Pharmacology Review: Drug Metabolism and Elimination in Horses

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For a drug to be absorbed and distributed through the tissues of a horse to the points at which it produces its pharmacological effects, it must be relatively lipid soluble. However, neither horses nor humans spontaneously eliminate lipid-soluble molecules, and Brodie[3] has calculated that the pharmacological effects of a dose of pentobarbital in man would last for up to 100 years if termination of its action was dependent on excretion of the parent drug alone. Both man and the horse, however, long ago solved this problem, and horses, as herbivores which are constantly exposed to unusual plant molecules, have very effective drug metabolizing systems.

Because it is the characteristic of lipid solubility which renders drugs difficult to excrete, the basic manouver in drug metabolism is to modify the drug in such a way as to render it more water soluble and less lipid soluble. Then, in its modified and more water-soluble form, the drug can be passed out of the body through the renal glomerulus or via the liver into the bile and not be reabsorbed. The modification of molecules into more water-soluble, less lipid-soluble forms is therefore the central process in drug metabolism.[7]

Although the term *drug metabolism* or *drug biotransformation* was once considered synonymous with the term *drug detoxification*, this is not always true. Many examples exist of drugs which are metabolized to more active and toxic forms. Perhaps the best example in equine medicine is chloral hydrate, which is rapidly metabolized by alcohol dehydrogenase to its active form, trichloroethanol.[8] The diuretic ethacrynic acid, which was specifically synthesized as a diuretic, produces its diuresis not as ethacrynic acid, but rather as its cysteine conjugate or metabolite. Similarly, codeine is thought to produce its pharmacological effects not as codeine, but only after metabolism to morphine, and oxyphenbutazone, an important metabolite of phenylbutazone, shares many of phenylbutazone's pharmacological effects. It is thus apparent that though drug metabolism commonly means drug inactivation, this is not always the case, and sometimes "drug metabolism" serves to make a drug more pharmacologically active or toxic.[1]

Drug metabolism occurs predominantly in the liver in most animals in which it has been studied. Some metabolism also occurs in the kidneys, lungs and intestinal wall. In the horse, which has relatively active plasma esterase enzymes, significant hydrolysis of drugs containing ester bonds may occur in the blood plasma.[15] Another source of unusual drug metabolites in the horse would be the intestinal tract, where microbial degradation of drug molecules probably gives rise to some very unusual drug metabolites.

In the liver cells, where drug metabolism has been studied in the most detail, the various reactions involved can occur in the mitochondrial, microsomal or soluble fractions of the cell. These reactions are most conveniently considered to occur in two phases, known as Phase I and Phase II. In Phase I of drug metabolism, the usual result is to put a suitable small substituent or "handle" on the drug. These reactions usually involve the addition of OH, NH_2 or COOH groups to the drug. These changes serve to increase the water solubility of the drug molecule and may also alter (increase, decrease, change) its pharmacological activity, or its toxicity, as outlined earlier. Further, these changes usually "set up" the drug molecule for the horse's second line of defense, which is conjugation. Conjugation consists of linking the drug with a large endogenous water-soluble molecule. Conjugated drug products are almost invariably pharmacologically inactive* and are likely to be rapidly excreted, either by glomerular filtration or sometimes by excretion in the bile. As a general rule, the probability of excretion increases as the drug molecule is transformed from its parent form by the process of drug metabolism (Figure 1).

* The only known exception is ethacrynic acid, mentioned earlier.

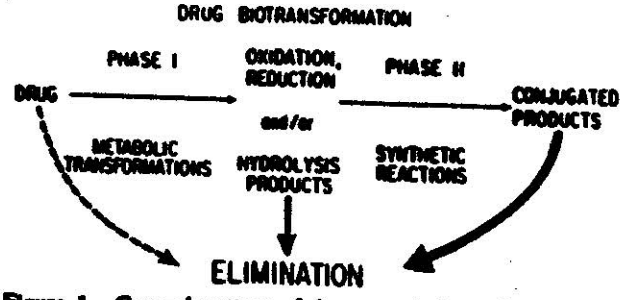

Figure 1. General pattern of drug metabolism. As the reactions proceed from left to right, the probability of elimination of the drug increases.[1]

