THE EFFECTS OF DRUGS ON EQUINE PERFORMANCE AND THE USE OF ELISA TESTS IN EQUINE MEDICATION CONTROL

Thomas Tobin

SUMMARY

Since the turn of the century stimulant drugs have been used in attempts to influence the performance of horses. More recently, non-steroidal anti-inflammatory drugs have been used with the goal of restoring normal performance in horses with minor, musculoskeletal problems. These uses of medication have led to attempts to experimentally define the effects of drugs on equine performance.

Classic performance trials in which small numbers (10) of horses are treated with drugs and their "maximal" performance compared with control values have yielded little useful data. This is because these experiments cannot detect less than a 4% improvement in equine performance, and none of the drugs tests have been able to produce a supra-maximal improvement in performance of this magnitude. However, if the test is made sub-maximal, such as gallop tests, then some workers have reported improvements in "performance."

The use of simple behavioral models allows one to readily characterize the effects of drugs in horses. However, like the sub-maximal output experiments, these experiments do not answer the question of whether or not these drugs affect performance. On the other hand, they may lay the groundwork for studies on larger numbers of racing horses, which appears to be the only satisfactory approach to studies on the effects of drugs on equine performance.

The increased potency of drugs used to affect equine performance has led to a need for extremely sensitive testing methods. We have recently developed a series of simple one step ELISA tests for drugs in racing horses that can detect drugs or drug metabolites at nanogram and subnanogram concentrations in equine blood and urine. These tests are particularly effective in pre-race testing and also increase the effectiveness of post-race testing for many medications in racing horses. Additionally, antibodies to commonly used therapeutic medications have been raised which may permithedevelopment of rapid, sensitive, and economical quantitative assays for many medications used in racing horses.

Author's address: The Gluck Equine Center, University of Kentucky, Lexington, KY 40511.

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. with approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #171 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center of Toxicology, University of Kentucky.

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

Table I. Various categories of medication in performance horses.

1. Medication to Win
   a) Acute: short-acting stimulants, amphetamines, cocaine, narcotics.
   b) Chronic: repeated dosing for weeks or months, vitamins or anabolic steroids.
   c) "Washy" horses: dosing with a small dose of depressantor tranquilizer to "take the edge off" an excitable horse.
   d) Always illegal and usually an "inside job"
2. Medication to Lose
   a) Depressants: large doses of a tranquilizer, sedative or depressant.
   b) Always illegal and usually an "outside job"
3. Medication to Restore Normal Performance
   a) Non-steroidal anti-inflammatory drugs, phenylbutazone, etc. Often permitted under controlled rules.
   b) Corticosteroids: administered intra-articularly to control joint pain; occasionally permissible.
   c) Local Anesthesia: nerve or joint blocks; always illegal
   d) Fluids and electrolytes: often permissible
4. Accidental or Inadvertent Doping
   The accidental occurrence of a positive
   a) Procaine from procaine penicillin
   b) Caffeine from coca husks in food pellets
   c) "Robaxin" from glyceryl-guaiacolate
   d) Botanical positive or false positives
5. Medication to "Mask" Other Drugs
   Administration of dipyrone or polyethylene glycol, thought to interfere with the detection of other drugs.
6. Medication to "Dilute" other Drugs
   Diuretics: furosemide, ethacrynic acid, hydrochlorthiazide.
7. Miscellaneous Mechanisms
   "Blood doping"
   "Bicarbonate doping"

INTRODUCTION

In the latter part of the nineteenth century a group of American trainers migrated to Europe and became an important influence in European racing. As a group, these trainers were known as the Yankee Alchemists for the simple reason that they were at the very least suspected of using medications in their horses. These suspicions led to the introduction of chemical testing in European racing in the early years of this century and, shortly prior to the advent of the Second World War, to the introduction of drug testing into American racing.[12]

With the increase in activity of the chemical and pharmaceutical industries in this century, the identification and synthesis of increasingly potent drugs, and the development of entirely new classes of pharmaceutical agents, the potential for the use and misuse of medications in racing horses has increased substantially.

Table I briefly reviews the different classes of drugs that have been used in racing horses and shows how they may be used to affect their performance. Since the rules on the legality of these medications are, in at least some cases, variable from jurisdiction to jurisdiction, I will comment briefly on the way that these medications are handled forensically, although principally from a North American point of view.

The classical concept of illegal medication is acute stimulant medication, the traditional "doping" or "hopping" of a horse. In this procedure, the horse is give a dose of a stimulant as close to post time as possible. The purpose of this is to ensure that the horse is maximally stimulated at the time of the race and therefore puts in a superb or "supra-maximal" performance, and wins the race. In theory this sounds easy, but in practice it is far more challenging than might appear at first glance. To effectively stimulate a race horse, one has to select the correct dose for that particular horse and administer the drug at the appropriate time prior to post time. For some drugs, selecting the right dose simply requires knowing the general pharmacology of the drug in the horse. For other drugs, however, there are large differences between horses in their responses to a particular drug or dosage.

