and its metabolites. The data was plotted as peak height vs μg/ml and analysed by linear regression (Figure II).

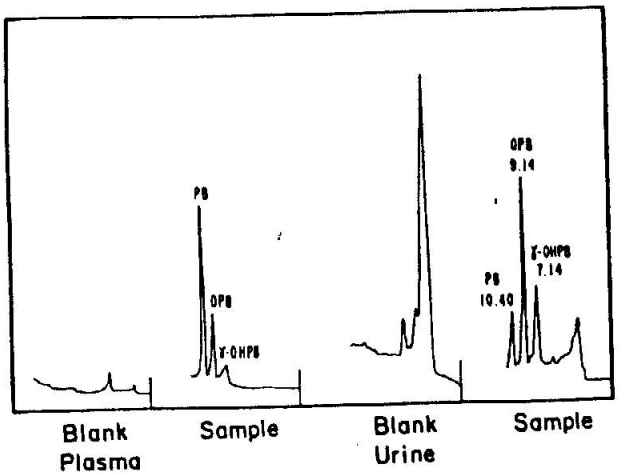


Fig. II

The pH of the urines was measured upon receipt of samples using a Fisher Accumet Mode 230 pH meter.

During the course of these studies it became apparent that a major factor determing urinary concentrations of phenylbutazone and its metabolites is urinary pH. In this communication, we report on the distribution of urinary pH values in horses at two race meets in Kentucky and show how urinary pH affects urine concentrations of phenylbutazone and its metabolites.

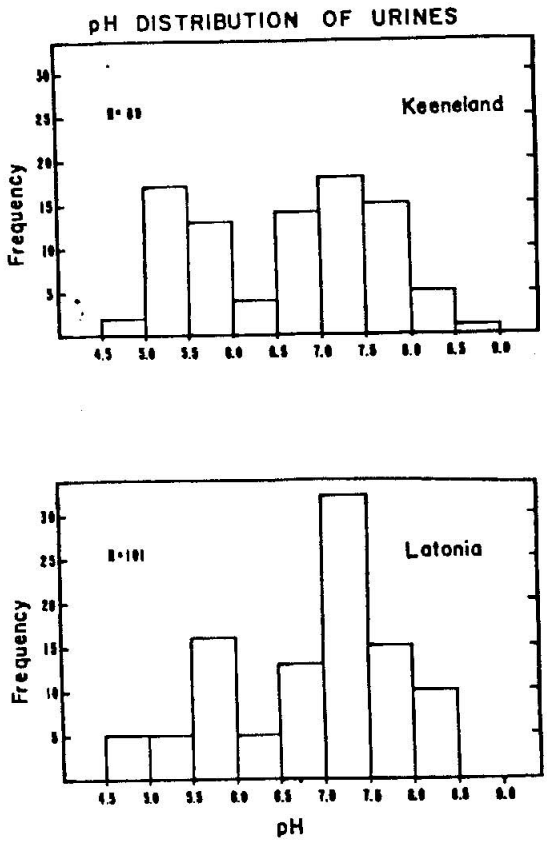


Fig. III

RESULTS

The distributions of pH values in urine samples from the Spring 1983 Keeneland and Latonia meets are shown in Figure III. In each case, the pH range extends from pH 4.5 to pH 8.5 - 9.0, and is apparently bimodal. The range of these distributions is in good agreement with those reported previously by English and Japanese investigators.[2] This wide distribution of pH values makes its likely that pH dependent effects on urinary concentrations of drugs are likely to be found for at least some drugs in the urine of racing horses.

