
The formal field of study which deals with the movement of drugs through the body of the horse is known as pharmacokinetics. Most drugs obey a number of relatively simple rules during their movement through the horse, so plasma concentrations of drugs can be quite predictable. Urinary concentrations of drugs, however, are less predictable, largely because the volume and composition of urine is varied as the animal maintains its internal environment constant. The easiest way to gain an understanding of the characteristics of the drug distribution in the horse is to follow a typical drug through the body of the horse.

When a veterinarian wishes to study the movement of a drug through a horse (i.e., its pharmacokinetics), its first experiment is to give a fairly large dose of the drug by rapid intravenous (IV) injection. Immediately after injection, the drug goes through a rapid mixing phase, which takes about 3 minutes. During this period the drug is evenly distributed throughout the blood stream and is entering into the next phase, which is movement out of the blood stream into tissues. If one draws a blood sample at the end of this period, i.e., at about 3 minutes, one finds the highest blood levels of drug that that particular dose can give rise to (Fig 1).

The next process, and the first one that pharmacologists are really interested in, is that of movement of the drug out of blood into the tissues. This is technically known as the alpha or distribution phase. In it, the drug moves out into the tissues until the tissues are saturated with the drug. This process can occur very rapidly for water soluble drugs, and slowly for others, and hardly at all for drugs which are rather poorly lipid soluble or carry a charged charge. Usually, this process takes between 15 minutes to 1 hour, and at the end of this period the tissues are saturated and can be with that particular dose of the drug in question.

From this point on, the rate at which body levels of the drug decline depends on the rate at which the drug is metabolized or eliminated. This is usually much slower than the distribution phase and is called the beta or metabolic or elimination phase. This phase continues until the drug can no longer be detected in the body of the horse and for a very long time thereafter.

Some typical plasma levels obtained after procaine was injected IV in horses are presented in Fig 1. The first plasma sample was drawn right after the mixing phase and shows about 0.6 µg/ml of drug in plasma. As the drug distributed out of plasma and into the tissues, the plasma levels fell very rapidly, and this portion of the curve is marked "distribution" phase. This was then followed by the metabolic phase, during which the blood levels were reduced by excretion and metabolism of the drug only. If one looks carefully at this curve, one sees that it is declining very slowly and that it looks as though it is never going to reach zero. In fact, this is what actually happens—after you give a drug to an animal its drug concentrations never reach zero.

Now, one might ask, how can anybody tell from the type of data in this figure when the phase of distribution stops and when metabolism begins, and how on earth can one be sure that the drug concentrations will never reach zero? These questions can be easily answered by plotting this data in a slightly different way, that is, by making the drug axis logarithmic. Since only 1 axis of the plot is logarithmic, it is called a semi-logarithmic plot.
If one plots the data of Fig 1 on this type of plot (see Fig 2), it turns out that the metabolic phase becomes as straight as an arrow, and the metabolic phase is usually taken as that portion of the curve which plots linearly. We know that halving takes about 3 minutes, so the period between halving and metabolism becomes the distribution phase. Because there is no zero on a log scale, which simply decreases in tenfold units forever, the blood levels of the drug can never reach zero and will continue to decline forever. In addition, there are a number of practical things to be learned from the semi-log plot, all of which are based on this “log-linear” relationship between drug concentrations and time.

Fig 3—Semi-log plot of distribution and elimination of procaine. The data from Fig 1 was replotted on the drug concentration on a logarithmic scale. Note that two to the log scale means that the time taken for the desired line portion of the curve to halve is constant.

This “log-linear” relationship is very important. In the first place, it turns out that the time required for a drug concentration to halve, i.e., to fall to 50% of its original value, is constant, no matter what point on the curve one picks. Thus, the half-time of procaine in the horse is about 52 minutes, as shown in Fig 2. Each drug has its own individual half-time in the horse, and this half-time is pretty constant for each drug. Thus, given this half-time, it is easy to calculate what drug levels were or will be in a given animal, if you know the concentration at any given time. Then, if you know the pharmacology of the drug you can state what the pharmacological effects at that time would have been.

This kind of data is also important in calculating blood levels from a given dose, and one can predict from this kind of information what the probable pharmacological effects from a given dose are likely to be.