One of the best examples of this is the response of horses to cocaine. Figure 1 shows that when measuring rates of behavior in operant conditioned horses, cocaine can induce both increases and decreases in horses' behavioral rates following variable doses of this drug. For example, we showed that there was up to a 100-fold range in doses of cocaine to maximally stimulate individual horses,[9] and to use this drug effectively in a horse one would have to know how sensitive the horse in question was to the drug. The basic message of this is that for certain drugs one needs to know reasonably well how an individual horse will respond to certain medications or doses to use them effectively, and this information is often not readily available.

Another approach to stimulant medication involves chronic administration of a drug for weeks or months prior to a race. The classic example of this type of medication is treatment with anabolic steroids. In this case, the trainer can likely monitor the response of the horse and can titrate the dose for optimal effect. This pattern of medication was widely used in parts of Europe before the introduction of chemical tests for anabolic steroids.[6] When these tests were first introduced, they uncovered evidence of abuse of these agents in about 10% of horses tested. This pattern of abuse dropped substantially in the following weeks with the advent of effective chemical testing.

A more subtle form of doping is the judicious use of tranquilizers on "washy" horses. A nervous or "washy" horse is a horse that gets excited in the paddock and dissipates his effort prior to the race. In addition, an overly excited horse can be difficult to control in a race, and such horses may respond

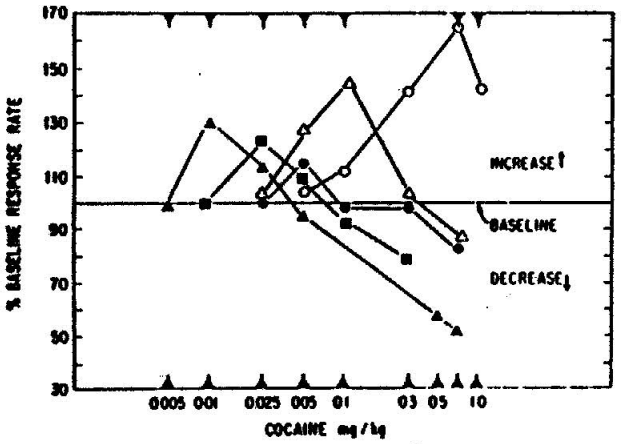

Figure 1. Acute effects of cocaine on operant conditioning responding schedule. The symbols represent the percentage of change in responding rates from control for each animal as the dosage of cocaine was increased. Reproduced with permission from Charles C. Thomas, Publisher, Springfield, Ill.

with an improved effort after a small dose of a tranquilizer. Tranquilizers, such as acepromazine, when used in this manner, are classified as stimulant medications, even though they are pharmacologically sedatives. Recently, a pattern of acepromazine abuse was detected in Illinois racing with the use of the newly developed enzyme-linked immunosorbent (ELISA) and particle concentration fluorescence (PCFIA) immunoassay technology.[4]

A much less subtle way in which depressant medications are used to influence the outcome of a race is to sedate or depress one or more of the horses. This generally involves someone "outside" the horse's stable who wishes to alter the outcome of an event by "stopping" the competition. For example there are available new xylazine-like compounds that are potent sedatives and that are currently difficult to detect.[1] A small dose of these agents administered to a horse could have a definite effect on an equine event and these drugs are being abused in some circles. While it is difficult to be sure that stimulant doping is actually affecting performance, it is much easier to demonstrate the effects of depressant medication in equine athletes.

Restoration of "normal" performance is another objective of medication. Generally this takes the form of anti-inflammatory drug administration to combat joint or muscle pain or the use of a diuretic to lessen the effects of exercise-induced pulmonary hemorrhage (epistaxis or "bleeding"). The use and effect of these "soft" drugs on equine athletes is a much discussed subject. For example, one of the surprises of the early work on equine performance was an apparent performance stimulating effect of phenylbutazone in supposedly sound horses. These horses improved after treatment with phenylbutazone, which has led to suggestions that the horses were actually subclinically unsound and were merely "normalized" by phenylbutazone.

Other forms of medication which restore normal performance include the intra-articular administration of corticosteroids. In this case the drug is injected directly into the inflamed joint and its performance effect is due to its anti-inflammatory action. If the joint is inflamed to the point that performance is adversely affected, these maneuvers are very effective and can restore normal performance. However, corticosteroid administration can interfere with the regeneration of articular cartilage and lead to degenerative changes in the joint surface and surrounding tissue.[11]

A similar effect can be obtained with the use of local anesthetics. These agents rapidly and effectively alleviate local pain and are widely used in the diagnosis of lameness. If a treatment is so clearly effective that it can be used to diagnosis lameness, it is likely to have a positive effect on an ailing athlete. Local anesthetics are, in fact, important therapeutic agents used in both equine and human sports medicine in the restoration of normal performance.

