# DETECTION TIMES AND CLEARANCE TIMES FOR DRUGS IN RACING HORSES

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Acknowledgements: The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published as Kentucky Agricultural Experiment Station Article No. 89-4-34 with approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #165 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky.

Supported by grants from the Kentucky Equine Drug Research Council and the Kentucky State Racing and Harness Racing Commissions.

Adapted from "Uncertainty in the Detection Times for Drugs in Horses," by T. Tobin (1986), published in *J. Equine Vet. Sci.* 6(3):124-128.

## SUMMARY

Major uncertainties exist with reference to the times for which drugs can be detected in the blood and urine of horses. As a general rule drugs are detectable in blood for much shorter periods than in urine, and some drugs are detectable in blood only with great difficulty. On the other hand, the great majority of drugs are detectable in urine, either as parent drug or drug metabolites, and the recent advent of sensitive immunoassays makes it likely that, in principle, virtually all drugs are detectable in urine.

The times for which drugs can be detected in blood or urine varies enormously, depending on three major factors. In the first place, horses are treated with drugs at one million-fold different doses, and they eliminate these doses at rates that vary about 300-fold. Secondly, the sensitivity of the tests that the analysts use to detect these drugs can easily vary up to 100-fold or more. Thirdly, horses treated with exactly the same doses of drugs can "spread out" or distribute the blood levels of these drugs about 50-fold in a skewed or irregular manner. In this distribution, a large proportion of horses show lower blood levels of drugs, but a small proportion of horses show relatively much higher blood levels of drugs. Beyond this, the different pH (acidity) values of urine samples can cause urinary levels of drugs to vary by 200-fold or more.

Because of these factors there are large uncertainties concerning the blood or urinary levels of drugs which are found even after the same doses of the same drug. These uncertainties lead to enormous variations in the times that it takes drugs to clear the blood and urine of horses and, therefore, to comparable technical difficulties in the regulatory process of medication control.

## INTRODUCTION

The problem of clearance times for drugs in racing horses is a central problem in equine medication control. In an ideal world all horses would be equal and all doses of

drugs to horses would be the same. These equal horses would then metabolize each drug at the same rate, and the times taken for drugs to clear horses could be determined and tabulated. These "detection time" tables would be made available to veterinarians and regulators, medication rules would be drafted based on these tables, and these rules would then be obeyed by horsemen. Unfortunately, as I will set forth below, the situation is far more complex than this idealized situation. This complexity, in turn, leads to multiple solutions to the problem of equine medication control. In this, as in other areas, the existence of multiple solutions means that no one solution is clearly superior. I know that you as practicing veterinarians have a sense of the complexity of the problem, but by the time that we finish these lectures I think you will have an even better understanding of the magnitude of the problem.

The first factor that you should be aware of is that virtually all drugs are much more difficult to detect in blood than in urine. The principal exceptions to this rule are the acidic drugs such as phenylbutazone and furosemide, which are highly bound to plasma proteins and are therefore present in high concentrations in blood (Tobin 1981). Additionally, these are generally less potent drugs, and are, at least at this time, administered in doses of between 0.5 and 2 g/horse. For this reason, these drugs are easily detectable in urine and also, in general, easily detectable in blood. In point of fact, phenylbutazone metabolites can be present in urine at concentrations that have been claimed by some to interfere with the detection of other drugs, and exhibit so called masking effects.

Once one begins to consider the basic drugs it turns out to be much more difficult to detect these drugs in blood than in urine. This is important because the basic drugs include the bulk of the hard or illegal medications whose use is uniformly proscribed in horses. These include stimulants, depressants, local anaesthetics and tranquilizing drugs that have been defined as the "hard" or illegal medications. These drugs are not bound to plasma proteins to the same extent as the acidic drugs and are therefore much more difficult to detect in blood. Beyond this these drugs can be very potent, being administered to horses at dose of 1 mg/horse or less. Under these circumstances the detection of these drugs in urine represents a substantial challenge, but their detection in blood is much more of a challenge and one that is not completely solved yet, even with the new and very sensitive immunoassay detection methods.