Table 1 shows the likely biotransformation pathways followed by drugs. Drugs with a simple ester or amide linkage are generally hydrolyzed by plasma or liver esterases. The first step in handling an aromatic group is usually ring hydroxylation. The likely pathway for phenolic or aromatic hydroxyl groups is glucuronide conjugation. Aliphatic amino groups tend to be deaminated, while aromatic amino groups are either conjugated or acetylated. In its patterns of drug metabolism, the horse broadly resembles other species in that most of the oxidations associated with microsomal enzymes in other species have been demonstrated. Reviewing drug metabolism in the horse, Moss[9] reports that with the possible exception of a methyl sulfoxide derivative of caffeine, no unique or even unusual metabolic pathway has been demonstrated in the horse.

TABLE 1

Probable biotransformation pathways of drugs.

Ester group:	**Hydrolysis**
Aromatic rings:	**Hydroxylation**
Hydroxyl (—OH)	
Aliphatic:	Chain oxidation; glucuronic acid conjugation
Aromatic:	Glucuronic acid conjugation; sulfate conjugation; methylation
Carboxyl (—COOH):	
Aliphatic:	Glucuronic acid conjugation
Aromatic:	Glycine conjugation; glucuronic acid conjugation
Amino ($—NH_2$):	
Aliphatic:	Deamination
Aromatic:	Acetylation; glucuronic acid conjugation; methylation (sulfate conjugation)

The simplest pattern of drug metabolism is that found with procaine, where plasma and liver esterases hydrolyze the drug to p-aminobenzoic acid (PABA) and diethylaminoethanol.[16] This process occurs quite rapidly in blood, so that at 37°C in equine blood, procaine has a half-life of about 8 minutes. In the horse, however, most procaine is distributed outside the blood and liver compartments, so the actual half-life of procaine in the horse, at about 50 minutes, is much longer than it is in the blood. It is important, however, to add oxalate and fluoride or other enzyme poisons to blood samples if one wishes to find procaine in them, for if this is not done the procaine present will be rapidly hydrolyzed by plasma procaine esterases.[14]

Since procaine is largely hydrolyzed by liver and plasma esterases, and since these are, to a greater or lesser degree susceptible to inhibition by organophosphate and carbamate anthelmintics and insecticides,[6] it is likely that the kinetics, plasma half-life, and rate of excretion of procaine in the horse are prolonged by exposure to these drugs, and this possibility should be considered by horsemen when estimating "clearance times" for procaine or procaine penicillin in horse urine.

The Phase I, metabolic reactions of drugs are carried out by enzymes that are located predominantly in the liver. When liver cells are fractionated, the drug metabolizing activity is found in what is called the "microsomal system," so the enzymes are called the microsomal oxidizing enzymes. These enzymes have a specific requirement for reduced nicotinamide adenine dinucleotide phosphate (NADPH) and molecular oxygen (O_2) and are classified as mixed-function oxidases. This system contains a cytochrome called "cytochrome P-450" and a flavoprotein which catalyses reduction of this cytochrome by NADPH, called NADPH-cytochrome-P450 reductase. Among the reactions catalyzed by these microsomal mixed-function oxidases are aromatic and side chain hydroxylations (as with phenylbutazone), N and O dealkylation, sulfoxide formation, N oxidation, N hydroxylation and deamination of primary and secondary amines.[1,6]

The metabolism of phenylbutazone in the horse provides a good example of ring and side chain hydroxylation of a drug (Figure 2). Hydroxylation on the ring yields oxyphenbutazone, which shares many of the pharmacological actions of phenylbutazone and is the most persistent of the phenylbutazone metabolites in horse urine. Hydroxylation of the side chain gives rise to γ-hydroxyphenylbutazone, which is the major metabolite of phenylbutazone found in urine for about the first 10 hours after a single dose. This side chain oxidized molecule is much less tightly protein bound than phenylbutazone, and this is the reason that it is excreted in high concentrations in urine during the first 24 hours after a dose of phenylbutazone.[5] Together, these two metabolites account for about 25% of a dose of phenylbutazone administered to a horse, with the remaining 75% of the dose currently unaccounted for.[5]