A very interesting and very important fact which becomes clear from this type of plot is the fact that the size of dose administered has very little effect on the “clearance time” for a drug. The “clearance time” for a drug, in the racing or forensic sense, is not the time taken for a drug to clear from the body (which is infinite), but the time which must elapse before an analyst can no longer find it. Now this time depends only on the analyst’s ability, and if the analyst’s methods are good, it is hardly affected at all by the size of the dose as shown in Fig 3. Here a horse has been dosed with a hypothetical drug, where plasma (or urine) levels fall away as indicated by the line labelled “dose x.” After 5 hours, the analyst can no longer detect this drug, so its “clearance time” is 5 hours. Now, if one gives 10 times the dose, which raises the blood level tenfold, the time to reach the clearance level is only extended by 1 hour, or by 20%. Thus, huge changes in dose are required to produce very small changes in “clearance times,” because of the logarithmic relationship between dose and time. Because doses of most drugs are usually administered at fairly well-defined dose rates, the effect on “clearance times” of the usual variation in therapeutic doses is trivial.

Fig 3—Relationship between dose and “clearance time.” Dose “x” of a hypothetical drug was given to a horse and generated the plasma levels shown in the lowest curve. At least 10 times the dose (“x” x 10) must be given to raise plasma levels of the drug tenfold to increase the clearance time by 1 hour or 20%. It can also be shown that the more sensitive the analytical method, the smaller the effect of dose on clearance time.
Finally, it is not easy to alter either the plasma level of a drug in the horse or the rate at which this level changes (i.e., the rate at which the drug is excreted). A number of experiments with furosemide in horses showed essentially no effect of this drug on plasma and urinary levels of a number of drugs. Similarly, drugs which might be expected to displace other drugs from plasma protein binding sites do not appear to have any effect on drug half-lives in the horse. By and large, then, plasma levels of drugs tend to be predictable, they fall at rates that are predictable for a given drug, they are not easily influenced by the administration of other drugs, and their times of clearance from plasma are relatively constant.

It is sometimes suggested that individual idiosyncrasies and the presence of other drugs will affect the rate of metabolism of drugs and thus their "clearance times." There are good reasons why these factors are considerably less important in racehorses than in other species. In a study on the effects of chloromycetin, quinidine, and oxyphenbutazone on phenylbutazone metabolism in Thoroughbred horses, our laboratory found minimal and clinically nonsignificant effects of these drugs on phenylbutazone metabolism. This is despite the fact that these drugs are potent inhibitors of drug metabolism in other species. Similarly, it must be remembered that, because racehorses are bred from a relatively small genetic pool and their care and nutrition are usually excellent, the probability of individual "idiosyncrasies" (genetically or environmentally determined) occurring are considerably less than in most populations. It seems reasonable, therefore, to conclude that the effect of these influences on plasma and urinary "clearance times" of drugs in horses are likely to be minimal.

The one supreme advantage for forensic purposes that plasma levels of a drug confer is that they can be directly and consistently translated into pharmacological effect. Given the appropriate research base, one can say with some certainty from a blood level whether or not the animal was under the pharmacological influence of the drug at the time of racing. This is usually an impossible extrapolation to make from urinary levels of a drug.

Despite this and other problems, urine testing is the backbone of drug testing today, and with good reason. The urine sample can usually be gotten without any major interference such as is required to draw blood samples, and most commissions and commission veterinarians are familiar with its collection. Urine also has a large number of apparent advantages for the analyst as a testing medium, which may appear as disadvantages to veterinarians, owners, and trainers.

Drugs enter the urine in 3 ways. At the renal glomerulus where the urine is first formed, all drug and drug metabolite molecules enter by the process of glomerular filtration. Two things may happen to these molecules. If they are poorly lipid soluble, whether they are drugs or drug metabolites, they are now essentially trapped outside the body. As the kidney reabsorbs water and essential nutrients from the glomerular filtrate, these trapped molecules are concentrated in the forming urine; and their final concentration in urine depends only on the degree to which the urine is concentrated. If the animal is conserving water and the volume of urine is small, the concentrations of these drugs in urine will be very high, theoretically up to 100 times their plasma levels. This is a very important consideration for the analyst, as there are a number of drugs which cannot currently be detected in plasma and are only detectable in urine as their water soluble, highly concentrated metabolites. The best example of a drug such as this is apomorphine. Apomorphine is very difficult to detect in equine plasma with current analytical techniques. In urine, however, it is excreted as a water soluble glucuronide metabolite which is greatly concentrated in urine. This renal concentrating effect is very important in that it enables most analysts to detect apomorphine in equine urine.

**HOW DRUGS ENTER THE URINE**

1. **Glomerular Filtration**
   - All drugs, one way only.

2. **Organic Acid and Base Transport**
   - Rapid transport,
   - Poorly lipid soluble drugs.