Analyzing this data, substantial pH dependent differences were found in the concentrations as oxyphenbutazone increased. Below a urinary pH of about 7, the concentrations of oxyphenbutazone in equine urine were all about 15 μg/ml or less (Figure IV). Above a pH of about 7, however, the concentrations of phenylbutazone in equine urine were substantially greater, and ranged from 20 to 80 μg/ml (Figure IV). This increase in urinary concentrations of oxyphenbutazone occurred despite a trend for plasma levels of oxyphenbutazone to decrease (Figure V). When these data are reported as oxyphenbutazone urine to plasma ratios against the pH of the urine, the effect of pH on the ratio is readily apparent (Figures VI, VII). These data are consistent with and support our previous results suggesting that urinary pH is the major determinant of oxyphenbutazone concentrations in the urine of racing horses.

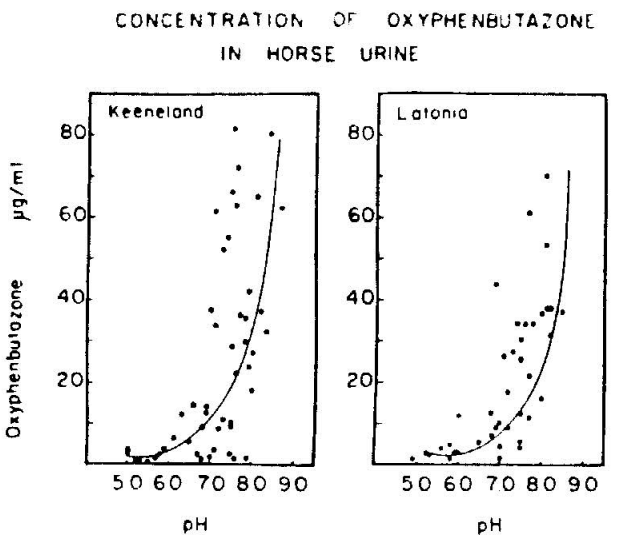


Fig. IV

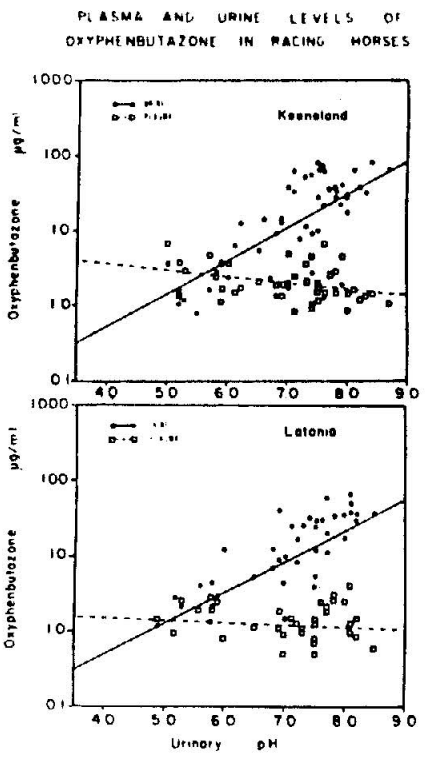


Fig. V

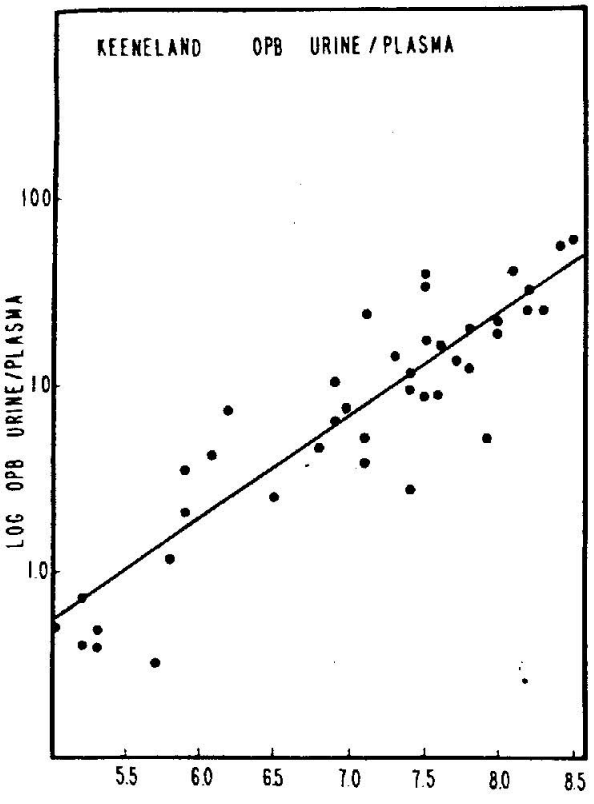


Fig. VI

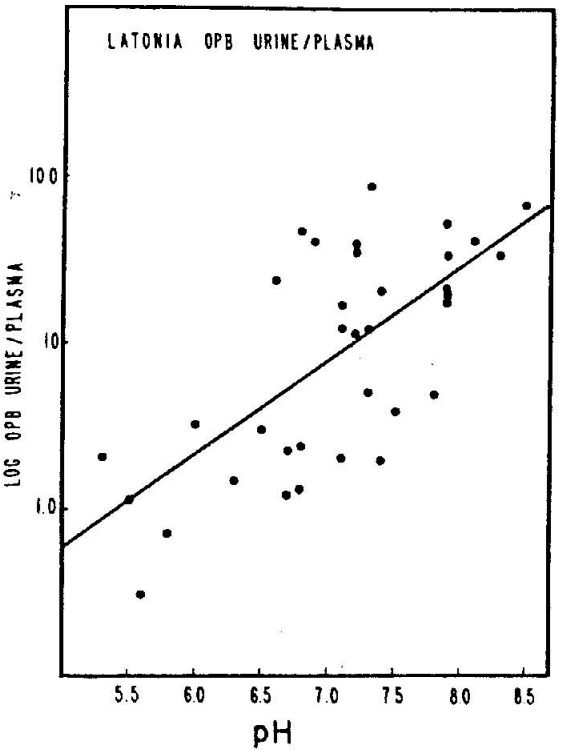


Fig. VII

Urinary concentrations of phenylbutazone also varied with the pH of the urine sample but less markedly than those of oxyphenbutazone. As shown in Figures 8 and 9, urinary concentrations of phenylbutazone average about 1 μg/ml or less below pH 6.0, while at pH levels above 8.0, concentrations greater than 10 μg/ml are not uncommon. When the ratios of urine: plasma phenylbutazone levels were plotted against pH (Figure X, XI) the ratio increased with increasing pH, but the pH effect was not so marked as with oxyphenbutazone.