## HOW HORSES ELIMINATE DRUGS

The technical complexity of the medication problem is remarkable. There are about 4,000 drugs in everyday use, and between 10 and 100 times this number of agents have been tested in laboratory animals. Beyond this, there are 63,000 chemicals in common use, which makes for an enormous number of chemicals likely to turn up in a racing horse. The number of agents likely to be detected in horse urine is remarkable.

Adding to the basic complexity due to the number of agents are the facts that different drugs are administered to horses at markedly different doses, and are eliminated by horses at widely different rates. The doses given to a horse can vary one million-fold, from a few micrograms (millionth of a gram) of a very potent substances, such as etorphine, to eight grams of a drug such as naproxen. This million-fold difference in amounts of drug injected makes for equivalent differences in the ease with which they can be detected, and also for very substantial differences in the time for which they can be detected in blood or urine.

To get a feeling for the complexity of this problem, let us follow the sequence of events when a dose of phenylbutazone is administered to a horse. The first thing of which one has to

be aware is that when one administers such a dose, an extremely large number of drug molecules are injected. One dose of phenylbutazone contains about $10^{21}$ molecules ie. 10 followed by 21 zeros. The smallest dose of drug that one is likely to inject contains about $10^{16}$ drug molecules, or about 45 µg of etorphine/ horse. A question that has bothered researchers for a very long time is how long it takes a horse to completely eliminate these very large numbers of drug molecules. Until recently, it was considered that drug excretion would continue indefinitely. However, inspection of the characteristics of drug elimination suggests that horses can "completely clear" from their bodies virtually any dose of any drug administered and that for some rapidly excreted drugs, this may occur in a matter of days.

Horses are able to rapidly excrete these very large numbers of drug molecules because they excrete drugs (as do all animals) by halving the amount of drug in their bodies in a relatively short period of time. The period of time that it takes to eliminate half of a dose of drug is called the half-life of that drug. You can mimic this process by taking a piece of paper and tearing it in half, and then in half, and then in half again. In the first half-life, most commonly about 3 to 10 hours, the horse elminates 50% of the drug in his body. In the next half-life, he halves the remaining amount of drug again, so now he has excreted 75% of the drug dose (Figure 1). This halving process continues until all of the

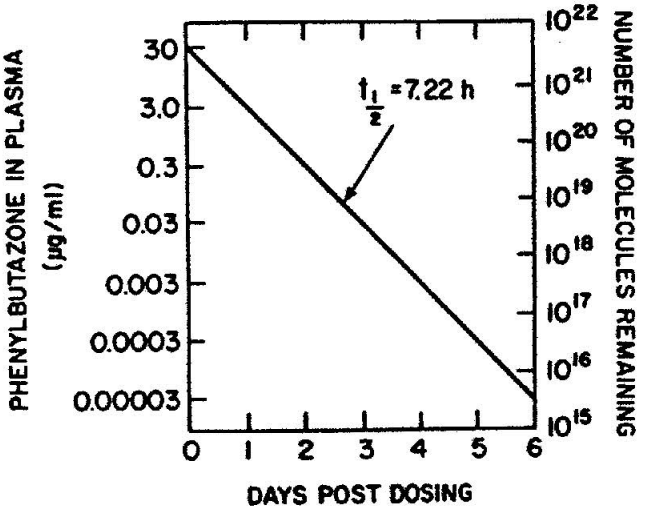


**Figure 1:**

**Elimination of a hypothetical dose of phenylbutazone by a horse. A dose of 3 g phenylbutazone/450 kg horse means that about 6 x $10^{21}$ molecules of phenylbutazone are injected into the horse. This dose will give an initial blood level of approximately 30 µg/ml. If the drug is cleared with a $t_{1/2}$ of 7.22 h, 90% of the administered drug will be eliminated every 24 h. By extrapolation, elimination of the last drug molecules will occur at about 21 days after dosing assuming that each drug molecule has the same probability of being eliminated by the horse, whether it is the first or last molecule eliminated.**

**This outcome requires only that elimination of the drug continue to follow first order kinetics and the same rate constant. Good experimental and theoretical grounds exist to support this suggestion. The model is quite independent of any conceptual pharmacokinetic compartments for interpreting the actual rate of decline in drugs levels. (Tobin 1986). Reproduced with permission from *J. Equine Vet. Sci.***

drug is eliminated. It is relatively easy to show that the average horse will excrete all the $10^{21}$ drug molecules of a dose of phenylbutazone from his body within about 70 half-lives. The actual number of half-lives that the process takes depends only on the number of drug molecules that were originally injected, and ranges between 66 and 77 half-lives, depending on the number of molecules injected.

