Pharmacology

Non Reviewed

UNCERTAINTY IN THE "DETECTION TIMES" FOR DRUGS IN HORSES

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SUMMARY

There are three major sources of uncertainty in the times for which drugs can be detected in a horse's blood or urine. First, horses are treated with drugs at one million-fold different doses, and they eliminate these doses at rates that vary about 300-fold. Second, the sensitivity of the tests that the analyst uses to detect these drugs can vary up to 100-fold or more. Third, horses treated with exactly the same doses of drugs can "spread out" or distribute the plasma 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 at least 200-fold. These factors cause large uncertainties concerning the blood or urinary levels of drugs which are found even after the same doses of the same drug. These uncertainties result in considerable technical difficulties for the regulatory process of medication control.

INTRODUCTION

The technical difficulties with medication control have three primary sources. The first of these is the large differences in the doses of individual drugs given to horses, and the large differences in the rates with which these doses are eliminated by the average horse. The second area is the sensitivity of the test that the analyst uses. The third problem area is horse-to-horse variability in the way that the same dose of the same drug is handled. Together, these three sources of variability make equine medication control technically challenging. In this article, we will detail the factors which affect the times for which drugs can be detected in horses.

HOW HORSES ELIMINATE DRUGS

The technical complexity of the medication problem is remarkable. There are about 4000 drugs in everyday use, and at least ten 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 sheer number of agents likely to be detected in horse urine is remarkable.

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 very potent substances such as etorphine[a] 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 in the time for which they can be detected in blood or urine.

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[a]Etorphine is the generic name for "elephant juice," a very potent narcotic and stimulant drug in racing horses.

TABLE 1

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

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

When a clinical dose of phenylbutazone is injected into a horse, an extremely large number of drug molecules are injected. One dose of phenylbutazone contains about 10^{21} molecules, that is, 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 large numbers of drug molecules. Until recently, it was considered that drug excretion would continue indefinitely. However, we now know that in fact, horses can "completely clear"[b] from their bodies virtually any dose of any drug administered and that for some rapidly excreted drugs, this can 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 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 eliminates 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 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, the chemist is no longer able to find any "trace" of the drug long before it is completely

[b]To completely clear a drug is to eliminate it to the point that there are no drug molecules whatsoever left in the horse.

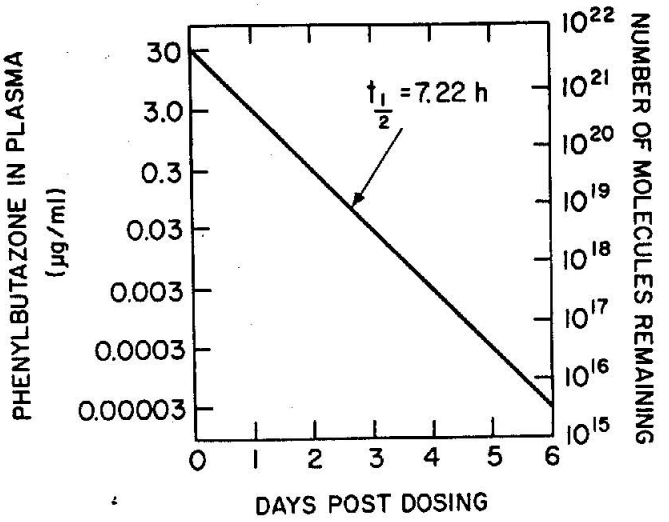

Figure 1. Elimination of a hypothetical dose of phenylbutazone by a horse. A dose of 3 g phenylbutazone/450 kg horse means that about 6 x 10^{21} molecules of phenylbutazone are injected into the horse. This dose will give an inital blood level of approximately 60 µg/ml. If the drug is cleared with a t1/2 of 7.22 h, 90% of the administered drug will be eliminated every 24 h. By extraplation, 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 drug levels.