While local anesthetics are often legal and permissible in human sports medicine, they are illegal in most equine sporting events. This is because of the potential for a horse to misstep with a blocked leg and cause a serious mishap. Such a mishap could lead to an accident that could put the lives of both horses and jockeys at risk. At this time, virtually all racing jurisdictions expressly forbid the use of local anesthetics.

The final category of medication methods to be discussed here is blood doping, or the administration of an animal's own blood cells prior to an event. In performing this procedure, one is attempting to mimic the animal's own splenic reservoir function. No clear evidence exists to suggest that this method actually is effective in improving the performance of a horse.

Because of the multiplicity of ways in which drugs can be used to affect equine performance and the sometimes large stakes depending on the outcome of these events, the question continually arises as to whether or not these drugs actually affect equine performance. Over the years a number of different experimental approaches to this problem have been taken, and none of these approaches has been particularly successful. More recently, however, because of the increasing interest in the effect of certain drugs on performance, interest in this question has again arisen, and I will briefly review the ways in which this problem has been approached, and the strengths and weaknesses of each approach. These approaches are the

Table 2. Results of maximum output performance tests.

Study #	Drug	Control	Test	Test Control	n	% Change	Conclusion
1	Furosemide	136.33	136.00	-0.33±1.59	5	-0.24	no sig. diff
2	Furosemide	146.7	145.2	0.5	6	-1.02	no sig. diff
3	Amphetamine				3	+2.4	no sig. diff
4	Nandrolone	72.8±1.8	75.8±1.76	3.0±2.5	6	4.1	no sig. diff

Only Study #1 presents actual performance times for each horse. Study #2 provides mean and range, with the notation that the effect is non-significant. Study #3 provides only maximum, minimum and mean percent changes. In Study #4, a cross-over study, the investigator simply notes that his second period times were 4.1% slower than his first period times. Based on the data of Study #1, testing at the 5% level and desiring a significant result 80% of the time, furosemide would have to produce an increment in performance of 5.08 seconds or of 3.75%.[14]

"maximal output performance" experiment, the "submaximal output" performance experiment, the "pharmacological" experiment and the "statisticians" experiment.

The Maximal Output Performance Experiment

The conceptually simplest approach to the study of equine performance is in the maximal output performance or Horseman's Experiment, so-called because horsemen are the people who usually suggest it. In this experiment, one runs about six horses, with or without the drug, for about a mile at top speed. The distinguishing characteristic of this experiment is that the control horses are run at maximal output, and the drug is being asked to produce a "supra-maximal" performance effect. Drugs studied in this type of experiment include amphetamine, furosemide and the anabolic steroids. Perhaps not unexpectedly, such a drug-induced supra-supra-maximal performance effect has yet to be demonstrated.

The problem with this experiment is that the drug effect is likely to be small, while the noise or background variability found in the controls may be large. We are aware of several such studies in racing horses, and all have yielded inconclusive results (Table II).

More recently, we analyzed the data from these experiments to determine the potential for these tests to produce statistically significant results.[14] Unfortunately, no other workers have presented individual data points or a mean and a statistical estimate of the variance encountered in their performance trials. However, on the basis of the variance reported in time trial work from our laboratory, one would need a performance improvement of about 3.75% on top of an already maximal performance for statistical significance. This is a large increment in performance to expect of any medication, and is unlikely to be observed in the small number of animals tested in maximal output performance experiments to date.

Sub-maximal Performance Experiments

The sub-maximal performance experiment is in essence a modification of the behavioral model experiment in which the horses are run at less than maximal output with and without the drug. Because the horses are not being tested at maximal output, there is a better chance of obtaining statistically significant changes in times than in the maximal performance experiment. Using this approach, statistically significant effects of drugs in horses have been reported, but whether or not these effects are important in a racing situation is unknown. Therefore, a major problem with this type of experiment is that one cannot know how the results from these experiments relate to a "supra-maximal" performance effect.

This experimental method[1] has been taken by Sanford in England[8] and by Fujii in Japan.[2] Some of Sanford's data, which are typical of the data generated by this approach, is presented in Table III. With this type of experiment, Sanford reported statistically significant effects of drugs in gallop tests, but how this data may relate to effects of drugs on maximal or near maximal performance is not clear.