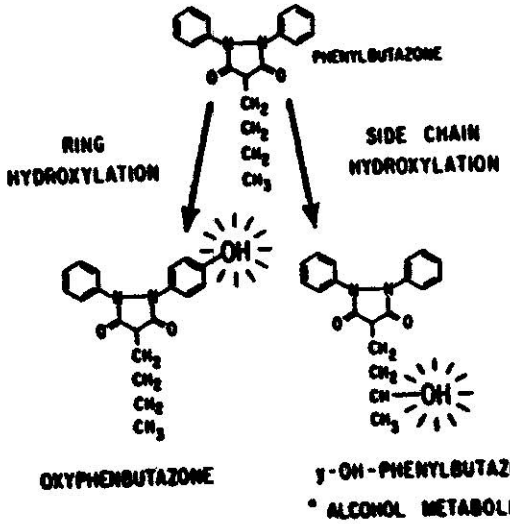

Figure 2. Ring and side chain hydroxylation of phenylbutazone. Two important products of phenylbutazone metabolism in the horse are oxyphenbutazone and γ-OH-phenylbutazone. These metabolic transformations are indicated by enlarged letters.

A good example of deamination by the horse is provided in the metabolism of amphetamine, where the NH_2 group at the two position is transformed to yield either 1-phenylpropan-2-ol, an "alcohol" metabolite, or 1-phenylpropan-2-one, a keto metabolite (Figure 3). Together these two metabolites make up about 40% of the known metabolites of amphetamine in the horse.[8]

While these typical Phase I type metabolic transformations usually increase the water solubility of a drug and accelerate its excretion, a Phase II type conjugation reaction invariably increases the rate of excretion of a drug.[1] In glucuronide formation, the parent drug, if it has a suitable acceptor group, or a drug molecule at the end of Phase I transformation, is linked to glucuronic acid. Glucuronides constitute a major proportion of the metabolites of many phenols, alcohols, and carboxylic acid-containing drugs in the horse. Many drugs, such as morphine, apomorphine, and pentazocine, are likely directly glucuronidated by virtue of their phenolic OH groups. Other drugs, such as the phenothiazines, must first be hydroxylated in a Phase I reaction (have a "handle" put on them) before they can be linked to glucuronic acid and excreted.[7]

DEAMINATION OF AMPHETAMINE IN THE HORSE

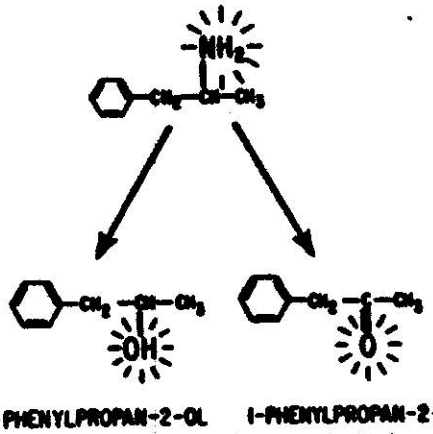

Figure 3. Deamination of amphetamine in the horse. Two important products of amphetamine metabolism in the horse are 1-phenylpropan-2-ol and 1-phenylpropan-2-one, with these metabolic changes indicated by enlarged letters.

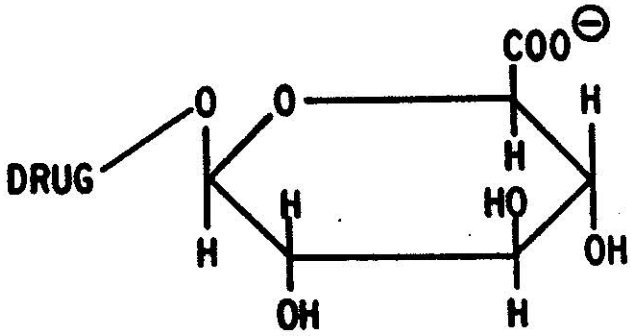

Figure 4. Drug-glucuronide complex. Because of the OH and COO^- groups on the glucuronide moiety, drug-glucuronide complexes are highly water soluble and poorly soluble in organic solvents.