3. **Equilibration Across Renal Tubule**
   - Operates in both directions and can be rapid.

**Fig 4**—The basic mechanisms by which drugs enter and leave the body.
Other drugs which are excreted in this way and concentrated in urine are metabolites of narcotic drugs such as morphine and pentazocine and the phenothiazine tranquilizers. For all of these drugs, their concentration in urine is very important for the analyst in that it enables him to detect these drugs in urine. On the other hand, any drug or maneuver which decreases the volume of urine (such as a diuretic) will act to decrease the concentration of these drugs in urine, and several examples of this have been reported.

Another major mechanism by which drugs enter urine is by means of the organic acid and organic base transport system. These are relatively nonspecific transport systems which actually secrete certain drugs into the urine. By far the best known drug which is secreted in this way is penicillin. Penicillin (an organic acid) is very rapidly secreted from the renal tubules and is found in very high concentrations in urine. In fact, in the early days of penicillin therapy, when the drug was extremely valuable, it was recrystallized from human urine for reuse! Though this is no longer done, penicillin is still excreted largely unchanged in high concentrations in urine. Another drug of particular interest to equine veterinarians with which this occurs is furosemide. Sixty percent of a dose of furosemide is excreted unchanged in urine within 1 and 4 hours in concentrations about 1000 times those observed in plasma. It is also found in urine for up to 3 days after it has been administered to the horse, and for long after its pharmacological effects have dissipated.

The third way in which drugs can enter urine is simply by diffusing through the walls of the renal tubules. To do this, the drug must first be lipid soluble. If this is the case, the drug can easily move from the kidneys into urine, and also from urine back into the kidneys. For lipid soluble drugs, the final concentrations of the drug in the urine appear to be primarily dependent on the pH of the urine and the pKs of the drug. For these reasons, urinary concentrations of lipid soluble drugs may vary up and down depending on the pH of the urine and are apparently not affected by changes in urine volume.

The concentration of drugs in urine also depends on the volume of urine. Since these factors are usually difficult to control, changes in urine volume on its hydration and acid base balance, and other factors affecting urinary concentrations of drugs are much more likely to be as variable while plasma concentrations of a drug tend to be stable. This is only reasonable, as it is by varying urine volume, pH, cation and anion content that the horse maintains a stable arterial blood volume. Therefore, the first problem with the urinary concentrations of drugs is that they are likely to be much more variable than plasma levels.

The second problem with urinary concentrations of drugs is that they are likely to be very much higher than plasma levels of the drug. While this increased concentration is initially not a problem, it becomes so with time, because urinary concentrations of the drugs or their metabolites are often maintained long after the drug has disappeared from plasma. Thus, furosemide can be found in urine at 1,000-fold higher concentrations that it is found in plasma, and it can be found there for 3 days without too much effort.

The pharmacological effects of furosemide, however, are notoriously brief and last only between 2 and 4 hours. Similarly, the major metabolite of pentazocine is found in equine urine for up to 5 days after the drug is administered, though it appears highly unlikely that pentazocine's therapeutic effects last for more than 6 hours. Procaine is always found in equine urine for long after its pharmacological effect has dissipated, no matter what time it is given by.

These drugs are all legitimate therapeutic agents, but they can give rise to "positive" for many days after their therapeutic effects have dissipated. There is no evidence, therefore, for any correlation between urinary levels of drugs and their metabolites or urine "clearence times" and the duration of the therapeutic effects of most drugs. This is, from everybody's point of view, the major problem with urine testing.

Another problem with urine testing is that some people believe that urinary concentrations of drugs can be extrapolated back to blood levels of drugs and conclusions about blood levels of drugs and times of dosing drawn from urinary concentrations of drugs. Some racing authorities have rules which state that not more than a certain concentration of phenylbutazone and its metabolites (165 1g/ml) can be found in urine postrace, because such a finding indicates race day medication. The experimental data on which this ruling was based has, to the author's knowledge, never been presented, so it is impossible to judge the relevance of the ruling. One thing, however, is clear. The urinary concentrations of phenylbutazone and its metabolites are highly sensitive to urinary volume. Therefore, all one has to do is administer a small quantity of furosemide (or even an osmotic diuretic) prior to urine sampling, or prior to the race, to reduce the urinary concentrations of phenylbutazone up to 50-fold or more.

There are also reports that the time of dosing with phenylbutazone can be determined by comparing the
relative levels of various metabolites in the urine sample. Again, the physiological basis for this ruling is unpublished and unconfirmed, and until this is done these claims are not scientifically acceptable.