The Pharmacologist's Experiment

The most effective way of obtaining information about the effects of drugs in horses is to test the horse's actions in simple behavioral models. For example, agonist narcotic analgesics in the horse produce a well-defined locomotor response which can be accurately measured by simply counting steps that the animal takes with its left front leg (Fig. 2). Using this model, one can generate classic dose and time response data for these drugs in the horse (Fig. 3) and demonstrate the likelihood of performance effects.[3] These models produce data qualitatively similar to that obtained with the submaximal output performance experiment, but which are far more detailed and informative. For example, these experiments can identify dosage rates and times post-dosing at which one obtains peak drug effects. They can also show that some drugs do not produce consistent behavioral effect in the horse, and that the effective doses of some drugs can vary up to 100-fold between individual horses. For these reasons,

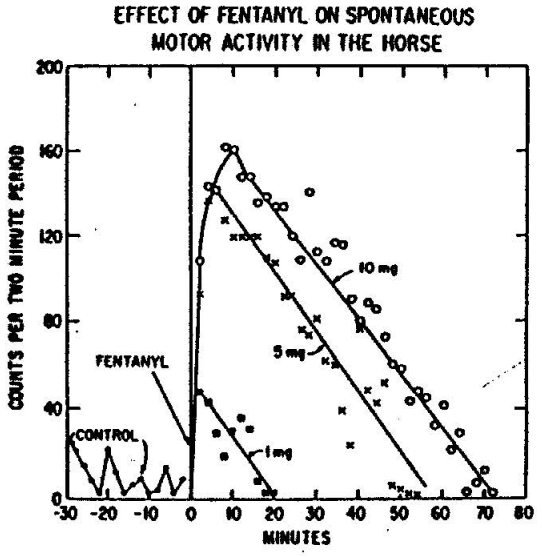

Figure 2. Effect of fentanyl on spontaneous locomotor activity in four horses. The lower panel shows the normal activity of a horse at rest in his stall, about 4 steps per two minutes. The top panel shows the locomotor response produced in horses by injection of 1, 5, and 10 mg of fentanyl per horse by rapid IV injection. Reproduced with permission from Irish Vet. J.

Table 3. Gallop test 2 x 200 m. Compounds suspected of increasing speed in Thoroughbred horses.

Compound	Dose (mg/kg)	Route	Number Tested
Methylamphetamine	0.1	i.m.	4
	0.2	i.m.	4
Methylphenidate	0.25	s.c.	4
	0.5	s.c	4
Pemoline	4.0	oral	3
	8.0	oral	3
Caffeine	2.0	oral	3
	4.0	oral	3
Phenylbutazone	8.0	oral	4
	6.6	i.m.*	4

*Injection made 23 hours before test. In these gallop tests, horses were run singly over a 200-meter course from a flying start. After an interval of about 5 min, during which period the horse returned to the start at a trot or slow canter, this gallop was repeated. No data on the dosing times, the actual performance times, or the variability in the performance times on which these conclusions were drawn were presented. Reproduced with permission from Blackwell Scientific Publications.

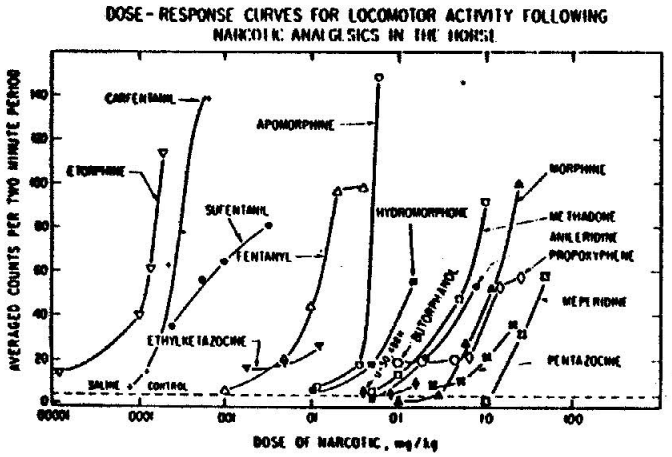

Figure 3. Dose-response curves for locomotor activity following narcotic analgesic administration in the horse. Horses were dosed with increasing amounts of the indicated drugs and the average number of steps taken during the peak two min period were plotted. The average counts per two min period for the saline control are shown by the dashed line near the bottom of the graph. Reproduced with permission from Equine Vet. J.

simple behavioral experiments to characterize the pharmacological effects of drugs in horses are necessary before performance experiments of any kind are attempted in horses.

The necessity for careful characterization of the pharmacological actions of drugs in horses was brought home to us by our experiments with fentanyl. Fentanyl is a highly lipid soluble narcotic analgesic, about 80 times more potent than morphine. It was reportedly widely used in racing horses in America during the 1970s. When we started our performance work on this drug, we used the dose and route of administration (0.25 mg/horse, 30 min before race time) reportedly used illegally on the racetrack. In this work, we saw no behavioral or performance effects due to fentanyl whatsoever.