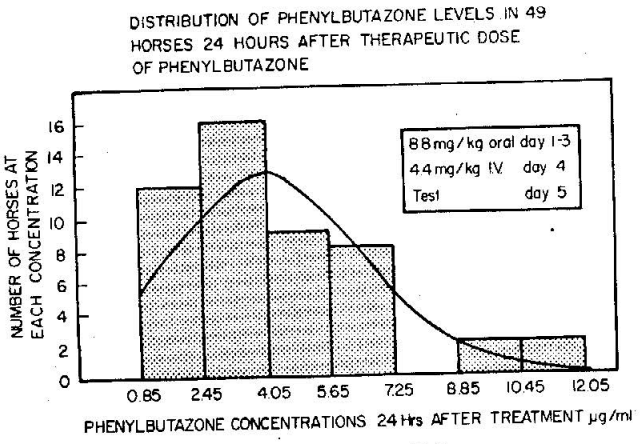

Figure 2. Phenylbutazone levels in 49 horses 24 hours after therapeutic doses of phenylbutazone. Forty-nine horses were dosed with 8.8 mg/kg (about 4.0 gm/1000 lb) of phenylbutazone orally for 3 days and then given 4.4 mg/kg (2 gm/1000 lb) on the fourth day. Twenty-four hours after the IV dose, blood 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.

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.

The half-lives of individual drugs in the horse vary about 300-fold, from half-lives of about 30 minutes for certain drugs which are rapidly metabolized and eliminated, to much longer half-lives (days) for drugs that are slowly eliminated. A list of the half-lives of some commonly used drugs in the horse is given in Table I. This table shows that the estimated time for drugs to completely clear the horse varies between 2 to 5 days for rapidly excreted drugs, to periods approaching years for drugs such as reserpine. Overall, therefore, the apparent range of half-lives and clearance times for drugs in the horse varies about 300-fold.

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

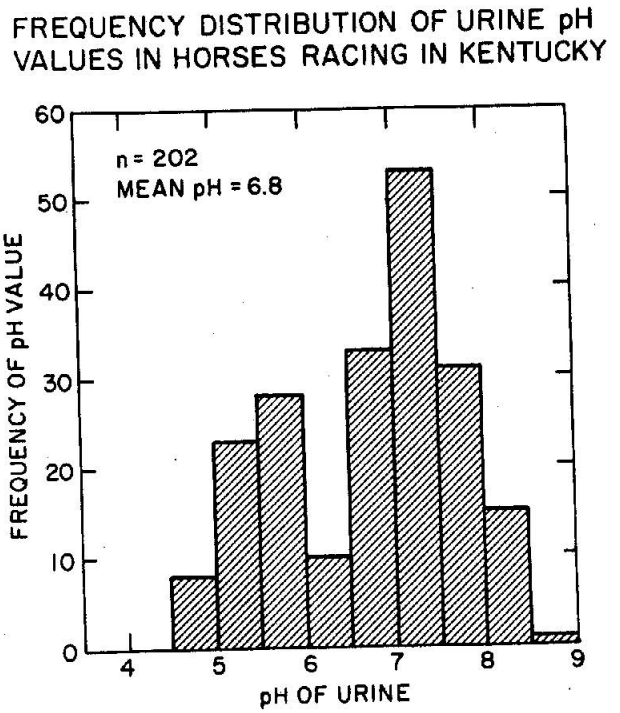

Figure 3. The hatched bars show the frequency of observed urinary pH values in 202 post-race urine samples.

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 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 the 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.

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. Figure 1 shows the elimination by a horse of a hypothetical dose of phenylbutazone (3 gm IV) with a half-life of about 7.2 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. He still, however, has about 10% of the dose left in 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, say, Ohio. By 48 hours after the dose, he only has 0.3 µg/ml in his blood, and