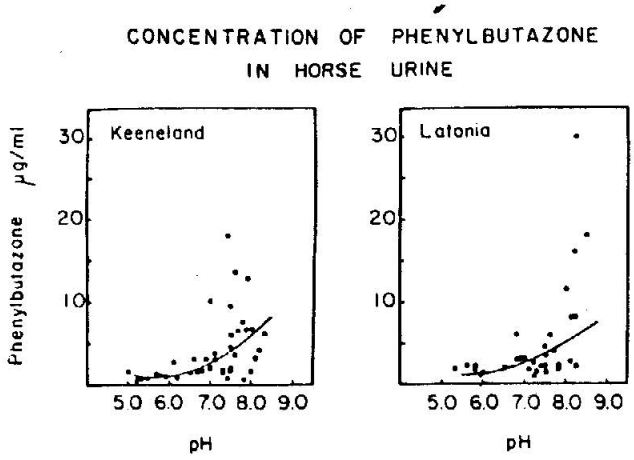


Fig. VIII

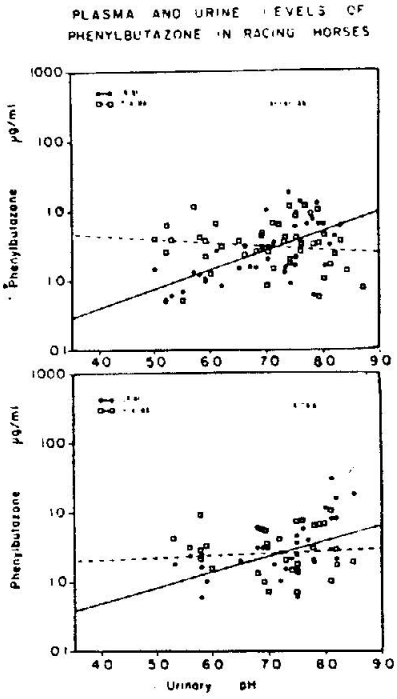


Fig. IX

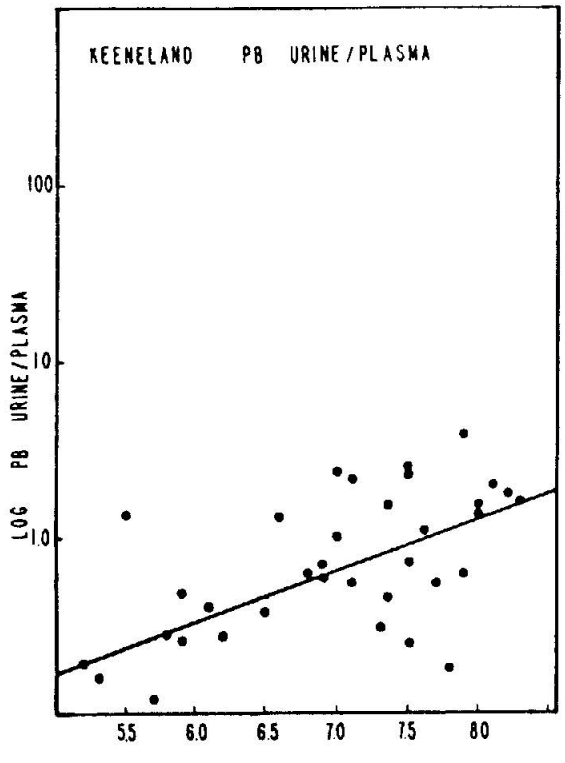


Fig. X

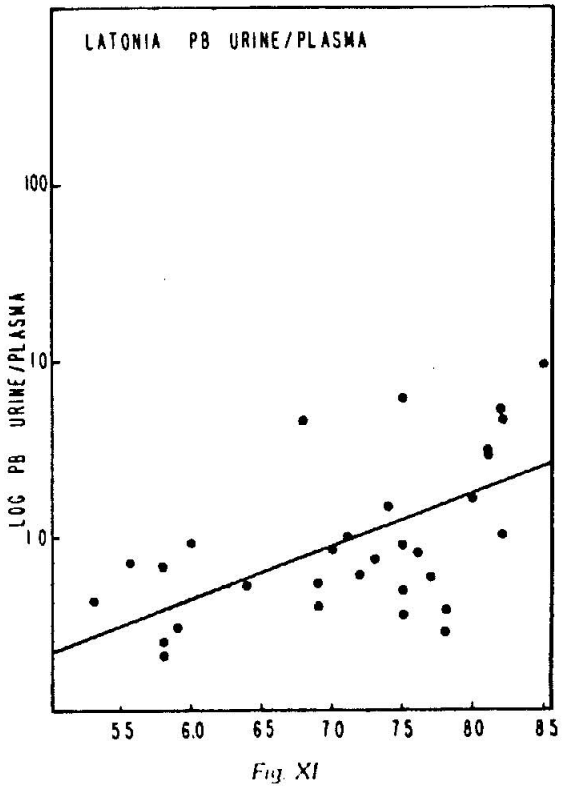


Fig. XI

The urinary concentrations of the alcohol metabolite of phenylbutazone were also affected by the pH of the urine sample (Figure XII). In horses racing during the Keeneland meet, the concentrations of the γ-hydroxyphenylbutazone or the alcohol metabolite in basic urines were much greater than those measured in acidic urines (Figure XIII). However, for reasons which are not clear, this effect was much less marked in horses racing in an earlier meet at Latonia where there was no clear-cut effect of pH on urinary concentrations of γ-hydroxyphenylbutazone.