Later, when we increased the dose of fentanyl for kinetic studies, we discovered the characteristic behavioral effects presented in Figure 2. It then became apparent to us that to obtain clear-cut behavioral effects with fentanyl requires a minimum dose of about 2-3 mg and the drug has to be given IV.[13] This lesson highlighted the necessity of defining carefully the pharmacology of a drug in racing horses before starting expensive performance experiments.

The Statistician's Experiment

The last type of experiment that we will discuss is the so-called statistician's experiment. In this type of experiment, the data are obtained by a study of the effects of approved medi-

Table IV. Effect of medication with furosemide on the performance of horses racing at Louisville Downs, Summer 1977.

	Number of Horses	Number of Trials	Mean Times	S.E.M.
Pre-furosemide F = 0.31	58	160	128.5925	0.2031
With Furosemide (F for significance should be > 3.0)	58	232	128.7366	0.1594

At this meet, furosemide was the only permitted medication, and its use was monitored by urinalysis. Horses could elect to go on furosemide at any time throughout the meet, but once on furosemide had to stay on it. Performance times for horses pre- and post-furosemide treatment were obtained from the meet programs and compared. Only times on good or fast tracks were taken. For the 58 horses selected, 160 pre-furosemide times were available and 232 post-furosemide times. A randomized block design was used where each horse represented a block. After adjusting for blocks (i.e. differences between horses), there was no significant difference between treatments (i.e. times on and off furosemide). Reproduced with permission from *J Equine Med Surg*.

Table V. ELISA screening of post-race urine samples followed by GC/MS analysis.

Sample Date	# Urine Samples	# Flagged by ELISA	# Positive by GC/MS Analysis	Drug Identified
10-3,4-87	34	5	3	Oxymorphone
10-4-87	16	1	1	Oxymorphone
10-11-87	8	1	1	Oxymorphone
10-17-87	36	3	2	Oxymorphone
10-17,18-87	27	3	1	Oxymorphone
10-20-87	21	4	4	Oxymorphone
10-27-87	24	1	1	Hydromorphone
TOTALS 9 Days Racing	166	18	13	

Post-race urine samples from two racing jurisdictions were screened for morphine and its analogs by the ELISA test and then subjected to gas chromatography/mass spectroscopy (GC/MS). The dates on which the samples were collected, the number of samples in each analysis batch, and the number of samples flagged "suspicious" by ELISA are presented in the first three columns. The results of GC/MS analysis of the flagged samples are shown in columns four and five. About 72% of the ELISA positives were determined by GC/MS to contain either oxymorphone or hydromorphone. For some of the unconfirmed ELISA positives, insufficient sample was available for complete GC/MS evaluation of their opiate status. Reproduced with permission from *Res Comm Chem Pathol Pharmacol*.

cation on actual track times of racing horses. This is potentially the most powerful of all the experimental methods available for answering questions about the actions of drugs in racing horses.

This type of experiment was first proposed by Mr. Carl Larsen of the Kentucky Harness Racing Commission, who pointed out that in 1977 the only drug permitted in harness racing in Kentucky was furosemide. He suggested that we study the differences in track times for harness horses racing at Louisville Downs with and without furosemide. Furosemide pre-race is recommended in racing horses for the treatment of exercise-induced pulmonary hemorrhage (epistaxis or "bleeding"). Whether or not it is effective in the treatment of this condition and whether or not it improves the performance of racing horses is unknown. We identified 232 times for these horses while they were on furosemide, compared with 160 times for the horses without furosemide. The results of this study (Table IV) suggest that the horses treated with furosemide were about one-tenth of a second slower after treatment with furosemide than before.[15] The numbers are large, the experiment undoubtedly relates to the performance situation, and statistically the answer is unequivocal. Furosemide treatment had no statistically significant effect whatsoever on the performance of Standardbred horses at this Louisville Downs meet.

In contrast with the small probability of obtaining statistically significant data from maximal output performance experiments, these racetrack experiments are much more promising. From the date of Table IV one can calculate that a true mean difference of 0.72 x (a 0.56% improvement) would be required to produce significant differences from controls at the 0.05 confidence level, assuming that it is desired to obtain a significant result 80% of the time. These are far more attainable figures than those developed from maximal output performance trials, and they suggest that this experimental approach should be pursued.

This approach has been taken a step further by Larry Soma of the University of Pennsylvania in his studies of Thoroughbred horses. Dr. Soma and his colleagues observed the effects of furosemide at Keystone Racetrack on horses where times had declined for three successive races and whose owners had them endoscopically examined. Those found positive for epistaxis (pulmonary bleeding) were then put on furosemide. The results showed that furosemide restored the performance of the epistaxis-positive horses to the level observed prior to their decline in performance. This experiment, therefore, suggests that the action of furosemide is to restore "normal" performance in racing horses. While there were difficulties with the controls utilized in this experiment, this work clearly points to the racetrack as the most satisfactory experimental tool for answering questions about drugs and racing performance.