In practice, of course, what happens is that the chemist is no longer able to find any "trace" of the drug long before it is completely cleared by the horse. In general, once the number of drug molecules in a horse drops below $10^{16}$, it becomes very challenging for the chemist to detect the drug. This shorter period, then, is the period for which the chemist can "detect" the drug in the horse and is spoken of as the "detection time" for that drug in the horse.

**TABLE I**
**Dose, Half-Lives and Estimated Number of Days to Completely Clear a Horse**

| Drug | Dose | Half-life (h) | Number of Half-Lives to Clear 99% of horses | Estimated Clearance Times (days) |
|---|---|---|---|---|
| Etorphine | 0.045 mg | — | 63 | — |
| Furosemide | 250. mg | 7.6 (Urine) | 76 | 24 |
| Methylphenidate | 400. mg | 3.4 (Plasma) | 77 | 11 |
| Pentazocine | 500. mg | 16 (Urine) | 77 | 51 |
| Phenylbutazone | 2. g | 7-20 (Urine) | 79 | 24-66 |
| Reserpine | 2.5 mg | 264 (Urine) | 68 | 748 |
| Procaine | 2.4 g | 24 (Urine) | 79 | 79 |
| Caffeine | 1.8 g | 17 (Urine) | 79 | 56 |
| Morphine | 45. mg | 5.98 (Urine) | 73 | 18 |

from Tobin (1986) Reproduced with permission from *J. Equine Vet. Sci.*

As well as depending on the dose of drug administered, the time for which a drug is detected depends to a large extent on the half-life of the drug in the horse. This is because the half-lives of drugs in horses can vary up to 300-fold, from about 30 minutes for certain short half-life drugs, to half-lives of days or longer for drugs that are slowly eliminated. The data of Table I shows a list of the half-lives of some commonly used equine drugs. As shown in this table, the time for some drugs to completely "clear" from the horse, the clearance time, can be as short as about 2-5 days, while that for a more slowly excreted drug such as reserpine can, at least theoretically, approach two years. Overall, therefore the apparent range of half-lives and clearance times for drugs in horses varies up to 300-fold or more between individual drugs in the ideal or average horse.

As a general rule, the detectability of a drug in a horse depends on both the amount of drug administered and the speed with which the horse clears the drug. If the drug is administered in gram amounts, such as with phenylbutazone or naproxen, and it has a long plasma half-life, then it, or its metabolites, will be detectable in blood or urine for relatively long periods. On the other hand, if the drug is given in very small amounts, in the

## THE LOG-NORMAL DISTRIBUTION

The first clear example of the marked variability in the way individual horses handle the same dose of a drug came in studies by an industry group on phenylbutazone. The committee was charged with determining what would be the highest blood level of phenylbutazone seen in horses 24 hours after dosing with a clinically acceptable dosage schedule. While it was clear that the average blood level of phenylbutazone in these horses would be about 4 µg/ml, our concept of the amount of "spread" that would occur in the blood levels between different horses was substantially off the mark. This author remembers the skepticism with which suggestions that 10 µg/ml of phenylbutazone might be an appropriate upper level were received. When the experiment was done, however, this suggested figure was far too conservative. In the 49 horses tested in this experiment, one showed a blood level of about 13 µg/ml, and a statistical projection of this data showed that one horse in a thousand would yield a blood level of about 23 µg/ml (Figure 2).