The last and most important subject to discuss is "clearance times." A "clearance time" is the period postdrug administration that must be allowed to elapse until a drug is no longer detectable in equine urine. The misconception exists that this period is in some way related to the metabolism of the drug in the horse and the rate at which horses eliminate a drug. Nothing could be further from the truth. If any one thing is true for all drugs given to horses, it is that the concentrations of any drug (or its metabolites) in plasma or urine merely get lower and lower, and lower, and lower, ad infinitum (Fig 2). The analyst's "clearance time" does not depend on the drug or its metabolism but only on when the analyst can no longer detect it and nothing else. For drugs which the analyst cannot detect, the "clearance time" is zero. For all other drugs, the effective "clearance time" is when the analyst loses track of it. If the analyst's methods improve, the "clearance time" extends. Good analysts give rise to long "clearance times." Poor analysts result in "clearance times" approaching zero, and an unusual number of drugs for which the "clearance time" is zero.

This author is only noted to call "clearance times" for drugs in horse, but the only drugs for which a "clearance time" can be cited with confidence are those which the analyst can no longer detect, for which the "clearance time" is zero. For any drug which the analyst can or should be able to detect, one can only offer guesses, no matter how much one knows about the drug in the horse. The reason for this is that one can never knows how good the analyst. An example of the reason for this is that the analyst's skill may be good but his is. He is usually only concerned with detecting the drug and can only guess at the lower limit of sensitivity of his methods for any particular drug. Because of this situation, none of the research on drugs in the horse now coming out of research laboratories can be applied to the very practical problem of "clearance times."

To demonstrate the overriding importance of the analyst's methods in determining drug "clearance times," Fig 5 shows some data on the clearance of furosemide from equine urine (taken from Reference 7). After 1 mg/kg of furosemide (IM), urinary concentrations of furosemide peaked at about 28 μg/ml, 6 hours after dosing (which time the pharmacological effects of the drug were over). Thereafter, the urinary concentration of furosemide fell with a half-life of about 5.3 hours and were followed in this experiment for 3 days. In this situation, if the analyst was only able to detect 5 μg/ml, the "clearance time" was about 18 hours, i.e., 12 hours since the drug had any pharmacological effect. If the analyst could detect 0.5 μg/ml, the "clearance time" would be 36 hours. This is about the sensitivity of the routine analytical procedure used in Kentucky, and it appears to be more than adequate for routine work. However, any analyst who wanted to use the methods from these experiments would have increased the "clearance time" to 72 hours. If a highly sensitive radioimmunoassay was used, such as is sometimes used for steroids, the "clearance time" could go up to 100 hours or more. Obviously, the most important determinant of "clearance times" is the analyst's methods.

Analysts often argue that because of the inherent differences between horses due to sex, age, build, urinary pH, size of dose, etc., "clearance times" are likely to be so variable that mean "clearance times" such as those presented in Fig 5 are not likely to be applicable in specific instances. This argument ignores both the fact that the veterinarian adjusts his dose to take these
factors into account and that in any event changes in dose have only a miniscule effect on "clearance times." While it is true that urinary volume and pH can produce very real effects on drug concentrations in urine, these effects are usually compared with the 10,000-fold range in drug concentrations demonstrated in Fig 5. Route or form of dosage can be important, but these factors can be specified. The sensitivity of the analyst's methodology is thus the primary and most important factor in determining drug "clearance times." As pointed out earlier, until analysts make known the sensitivity of their tests for legitimate therapeutic agents, none of the research now being done on these agents can be applied to the very practical problem of drug "clearance times." Until then, drug "clearance times" will continue to be handled on a trial and error basis, with veterinarians, trainers, and owners making the errors and going to trial.

As analytical methods improve, medication rules, as currently formulated, will become even more troublesome. To draw a parallel from everyday life, the veterinarian who treats an animal prereared with a drug is in the position of a man driving into a very strange state. In this state, none of the speed limits are posted because nobody knows what they are. E.g., nobody knows how good the traffic methods are. The speed limits may also be changed without notice (the analyst may change his methods). Further, these unknown and variable speed limits bear no relationship whatsoever to the highway conditions (the "clearance time" is in no way related to the pharmacology of the drug, only to whatever methods the analyst happens to use). No court which has any pretensions toward being a court of justice could enforce speeding tickets issued under these circumstances. Yet, this is the situation in which veterinarians and trainers find themselves daily and will continue to do so until they can encourage the racing community to decide on drug levels which may be considered irrelevant.

References