Immunoassay based drug testing

Until very recently control of the use of illegal medica-

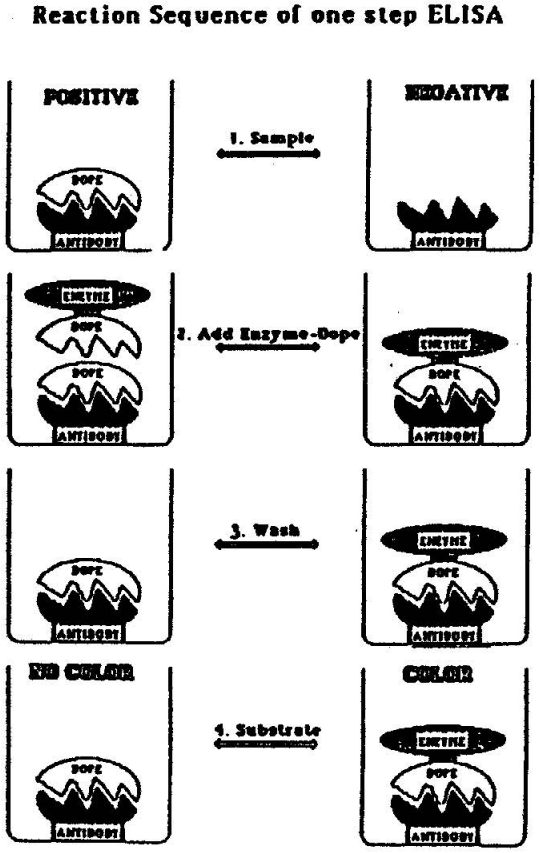

Figure 4. Reaction sequence of the one step ELISA test. Antibody to the drug is bound to the well, and test and control samples are added directly to the well. In control samples those sites remain free and bind the drug-enzyme conjugate when this is added. In "positive" sample wells the drug-enzyme conjugate cannot bind, because the antibody sites are already occupied. Unbound drug-enzyme is removed by the wash step and substrate added to develop the test. An absence of color, indicating that no drug-enzyme complex bound to the antibody, represents a positive test. Reproduced with permission from *Res Comm Chem Pathol Pharmacol.*

tion in horses in North America depended on thin layer chromatography (TLC). Post-race urine samples taken from racing horses were shipped to post-race laboratories, extracted by liquid/liquid extraction, and subjected to TLC. Samples showing evidence for the presence of drugs were subjected to further testing, including gas chromatography/mass spectroscopy (GC/MS) confirmation. With minor exceptions, medication control in racing horses in North America has depended on the TLC technology outlined above. More recently, however, we have developed a panel of ELISA based tests for use in equine drug testing, and these ELISA tests can provide very sensitive and effective screening for drugs abused in performance horses.[17]

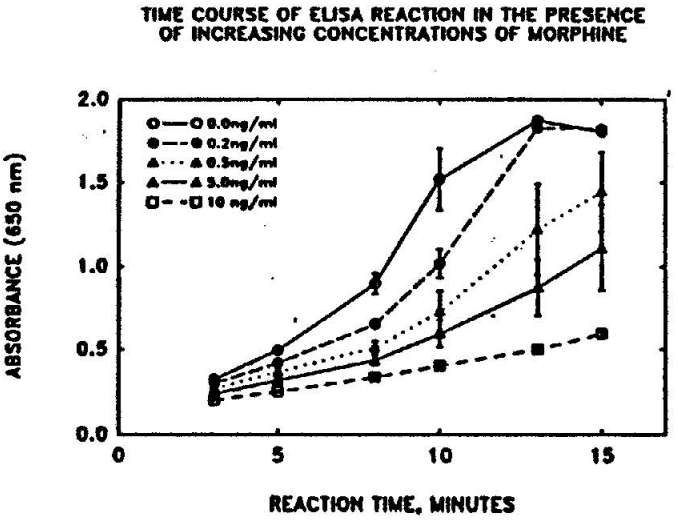

Figure 5. Time course of ELISA reaction in the presence of increasing concentrations of morphine. The symbols show the time course of the ELISA reaction in the presence of the indicated concentration of morphine. Reproduced with permission from *Res Comm Chem Path Pharmacol.*

These ELISA tests are based on those described by Voller.[18] Briefly, the anti-drug antibody is linked to flat bottom microtiter plates and drug-hemisuccinate is linked to horse radish peroxidase (HRP) to give rise to covalently linked drug-HRP complex. The assay is started by adding the standard, test, or control samples to each well, along with the drug-HRP solution. During this step, the presence of free drug or cross-reacting metabolites competitively prevents the antibody from binding the drug-HRP conjugate. The degree of antibody:drug-HRP binding is therefore inversely proportional to the amount of drug in the sample. After incubation the fluid is removed from the microtiter wells and the wells washed. Substrate is then added to all wells and their absorbance read in a microwell reader. A diagram outlining this sequence of events is presented schematically in Figure 4.