Inspection of the gucuronic acid molecule in Figure 4 shows it to carry a free COOH (carboxyl group) and numerous OH groups. These structures render a drug-glucuronide complex highly water soluble and greatly facilitate its excretion. Once cleared by glomerular filtration, a drug-glucuronide complex is highly unlikely to be reabsorbed across the renal tubules. Further, by virtue of its free carboxyl group, all glucuronides may be excreted by the organic acid transport system of the renal tubules, which further accelerates their excretion. Glucuronide conjugates may also be excreted in the bile, and this can be an important route of excretion if the molecular weight of the complex is above 300 or so.[1,7] Drug-glucuronide complexes, however, may be hydrolyzed by bacterial glucuronidases in the gastrointestinal tract and the parent drug reabsorbed, giving rise to the so-called "entero-hepatic" circulation of drugs. This entero-hepatic circulation of drugs can greatly prolong the time taken for some drugs to clear from the body.[1]

These same properties which make drug-glucuronide complexes so easy to excrete also make it difficult for the analyst to extract gucuronides from plasma and urine. Even though the charge on the carboxyl (COO^-) group can be eliminated by extracting under acidic conditions, the numerous OH groups on the glucuronide molecule prevent movement of the

drug-glucuronide complex into the analyst's extracting solvents. In experimental work with radioactive drugs the fraction of radiolabel in urine which will not readily extract into organic solvents is usually assumed to represent conjugated drug.[7] If a large proportion of a drug is excreted as a glucuronide complex, the normal extraction techniques that an analyst uses will not serve to recover the drug-glucuronide complex from plasma or urine.

The analyst's approach to this problem is to expose the plasma or urine sample to an enzyme called β-glucuronidase or to highly acidic conditions. Under these conditions the drug-glucuronide bond is split, and the parent drug or the Phase I metabolite which is a slightly modified drug is recovered. Most analysts run this hydrolytic procedure for about two to three hours. At the end of this period the analyst "extracts" the "hydrolyzed" urine and examines his extract for drugs. By either acidic or enzymatic hydrolysis it is likely that only a fraction of the drug present is released, and the acidic hydrolysis of horse urine is particularly likely to release extraneous material which does not make the analyst's job any easier.

It turns out that for reasons which are not clear, glucuronide drug metabolites are picked up in urine for relatively long periods after drug administration. Thus, the glucuronide metabolite of pentazocine[b] has been found in horse urine for up to five days, the glucuronide metabolite of apomorphine for up to 48 hours, and the gucuronide metabolites of phenothiazine tranquilizers for up to four days.[c]

Horsemen should be aware of these prolonged excretion times for drug-glucuronide complexes and be careful about the use of such drugs before competitive events.

Another factor which has the potential to greatly affect the clearance times for some drugs in horse urine is the urinary pH. Most drugs or drug metabolites in urine can be highly dependent on urinary pH.[1,7] Because acidic drugs will be charged (ionized) in a basic urine, and basic drugs will be charged in an acidic urine, they tend to "trap" in urines of the opposite characteristic, *i.e.* basic drugs "trap" in acidic urines and acidic drugs "trap" in basic urines.[1] This phenomenon has two potential effects on drug clearances, namely that it may increase or decrease the concentrations of drugs in urine and it may accelerate or retard the rate of excretion of drugs by horses.

[b] Talwin®-V. Winthrop Laboratories, New York, NY.

[c] Dr. J. W. Blake: *personal communication*, University of Kentucky.

There is very good evidence that urinary pH can both vary widely in racing horses and markedly influence the concentration of drugs in horse urine. Examination of the pH of urine samples received from the track in both Japan[10] and England[6] have shown a range of urinary pH values from pH 4.5, which is relatively acidic, to pH 10.0, which is quite basic (Figure 5). In each case, the frequency distribution curve was biphasic, with the greatest number of urines having a pH on the acidic side of about 5.0, but another peak on the basic side showed that a large proportion of the urines had a pH of 8.0. Both distributions, however, show that the range of urinary pH values varies from a low of about 4.5 to a high of about 10.0. Because urinary pH is measured on a log scale, this is close to a one-million-fold range, from 10^{-4} M hydrogen ions (pH 4.0 acidic) to 10^{-10} M hydrogen ions (pH 10.0 alkaline). Based on this range of possible urinary pH values and given a certain plasma level of drug such as procaine, one can calculate the possible range of procaine concentrations in equine urine using the Henderson-Hasselbach equation.[d] It turns out that because of the urinary pH factor, there is a rather mind-boggling 9,000-fold possible range in urinary procaine concentrations, given a single, fixed plasma level of the drug. This huge range of possible procaine concentrations in horse urine leads this author to the conclu-

[d] See Appendix for details of this calculation.