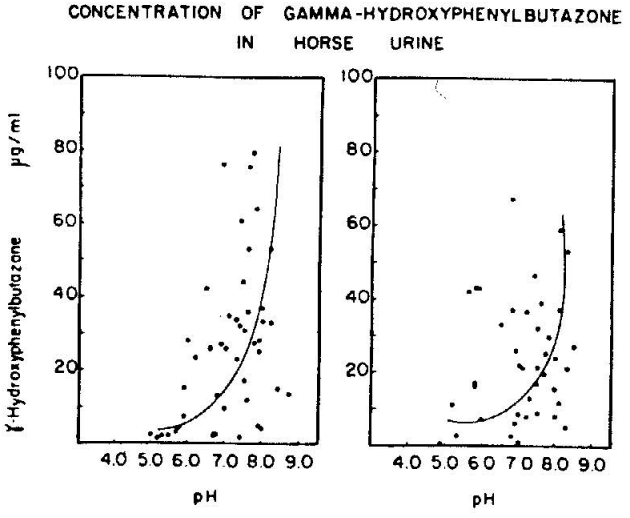


Fig. XII

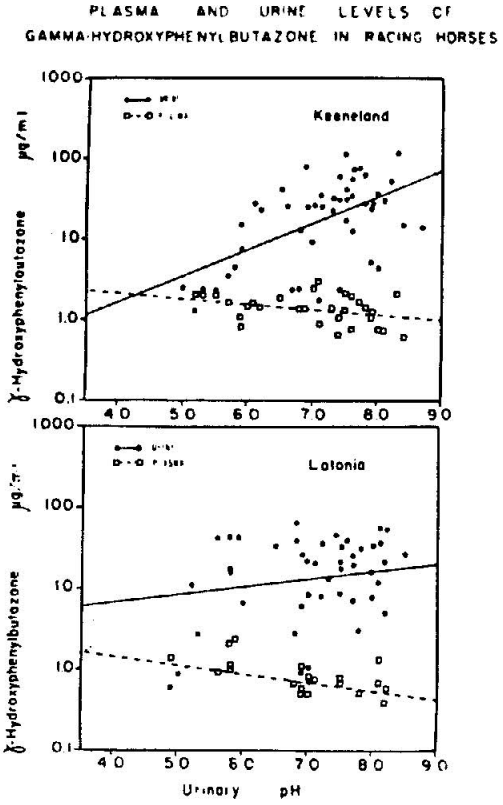


Fig. XIII

DISCUSSION

These results therefore confirm and extend our previous findings with respect to oxyphenbutazone. Concentrations of this metabolite in equine urine varied about 500-fold, depending on the pH of the urine sample, and the effect was consistent throughout both racing meetings examined.

The data for phenylbutazone also suggest an effect of pH on urinary concentrations of this agent. The effect was found in the horses running in both meets and amounts to about a 10-fold increase in urinary concentration of phenylbutazone as the pH of the urine increases. However, the actual concentrations of phenylbutazone in equine were small, the contribution of phenylbutazone to a total value of phenylbutazone and its metabolites is generally small.

In contrast to the small contributions of parent drug, the alcohol metabolite (γ-hydroxyphenylbutazone) makes a major contribution to total urinary levels of phenylbutazone and its metabolites. For data collected at both the Keeneland and Latonia meetings, the concentrations of γ-hydroxyphenylbutazone ranged up to 70 μg/ml. For reasons which are not clear, the Keeneland data appeared influenced more by pH than the Latonia data. Nevertheless, the data clearly show that under some circumstances urinary concentrations of μ-hydroxyphenbutazone can be influenced by the pH of the urine sample.