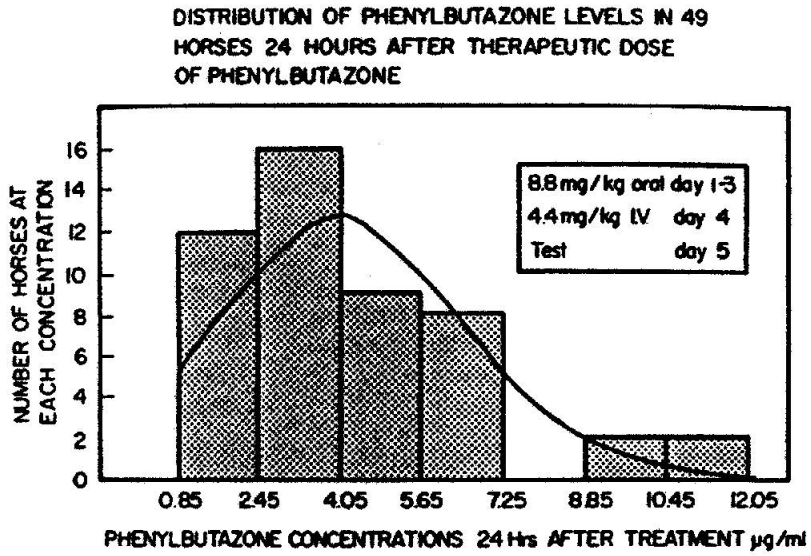


**Figure 2:**

Phenylbutazone levels in 49 horses 24 hours after therapeutic doses of phenylbutazone. Forty-nine horses were dosed with 8.8 mg/kg (about 4.0 g/1000 lb) of phenylbutazone orally for 3 days and then given 4.4 mg/kg (2 g/1000 lb) on the fourth day. Twenty-four hours after the IV dose, blood samples were drawn from each and assayed for phenylbutazone. The vertical bars show the number of horses found with the indicated blood levels of phenylbutazone, while the solid line represents a population curve fitted to these data. These data are reproduced with the permission of the National Association of State Racing Commissioners. The experimental protocol was prepared by the NASRC Blue Ribbon Medication Committee. The horses were dosed and analytical facilities were provided by Dr. George Maylin, Cornell University; Dr. Cliff Woodward, Pennsylvania; Dr. Richard Sams, The Ohio State University; and Dr. Thomas Tobin, University of Kentucky. Data analysis was performed at Cornell and Kentucky. These experiments were completed in the spring of 1980 (Tobin 1986). Reproduced with permission from *J. Equine Vet. Sci.*

order of a milligram or so, and if it tends to be rapidly excreted, then it may only be detectable for relatively short periods in urine, or it may not be detectable at all. For many years, fentanyl, which was administered in amounts of 1 mg or less, was virtually undetectable. Now, however, fentanyl is relatively easily detected in horse urine and its use in racing horses is well controlled (Tobin *et al* 1988).

## THE SENSITIVITY OF THE ANALYST'S TESTS

The second factor which affects the period for which one can detect a drug in blood or urine is the sensitivity of the analyst's tests. To return briefly to Figure 1, these data represent the elimination by a horse of a hypothetical dose of phenylbutazone (3 g IV) with a half-life of about 72 hours. The horse starts with a blood level of about 30 μg/ml at the point that the drug is injected, and in one day he has cleared 90% of the drug from his body. At this point, he is close to the recommended tolerance in blood (2 to 5 μg/ml, depending on the jurisdiction) of phenylbutazone in the horse, and he would pass a blood level tolerance test in many American states. By 48 hours after the dose, he only has 0.3 μg/ml in his blood, and while the drug is still easily detectable, he would likely pass most American rules. He would not, however, pass in Australian, Canadian or English racing, since the drug is still easily detectable in his blood and urine, and these jurisdictions do not allow any detectable level of drug. Since the drug will be in the horse for 21 days, it will be detectable for at least another 3 or 4 days, depending only on the sensitivity of the tests that the analyst uses. Since there is usually no stipulation for the analyst to use any particular type of test, the sensitivity of the test can be changed by the analyst at will. This is the problem with "no detectable level" rules, namely that the detectable level depends to some extent on how hard the analyst tries to detect the drug, and also on whether he is looking for the drug in blood, which, as pointed out above, is difficult, or in urine, in which the drug can be much more readily detected. In general, therefore, if the rule in question is a "no detectable level" rule, it becomes very difficult to stipulate a time for which the drug can be detected because one has virtually no idea as to the sensitivity of the method that the analyst will be using.

The solution to this problem is simply to specify the "residue level" or "test level" at which an offense occurs. If the rule specifies a test level, then the type of test the analyst uses does not matter, since if the concentration of drug is below the specified level, it is irrelevant.