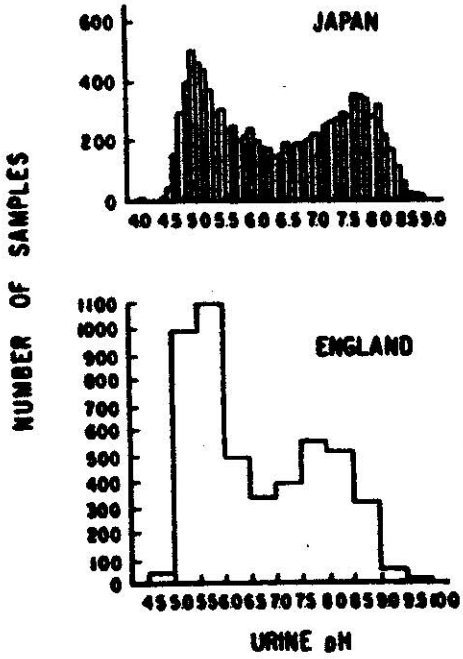

Figure 5. Range of urinary pH values in racehorses. The upper histogram shows the range of urinary pH values of about 10,000 postrace urines from Japan,[10] while the lower histogram shows the range of urinary pH values from official postrace urines from England in 1974.[6]

sion that anybody who tries to estimate a time of procaine administration from a urinary drug concentration cannot be doing more than making wild guesses against tremendous odds.

These theoretical predictions concerning procaine concentrations in horse urine were tested by Evans and Lambert[4] who presented evidence that even horses and horse urines obey the laws of chemistry. Evans and Lambert injected horses with procaine or procaine penicillin and followed urinary concentrations of procaine. They then dosed their horses with ammonium chloride to change their urine to an acidic pH, and followed the changes in urinary pH and urinary levels of procaine. They found (Figure 6 and other experiments) that a decrease in urinary pH was associated with an increase of about five-fold in urinary procaine concentrations. Further, because Evans and Lambert did not stabilize their plasma procaine concentrations, these pH-induced increases in urinary procaine concentrations were occurring in the face of declining plasma levels of procaine. It appears, therefore, that this mechanism of highly pH-dependent urinary levels of the drug also operates in the horse *in vivo*, and there is absolutely no reason to believe that it does not.

Although the actual concentrations of procaine in horse urine may vary markedly, it does not appear likely that changes in urinary pH will, in general, affect the plasma half-life of procaine. This is because procaine is very widely distributed in the horse and its plasma half-life is determined largely by enzymatic hydrolysis of the drug. As far as the kinetics of procaine are concerned, one might view the horse as a very large compartment in which procaine is hydrolyzed by enzymes in equilibrium with a very small compartment, the bladder, in which the level of procaine is highly pH-dependent. Because so little (less than 1%) of a dose of procaine ever appears in the urine, relatively large variations in urinary concentrations of the drug likely occur without any significant changes in the plasma half-life of the drug. This, however, is largely supposition and needs to be checked by rigorous calculations or, more satisfactorily, by experimentation.

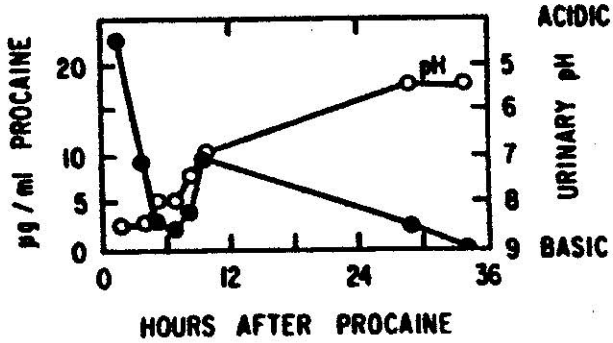

Figure 6. Effect of urinary pH on urine procaine concentrations. A horse was injected with 4.5 million units of procaine (solid circles ●-●) and urinary pH (open circles O-O) followed. Note the increase in urinary procaine concentrations during the period of sharp increase in urinary pH.[4]