Since all these samples were racing samples, the doses of phenylbutazone or other drugs which may have been administered to these horses are not known. However, the plasma levels of phenylbutazone and its metabolites observed in these experiments are relatively low and do not vary significantly with pH. On the other hand, urinary levels of phenylbutazone and its metabolites varied directly with pH and in a manner quantitatively consistent with that predicted by pH theory. The data, therefore, strongly suggest that urinary pH is a major determinant of urinary concentrations of phenylbutazone and its metabolites in horses.

The data reported here also shed light on the problem of "masking". "Masking" is thought to occur when high levels of phenylbutazone and its metabolites in a urine sample interfere with the detection of the drugs. One of the principal objections for the use of phenylbutazone in racing horses is that horsemen can use it to "mask" the presence of other drugs. However, these data show no evidence for high blood levels of phenylbutazone, as might be expected if horsemen were attempting to cause "masking" problems. Rather, these results suggest that high urinary concentrations of phenylbutazone and its metabolites are found in horses forming

alkaline urine, in which the ionized form of these drugs and metabolites are "trapped". High urinary concentrations of phenylbutazone and its metabolites are, therefore, more likely caused randomly by the physiology of the horse than by any ability or intent of horsemen to "load" urines with high or "masking" levels of phenylbutazone or its metabolites.

These observations are forensically important, since many racing jurisdictions regulate the use of phenylbutazone by measuring urinary levels of phenylbutazone and its metabolites. Most commonly, racing authorities have rules which state that medication with phenylbutazone within 24 to 48 hours of race time is not permitted. Some authorities enforce these rules based on the assumption that urinary levels of phenylbutazone and its metabolites are directly related to the time of administration of the last dose of phenylbutazone. In support of such rules, pharmacokinetic experiments on small numbers of horses (usually 6 or less) have been performed and reported in the literature[3] and elsewhere[4,5]. However, no investigators have studied the urinary kinetics of phenylbutazone and its metabolites over the full range of physiological pH values of equine urine. Because of this, the validity of these studies as a basis for regulatory and forensic decisions on racing horses is, at best, questionable.

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FOOTNOTE

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DISCUSSION

SNOW: May I ask if the bimodal distribution of urinary pH reported is observed with only post-race samples, or have they also examined pre-race samples? Studies that were carried out with David Crone, Hong Kong Jockey Club indicated a difference in distribution of urinary pH between pre and post-race samples.

HOUSTON: I never really looked at that, it's just all post-race testing, because that's all we do in Kentucky.

SNOW: I would like to suggest that the race will influence post-race urine pH.

HOUSTON: In terms of increase in lactic acid?

SNOW: Yes, this will result in an increase in hydrogen ion excretion in urine, and this could influence the excretion of drugs in the period following the race and micturition.

HOUSTON: That's true.

SNOW: Perhaps what you have reported reflects the degree of emptying of the bladder prior to racing. The pH of a post-race urine sample will reflect the degree of urine present in the bladder prior to racing, generally having a basic pH, and that formed following racing, and being acidic in nature. Also the volume of urine formed will influence the findings.

HOUSTON: No I really can't say that I know.

SNOW: For the data given for Kentucky, was the dose of PBZ administered by injection or orally?

HOUSTON: Generally the dosage was about 2 grams, that's an oral dose, I'm thinking more in terms of an oral dose, in that case it's about 2 grams. There wasn't any excess of the general therapeutic amount that is given to a horse.

SNOW: Prior to the pre-race dose, was the drug administered?

HOUSTON: It is possible, that I do not know. I do not have the records of what kind of chronic dose the horses were given.

WEBER: You indicated the samples were forwarded to your laboratory sometimes 24 to 48 hours after collection. Were pHs done at that time or at the time of collection?

HOUSTON: The pHs were done at the time they were received in the laboratory.

WEBER: One other thing, could you give an indication of how long after a race is made official that the urine and/or blood sample would be taken from the horse?