The ELISA tests outlined above are particularly effective. For example, Figures 5 and 6 show respectively the time course and sensitivity of the morphine ELISA, a typical "run" on a series of track samples and, in Table V, the results of the introduction of this test into routine post-race testing. As shown in Table I, of 166 samples screened in the Western United States, 18 were "flagged" by ELISA and of these, 13 confirmed positive on GC/MS.[5]

Table VI. Efficacy of PCFIA and ELISA tests.

Drug	State	TLC Status	Immunoassay Positives
Buprenorphine	New Mexico	No test	Multiple (>50)
xymorphone	New Mexico	Poor sensitivity	Multiple (>30)
Sufentanil	Oklahoma	No test	10/300*
Mazindol	Western States	Very poor sensitivity	Multiple (>20)
Cocaine	California	Poor sensitivity	2/83*
cepromazine	Illinois	Fair sensitivity	Multiple**(>25)

The table compares the TLC and immunoassay status of 6 drugs for which immunoassay tests have been introduced since August 1987. Figures marked by an asterisk (*) represent the ratio of positives called to total number of samples tested. **Acepromazine initially detected in pre-race samples. Reproduced with permission from *Res Comm Chem Pathol Pharmacol.*

Similar patterns of positives were seen across the Western United States wherever these tests were introduced. In general about 1% to 5% of the early samples tested were positive for a narcotic analgesic. Similarly, when the mazindol test was introduced in early 1988 about 2 to 5 % of the early samples were positive when confirmed by GC/MS.[7] The efficacy of these ELISA tests in racing chemistry had been dramatically established and a major false negative problem with TLC based screening had been exposed.

Comparative efficacy of TLC and Immunoassay Screening

Establishing the efficacy of PCFIA and ELISA based immunoassays (Table VI), exposed major deficiencies in TLC as a screening methodology. No TLC method for buprenorphine existed, so use of this drug was completely uncontrolled. Similarly, sufentanil abuse was uncontrolled and even "bragged on" by horsemen until the advent of this technology (Tobin et al., 1988b). While TLC methods for cocaine, oxymorphone and mazindol existed these methods were unable to detect the small doses of these drugs being used in horses. This was especially so for mazindol, where the TLC dose was about 400 mg/horse, while the dose used on the track was about 4 mg/horse.[7] Overall, the great sensitivity and speed of the ELISA tests rendered them highly effective screening tests and far superior to the old TLC screening methods. The ELISA tests established the efficacy of this technology in post-race testing and similar events soon took place in pre-race testing.

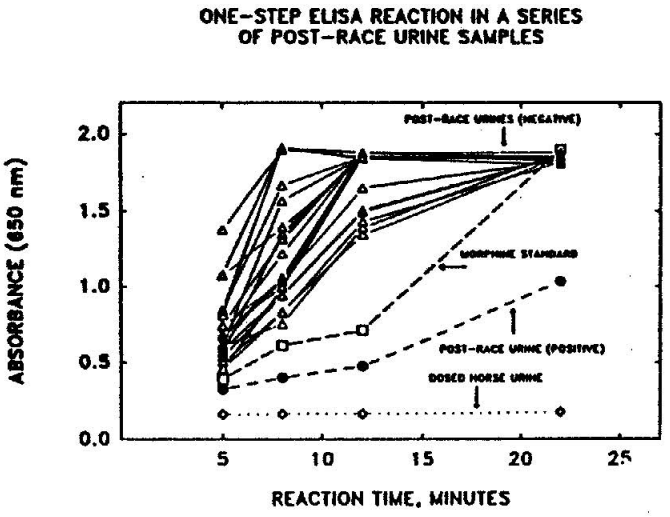

Figure 6. One step ELISA reactions in a series of post-race urine samples. The open triangles (△) show the activity of this ELISA test. The open squares (□) show the effect of 0.5 mg/ml of morphine added to this system. The open diamonds (◇) show ELISA activity in a dosed horse urine from and the solid circles (●) show ELISA activity in a sample subsequently determined to contain oxymorphone. Reproduced with permission from Res Comm Chem Pathol Pharmacol.