This apparent ability of urinary pH to affect the concentration of drugs or drug metabolites in horse urine with little effect on their plasma half-life may also hold for other drugs. Studying the urinary elimination of C^{14} administered as C^{14} phenylbutazone in horses, Moss and Haywood[9] showed that the C^{14} was excreted over a much longer period into an acidic urine rather than a basic urine. In other experiments, however, Piperno and co-workers[11] showed that changes in urinary pH had no effect on the plasma half-life of phenylbutazone. Again, metabolism is the primary factor which determines the plasma half-life of phenylbutazone, and although the concentration of phenylbutazone or phenylbutazone metabolites in urine may vary, the amounts of drug lost by this route are apparently not large enough to significantly influence the plasma half-life of the drug.

Experiments by Baggot[2] and co-workers have shown similar results with amphetamine, where changing the pH of horse's urine did not affect the plasma half-life of the drug but may affect its urinary levels.[8] The message appears to be that, as a general rule, the rate of decline of plasma levels and drug action in the horse is determined by drug metabolism, and while urinary drug concentrations may change dependent on urinary pH, they do not affect the rate of decline of plasma levels of drugs.

Another factor which might affect the plasma half-life of drugs in horses is the presence of other drugs. In our laboratory we examined the possibility that drugs such as chloramphenicol, quinidine and oxyphenbutazone, which have been shown to be potent inhibitors of drug metabolism in other species, might inhibit drug metabolism in the horse and thus give rise to unusually prolonged urinary clearance times for drugs. Of these three drugs, only oxyphenbutazone was found to inhibit phenylbutazone metabolism, and that only to a very small degree. We therefore concluded that clinically (or forensically) significant inhibition of drug metabolism was not likely to occur in the horse due to any of these drugs.[16]

It has been widely shown in laboratory animals that pretreatment with certain drugs can induce the liver drug-metabolizing enzymes, and chronic administration of phenylbutazone to dogs can increase the rate of metabolism of phenylbutazone itself and also of other drugs.[1,7] To this author's knowledge, no studies

on the possible effects of hepatic enzyme induction on the rate of clearance of drugs from equine plasma and urine have been made. However, it seems likely because of the large number of plant constituents which the horse is normally exposed to, that the effects of "normal" medication in the horse may not have any significant extra inducing effects on drug metabolizing enzymes.

Another factor which might be expected to alter the plasma half-life of drugs in the horse is alteration in blood flow to the liver, reducing the rate of delivery of drugs to the drug-metabolizing systems. Studies in man have shown that exercise can lead to a decrease in hepatic blood flow, and it is probable that similar effects occur in the horse. This possibility led Powis and Snow[12] to study the effects of exercise in the horse on plasma levels of propanolol, a drug which is principally cleared by hepatic metabolism and which has few hemodynamic effects at low doses. Powis and Snow found rather large increases in plasma levels of propanolol occurring during exercise, and the changes observed were too great to be accounted for by reduced drug metabolism. These workers further found that approximately similar changes were produced by adrenaline, at doses which produced an increase rather than a decrease in hepatic blood flow. Snow was unable to explain the marked alteration in plasma levels of propanolol seen in his experiments. As a practical matter, however, the plasma levels and rates of clearance of propanolol returned to control rapidly after exercise had ceased. Because of the very brief period of exercise in which most racing horses are involved, it does not appear that exercise is likely to affect drug metabolism or drug plasma and urinary levels in a way that would significantly affect pre- or postrace drug testing.

In summary, drug metabolism is by and large the most important mechanism in decline of plasma levels of drugs and termination of drug action in horses. Drug metabolism in the horse proceeds as in other species, with Phase I metabolic transformations followed by Phase II synthetic reactions. The types of metabolic transformations seen are qualitatively similar to those seen in other species, although there may be quantitative differences. While exercise and administration of other drugs have been shown to produce changes in the plasma levels of drugs in the horse, these effects are either small or transient and unlikely to be of clinical or forensic significance. Postrace urines from horses show a very wide, almost one-million-fold range, in pH (hydrogen ion) values. This variability in urinary pH can cause very large changes in urinary drug concentrations, but appears to have minimal effects on plasma levels or plasma half-lives of drugs. However, this large range of urinary pH values in postrace urines makes it essentially impossible to estimate times of dosage or dosage forms of drugs in horses from single urinary drug concentration measurements.