Having determined the drug level, let us say 5 μg/ml in blood for phenylbutazone, one might then conclude that it would be easy to determine the period of time before dosing at which the drug should be withdrawn. While the "residue level" or "test level" approach is the best and most satisfactory approach, it is still not a straightforward approach, because of the biological variability between horses, which results in very substantial "spreading out" of the blood or urinary levels of drugs in horses.

## BIOLOGICAL VARIABILITY: HOW HORSES "SPREAD OUT" BLOOD LEVELS OF DRUGS

The factors discussed up to now, dose, half-life, and test sensitivity, affect the detectability of drugs and the period for which they are detectable after dose. I want to speak next, however, about a major factor which affects the period for which a given drug can be detected in different horses; this is, the variability between individual horses in the way in which each handles a given drug. This is a very important factor, for these differences are easily underestimated, and experiments done in small numbers of horses will not accurately reflect the variability seen in horses that are screened in routine racetrack testing. We are aware of two major contributions to this variability. The first of these is the tendency of plasma levels of drugs in horses to be log-normally distributed, and the second is the effect of urinary pH on urinary concentrations of drugs.

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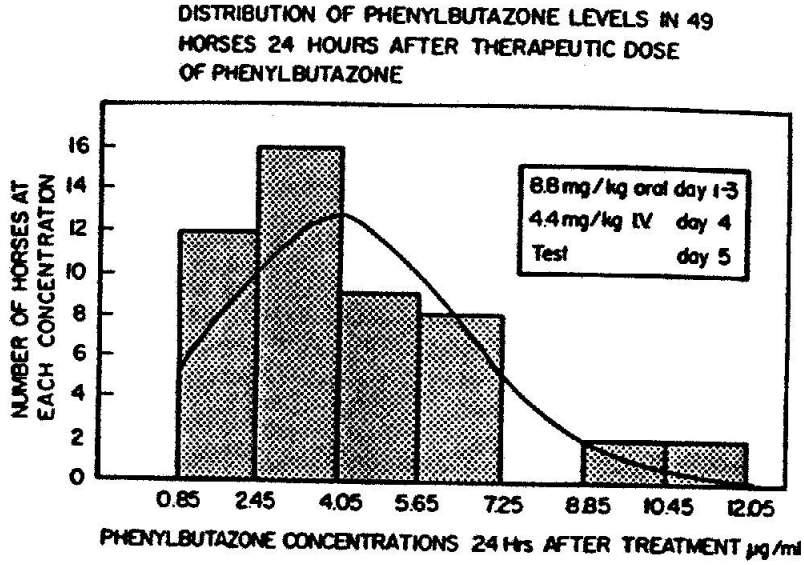


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While the wide spread of this data was considered surprising in 1980, the experiment has since been repeated several times. It turns out that when you dose horses with a drug and follow the blood or urinary levels of the drug, the horses (or humans, or presumably any other species) spread these levels out in a peculiarly skewed distribution, with a cluster at the lower end of the distribution, but a longer tail at the higher concentrations. This type of distribution is called log-normal distribution, for the very good reason that it becomes a "normal" or bell curve distribution if you convert the horizontal axis to logarithmic units. The problem with this type of distribution, however, is that if you estimate blood levels or "detection times" for drugs in small numbers of horses, you will tend to miss the rare horses that contribute to the high concentration "tail". These horses, of course, are the ones that will tend to show "positives" in post-race tests, since they are the ones that show high blood or urinary levels of drugs. When I say small numbers of horses, I mean ten or fewer horses, and it appears that to get a "grip" or estimate of the skewdness of a plasma level distribution, you have to test about 50 horses or more.

## URINARY pH AND URINARY DRUG CONCENTRATIONS

While it appears that this log-normal distribution pattern shows up in both blood and urine, there are further complicating factors which affect the urinary concentrations of drugs. These factors are urine volume and pH, and, of these factors, urinary pH is likely to be of by far the greater importance.

The term pH means whether the urine that the horse is putting out is acidic or basic. It turns out that racehorses put out urine of unusually wide pH values, varying from relatively acidic (pH 4.5) to quite alkaline (pH 9.0) (Houston *et al* 1985). This is an unusually wide pH range, and since pH is measured on a logarithmic scale, the actual acidity difference between an acidic and a basic urine can be a 500,000-fold difference. Figure 3 shows the range of pH values found in the post-race urines of horses racing in Kentucky, with one group of horses putting out acidic urines, and one group of horses putting out basic urines. This bimodal distribution is characteristic of post-race urines, and has been noted in urine samples from England, Japan, and Hong Kong as well as Kentucky.