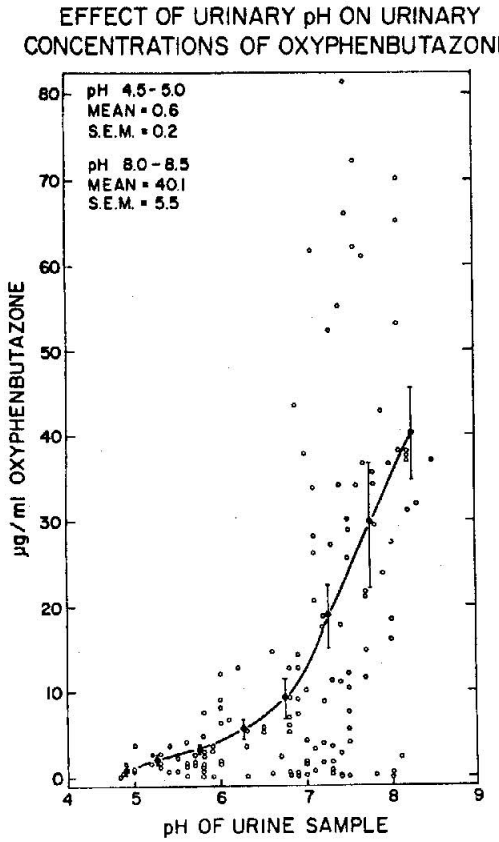

Figure 4. The open circles (0-0) show urinary concentrations of oxyphenbutazone (from Figure 4) plotted against urinary pH. The solid circles (0-0) show the mean urinary concentrations of oxyphenbutazone for each half-pH unit epoci ± SEM. The line connnecting the solid circles was fitted by eye.

while the drug is still easily detectable, he would probably pass most American rules. He would not, however, pass in 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 to 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 test, the sensitivity of the test can be changed by the analyst at will. This is a problem with "no detectable level" rules, and is a potential defense for horsemen racing under these rules. The solution to this problem is simply to specify the 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.

Biological Variability: How Horses "Spread Out" Blood Levels of Drugs

These factors of dose, half-life, and test sensitivity affect the detectability of drugs and the period for which they are detectable after dosing. 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, ie, 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 commonly 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.

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 the NASRC Vet-Chemists Advisory Committee 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 we all knew 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 sadly off the mark. This author remembers the skepticism with which his suggestion that 10 µg/ml of phenylbutazone might be an appropriate upper level was received. When the experiment was done, however, my 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).

The wide spread in this data came as a big surprise to all of us in 1980, but 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 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 a 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 kicker in 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" on 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 even further complicating factors affecting 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 greatest 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 urines of unusually wide pH values, varying from relatively acidic (pH 4.5) to quite alkaline (pH 9.0). 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 is a 500,000 difference. Figure 2 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 urinary 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. This follows what pharmacologists call the trapping rule, which states that acidic drugs "trap" in a basic urine, while 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 it by forensic 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. The maximal theoretical effect for phenylbutazone is about 3000-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 virtually impossible to relate a urinary drug concentration to pharmacological effect or a time of drug administration.

In summary, therefore, there are at least 4000 drugs in common use in horses which may be found in horse urine. The amounts of these drugs administered to horses to produce a pharmacological effect can vary up to one million-fold. The rate at which individual drugs are metabolized by the average horse can vary up to 300-fold. The difference in plasma or urinary levels of these drugs found after administration of the same dose of drugs to a horse can vary at least 50-fold in plasma. Urinary pH and urine volume and flow rate will make the variability in urinary concentrations of drugs even greater than that in plasma, but the actual extent of this effect is not clear. Good evidence for a 200-fold effect of urinary pH on urinary concentrations of phenylbutazone has been obtained, and the possible theoretical limit of this effect appears to be up to 9,000 fold.

In conclusion, urinary quantitation of drugs is likely to be virtually meaningless as a regulatory tool, and plasma level quantitation appears to be the only feasible approach to this problem.

THE URINE TRAPPING RULE

The trapping rule is the converse of the extraction rule. The extraction rule was based on the movement of drugs from urine into a fatty environment (Dichloromethane). On the other hand, the trapping rule requires that the drugs stay in the urine, and not go into the kidney (a fatty system). The trapping rule therefore holds that acidic drugs trap in basic urine, while basic drugs trap in acidic urine, which is (and logically so) the converse of the extraction rule.

Horse-to-Horse Variability and Forensic Chemists

From the data outlined in this article, there is obviously very substantial uncertainty as to the blood or urinary concentrations of drugs that will be found after dosing with the same amount of drugs in different horses. Because this complicates the regulatory process, these data and concepts tend to be viewed sceptically by regulatory chemists. For example, even though a well-known European analyst has published work which strongly suggested that urinary pH affects the elimination of phenylbutazone, he is apparently reluctant to accept the forensic implications of this effect. Beyond this, another regulator has suggested that "all the variability, the standard deviation in the horse has been shown to be 15% repeatedly, in many publications." This statement is extremely difficult to reconcile with the work reported here, and indeed with common experience on horse-to-horse variability.