Appendix

The pH-dependent ratio for drug concentrations on opposite sides of a biological membrane may be calculated from the following transformation of the Henderson-Hasselbalch equation (1):

$$\frac{[\text{Plasma}]}{[\text{Urine}]} = \frac{1 + \text{antilog}\,(\text{pH}_a - \text{pH}_p)}{1 + \text{antilog}\,(\text{pKa} - \text{pH}_u).}$$

Given that the pKa of procaine is 8.7,[13] and assuming an acidic urine of pH 4.7, a basic urine of pH 9.7, and a plasma pH of 7.4, in the basic urine:

$$\frac{[\text{Plasma Drug}]}{[\text{Urinary Drug}]} = \frac{1 + \text{antilog}\,(8.7 - 7.4)}{1 + \text{antilog}\,(8.7 - 9.7)} = \frac{21}{1.1} \approx \frac{19}{1.}$$

Therefore, this basic urine will contain 1/19 of the procaine that the plasma contains.

In the acidic urine:

$$\frac{[\text{Plasma Drug}]}{[\text{Urinary Drug}]} = \frac{1 + \text{antilog}\,(8.7 - 7.4)}{1 + \text{antilog}\,(8.7 - 4.7)} = \frac{21}{10{,}001} \approx \frac{1}{476.}$$

Therefore, this acidic urine will contain 476 times more procaine than the plasma.

The possible theoretical range of urinary procaine concentrations given a single plasma concentration is therefore 19 × 476 = 9,044 or a more than 9,000-fold possible range in urinary procaine concentration.

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Letters to the Editor

Dear Sir,

Referring to the article entitled "Pharmacokinetics of Digoxin in the Horse," *J Eq Med Surg* 2, (1978): 384-388, I have the following queries:

a) In Figure 1, the authors have plotted the mean β phase disappearance of digoxin from horse serum using two exponential lines instead of the more usual single line. For this reason their definition of β as "slope of monoexponential declining line" is confusing. If two distinct lines with differing slopes are required to represent the excretory phase of the drug, surely two exponents and not one are involved?

b) Most digoxin radioimmunoassays, including the one used by the authors, are considered accurate to ± 0.2 ng digoxin/ml (the mass equivalent of twice the standard deviation of the zero binding).* Calculations based on Figure 1 indicate that mean serum digoxin levels fell below 0.1 ng/ml between 72 and 96 hours. The mean serum value at 168 hours, for example, (approximately—log 2.24) is 5.8 pg/ml. For the reasons stated above, the accuracy of measurements made from 96 to 168 hours may be questionable.

Sincerely,

K. Button, B.V.Sc., M. Med. Vet. (Med.)
Department of Veterinary Physiology
and Pharmacology
College of Veterinary Medicine
Texas A & M University

* Digoxin 125 radioimmunoassay kit (solid phase). New England Nuclear, North Billerica, MA: (manufacturer's technical data).

Dear Sir,

Concerning Dr. K. Button's remarks on our paper "Pharmacokinetics of Digoxin in the Horse," we would like to make the following comments:

a) The curve represented in Figure 1 was drawn using a curve-sketching program which connects the data points. The model fit by the nonlinear decision program selected only one exponential for the terminal phase. Any deviations observed from a single exponential curve are the result of reproduction and point to point curve-sketching done by the computer program.

b) It is true that most of the six currently used assay methods for determing serum or plasma concentrations of cardiac glycosides are considered accurate to about 0.2 ng/ml. The RIA method is, though, the simplest, most rapid, and the most sensitive.* Thus, it seems likely at the present moment no assay is available that would go beyond the sensitivity of the RIA. Results are then presented and valid for the assay used.

Sincerely,

W. M. Pedersoli, D.V.M., Ph.D.
Assist. Prof. of Pharmacology
School of Veterinary Medicine
Auburn University

* Butler, V. P., Jr: Assays of Digitalis in the Blood. *Prog Cardiov. Dis.*, 14, (1972): 571-600.