REFERENCES

1. Clarke KW, Taylor PM: Detomine: A new sedative for horses. *Equine Vet J* 18, 366-370, 1986

2. Fujii S, Yoshida S, Kusanagi C, Mima K, Natsuno Y: Pharmacological studies on doping drugs for race horses. Transoxocamphor. *Jpn J Vet Sci* 32, 307-317, 1970

3. Kamerling S, Wood T, DeQuick D, Weckman TJ, Tai C, Blake TW, Tobin T: Narcotic analgesics, their detection and pain measurement in the horse: A review. *Equine Vet J* 21, 4-12, 1989.

4. Kwiatkowski S, Sturma L, Dai MR, Tai H-H, Watt DW, Tai CL, Woods WE, Weckman TJ, Yang J-M, Wood T, Chang S-L, Blake JW, Tobin T, Prange CA, Brockus C, Stobert D, Wie S, Chung RA, McDonald J, Bass VD, Merchant S, Artemendo M: Immunoassay detection of drugs in racing horses. VII. Detection of acepromazine in equine urine and blood by ELISA and PCFIA. *Res Comm Chem Pathol Pharmacol* 61, 391-412, 1988.

5. McDonald J, Gall R, Wiedenbach P, Bass VD, DeLeon B, Brockus C, Stobert D, Wie S, Prange CA, Ozog FJ, Green MT, Woods WE, Tai CL, Dai MR, Weckman TJ, Tai H-H, Yang J-M, Chang S-L, Black JW, Tobin T: Immunoassay detection of drugs in racing horses. III. Dectection of morphine in equine blood by a one step ELISA assay. *Res Comm Chem Pathol Pharmacol* 59, 259-278, 1988.

6. Moss MS, Haywood PE: Survey of positive results from race-

horse anti-doping samples received at Racecourse Security Services Laboratories. *Equine Vet J* 16, 39-42, 1984.

7. Prange CA, Brockus C, Stobert D, Wie S, McDonald J, Gall R, Wiedenbach P, Bass VD, DeLeon B, Ozog FJ, Green MT, Woods WE, Tai CL, Dai MR, WEckman TJ, Tai H-H, Yang J-M, Chang S-L, Black JW, Tobin T: Immunoassay detection of drugs in racing horses. V. Detection of mazindol in equine blood and urine by a one step ELISA assay. *Res Comm Subst Abuse* 9, 13-30, 1988.

8. Sanford J: Medication affecting the performance of racehorses and its control. *Proc 19th World Vet Cong* pp 382-385, 1971.

9. Shults T, Combie J, Dougherty J, Tobin T: Variableinterval responding in the horse" A sensitive method of quantitating effects of centrally acting drugs. *Am J Vet Res* 43, 1143-1146, 1982.

10. Soma LR, Laster L, Oppenlander R, Barr-Alderler V: Effects of furosemide on the racing times of horses with exercise-induced pulmonary hemorrhage. *Am J Vet Res* 46, 763-768, 1985.

11. Tobin t: Pharmacology Review: The corticosteroids. *J Equine Med Surg* 3, 10-15, 1979.

12. Tobin, T: *Drugs and the Performance Horse.* Charles C. Thomas, Springfield, IL. pp 11-26, 1981.

13. Tobin T, Combie J, Miller JR, Crisman MW, Blake JW: The pharmacology of narcotic analgesics in the horse. II. Studies on the detection, pharmacokinetics, urinary clearance times and behavioral effects of pentazocine and fentanyl in the horse. *Irish Vet J* 3, 169-176, 1979.

14. Tobin T, Kamerling, SG and Anderson RL: Drugs and racehorse performance. Trends Pharmcol Sci 6, 129-132, 1985.

15. Tobin T, Roberts BL, Swerczek TW, Crisman M: The pharmacology of furosemide in the horse. III. Dose and time response relationships, effects of repeated dosing, and performance effects. *J Equine Med Surg* 2, 216-226, 1978.

16. Tobin T, Tai H-H, Tai CL, Houtz PK, Dai MR, Woods WE, Weckman TJ, Yang J-M, Chang S-L, Black JW, McDonald J, Gall R, Wiedenbach P, Bass VD, DeLeon B, Ozog FJ, Green MT, Brockus C, Stobert D, Wie S, Prange CA: Immunoassay detection of drugs in racing horses. IV. Detection of fentanyl in equine blood and urine by a one step ELISA assay. *Res Comm Chem Pathol Pharmacol* 60, 97-115, 1988.

17. Tobin T, Watt, DS, Kwiatkowski S, Tai H-H, Blake JW, McDonald J, Prange CA, Wie S: Non-isotopic immunoassay drug tests in racing horses: A review of their application to pre-race and post-race testing, drug quantitation, and human drug testing. *Res Comm Chem Pathol Pharmacol* 62, 371-395, 1988.

18. Voller A, Bidwell DE, Barlett A: The enzym linked immunosorbent assay (ALISA) *Bull Wld Hlth Org* 53, 55-56, 1976.