HOUSTON: All I know, is, it's within a certain hour time, because they just wait till the horse voids, and there's no specific time that I know of when the sample was taken.

WEBER: You don't have a cut off time after which you would abandon a try for urine?

HOUSTON: No I really don't have that information.

McDONALD: You referred to masking. TLC masking, do you experience any other problems with interference of large amounts of bute with electron capture, mass spec., radio immuno assay in your laboratory?

HOUSTON: No sir, we don't.

McDONALD: Have you investigated it?

HOUSTON: Of the samples we run, we usually run thoroughbred tracks, we do not see any interference with the phenylbutazone in any of the procedures that we have used.

McDONALD: You've done a lot of electron capture I presume

HOUSTON: We've done a lot of that and a lot of mass spec. data.

McDONALD: I'm kind of concerned because Sams as of last week noted significant interference with an electron capture assay for ethacrynic acid and Maylin has noted significant interference with huge amounts of phenylbutazone interacting with the charcoal in the RIA kit and consistently your laboratory says there are no problems with inordinately high amounts of phenylbutazone and its decomposition products, etc.

HOUSTON: That's right we have not seen anything that inteferes with our detection methods.

McDONALD: Well aren't your detection methods the same as the NASRC detection methods which Sams, which is a quality assurance lab. for you, monitors?

HOUSTON: Yes, we use mass spec. as one of our procedures, the only one we do not use is RIA, we do not use that as a routine analysis in our lab., but we have a lot of phenylbutazone in the thoroughbreds samples that come in everyday, we are used to dealing, with seeing phenylbutazone and in no case have we seen such an excessive amount that it has interfered as far as we know

with any detection of any other drug. I think our detection of any of the illicit or positive of illicit drug has been quite good, we seem to be able to stay up with any other lab. in the country.

McDONALD: Why is there this divergence of opinion between a quality assurance lab. that you work with and your lab. on the issue?

HOUSTON: I'm aware of the situation, and they are mainly differences of opinion I would suspect.

SMITH: I'm really puzzled by your findings on the basis of the interpretation of the clearance pharmacokinetics. When a thing appears in the urine you normally expect it to disappear from the plasma, your data doest't seem to show that, you see you've got no correlation between the plasma levels and the enhanced appearance of the two metabolites in urine with respect to urine pH. That's correct isn't it? I don't understand that situation.

HOUSTON: Well the plasma is less yes, we found a lower amount, that we observed in the data.

SMITH: You showed no correlation as I understand between the plasma levels and appearance in the urine despite the fact of there being large amounts of metabolites in the urine. That wasn't reflected by changes in plasma level.

HOUSTON: Right, it was not.

SMITH: I'm puzzled why, it's against normal clearance pharmacokinetics.

HOUSTON: It's the interpretation of data. This is strictly the raw data, analysis of over close to 300 horses, and running the HPLC of the plasma, and then again of the urines, most of the metabolites and everything was definitely in the urines and the plasma were reasonably low. I don't think plasma levels were really in excess or any different from those reported by a number of other labs.

SOMA: If these animals had been given lasix wouldn't that artificially change the pH, wouldn't they have a lower pH? And one other statement, urine samples standing over night would lose carbon dioxide so the pH measured the next day would not necessarily reflect the pH taken at sampling time.

HOUSTON: On the latter, that's true but again I think the fact is that I looked over a considerably large number of samples. I think that has to be taken in effect. The other question in terms of the use of lasix. I looked at dosing some horses with lasix and observing, monitoring their pH over a period of time to see if there was any significant change or any change in the pH in the presence of the furosemide and based on what I saw there was very little effect in pH change. In the data that I have of some of the horses which were dosed with lasix at time of racing, the PB, OPB and gamma alcohol levels I do have, they are a little bit lower than data with horses that are non-lasix horses. The data with the furosemide shows the same general trend but the values are slightly lower but it does show the same trend with the pH.