Any pharmacologist or veterinarian worth his salt will tell you that differences in urine pH are well known to affect the urinary concentrations and rates of excretion of certain drugs. Since there is no reason to think that the horse is any different than other animals, we examined the urinary concentrations of phenylbutazone and its metabolites in samples from horses racing in Kentucky. In this analysis, we noted that the concentrations of oxyphenbutazone were much higher in basic urines, as compared with its concentrations in horses putting out an acidic urine (Figure 4). This follows what pharmacologists call the trapping rule, which states that acidic drugs "trap" in a basic urine, while the basic drugs "trap" in acidic urines.

What the ultimate forensic significance of this effect is, is not clear, but it is certainly much larger than the minimal value currently assigned to it by some scientists. Our studies with phenylbutazone have shown that the "ion trapping" effect in basic urines was at least 200-fold for phenylbutazone, 60-fold for oxyphenbutazone, and an apparent 30-fold for the alcohol metabolite (Houston *et al* 1985). The maximal theoretical effect for phenylbutazone is about 3,000-fold, while the theoretical effect for procaine, a commonly detected basic drug is about 9,000-fold. These are very large effects indeed, and along with the currently unknown effects of urine volume make it difficult to relate a urinary drug concentration to pharmacological effect or a time of drug administration with any degree of accuracy or confidence.

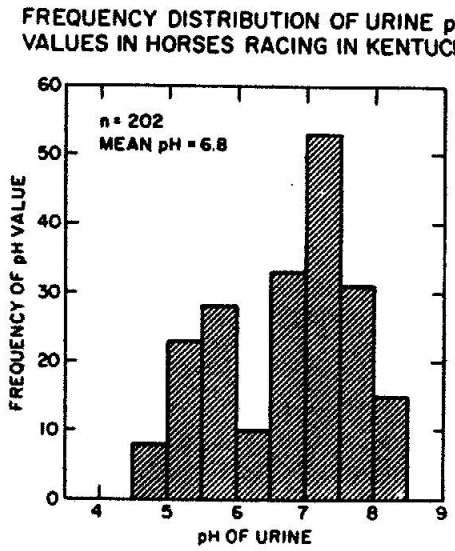


**Figure 3:**
The hatched bars show the frequency of observed urinary pH values in 202 post-race urine samples (Tobin 1986). Reproduced with permission from *J. Equine Vet. Sci.*

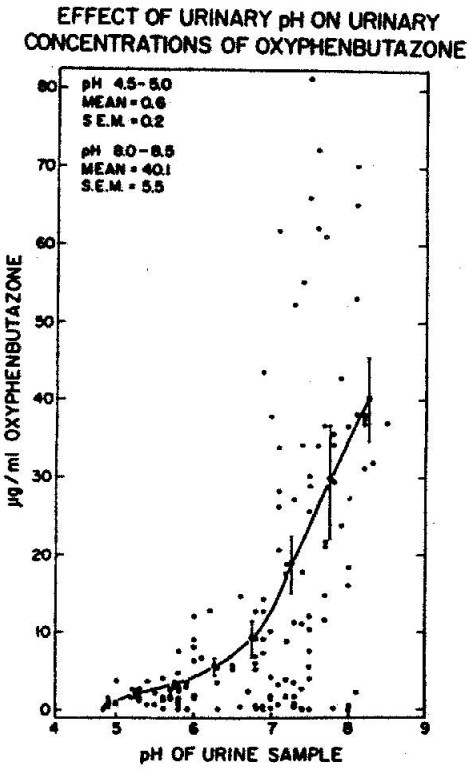


**Figure 4:**
The open circles (O-O) show urinary concentrations of oxyphenbutazone plotted against urinary pH. The solid circles (●-●) show the mean urinary concentrations of oxyphenbutazone for each half-pH unit + SEM. The line connecting the solid circles was fitted by eye (Tobin 1986). Reproduced with permission from *J. Equine Vet. Sci.*

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