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MEDICATION OF PERFORMANCE HORSES*
by T. Tobin

The medication of horses prior to an event in which the medication may directly or indirectly influence the animal's performance is no longer uncommon. In some events, such as races in certain jurisdictions or trail rides, medication in the form of fluid therapy or anti-inflammatory drugs is an accepted practice. However, in other circumstances, such as sales, or in other jurisdictions, medication is explicitly forbidden and compliance with the rules is monitored by chemical analysis of body fluids (Table 10). The medication of performance horses has become an important concern for veterinarians because of the increasingly widespread use of medication in race horses prior to race time and parallel increases in the sophistication of chemical analyses.[1] Further, this area is likely to become of increasing importance to veterinarians if federal legislation makes medication of any race horse a felony.

Medication of some form was, until recently, permitted in horses in most US racing jurisdictions, the principal exception being New York state. The American Horse Shows Association bans the use of most drugs, especially stimulants and tranquilizers, in horses being shown. A related problem is the use of drugs, in horses examined for sale, to mask lameness or undesirable behavior. Because these problems are likely to become more important rather than less in the future, the importance of the veterinarian's understanding of the principles of drug metabolism, elimination and medication testing in horses is also likely to increase rather than decrease in the future.

The medication of performance horses may be divided into several distinct categories (Table 11). Medication to win, or stimulant medication, occurs when a horse is dosed shortly before a race with a stimulant, such as amphetamine, caffeine, morphine or apomorphine, to increase its speed.[22] Such stimulant medication is commonly referred to as "doping." Medication to win also includes the use of very small doses of a tranquilizer to enable an excitable or "washy" horse to perform well. Another, perhaps more subtle, form of medication to win is treatment with anabolic steroids prior to an event or sale to increase muscle mass, aggressiveness and possibly performance.

All of these forms of medication are prohibited by the rules of racing of which I am aware in all jurisdictions. Since effective stimulant doping requires a good knowledge of the pharmacodynamics of the particular drug and the horse's response to the drug, as well as access to the horse shortly before the race, it is usually considered an "inside job" performed by somebody with relatively free access to the horse.

Medicating to lose, or depressant doping, occurs when a horse is given a tranquilizer or depressant, such as acetylpromazine, to make it run more slowly. Because there are many other ways of "stopping a horse" without administering a drug, doping in this fashion is usually considered as an "outside job." This was the "classic" form of interfering with a horse usually done in an effort to bring off a betting coup. Using the appropriate drug at the proper dosage, this approach can, apparently, be successful, although one more commonly hears of races in which horses have been heavily tranquilized to the point that their condition is obvious and they are scratched. Depressant medication is also illegal under the rules of all racing jurisdictions of which I am aware.

Another common type of medication is medication to restore normal performance. Predominant among this class of drugs is phenylbutazone, which of course cannot improve a horse's performance beyond its so-called "innate"

[1]Kentucky Agricultural Experiment Station article 79-4-176. All figures by permission of the Journal of Equine Medicine and Surgery.

Table 10. Incidence of Drugs as Reported by Members of the Association of Official Racing Chemists, 1947-73

Drug	Times Reported	Drug	Times Reported
procaine	661	mephenesin	6
caffeine	482	pemoline	6
amphetamine	452	pyrilamine	6
phenylbutazone	267	acetylsalicylic acid	5
methylphenidate	193	butacaine	5
theobromine	127	imipramine	5
methamphetamine	121	methoxamine	5
dipyrone	116	propiomaxine	5
polyethylene glycol	111	tetracaine	5
phenobarbital	86	chloroquine	4
oxyphenbutazone	66	hydrocortisone	4
morphine	62	levorphanol	4
ephedrine	59	prednisone	4
strychnine	50	mefenamic acid	4
thiamin	50	meperidine	4
pentazocine	49	cinchonidine	3
nikethamide	44	propoxyphene	3
barbiturates	43	sulfanilamide	3
promazine	38	sulfaphenazole	3
methapyrilene	35	thiabendazole	3
nicotine	33	acepromazine	2
indomethacin	26	antipyrine	2
ethylaminobenzoate	23	barbitone	2
atropine	22	codeine	2
pipradrol	21	chloral hydrate	2
phenothiazine (derivative)	18	dibucaine	2
lignocaine	16	doxapram	2
chlorpromazine	15	guaiacol	2
prednisolone	13	phemitone	2
theophylline	12	meprobamate	2
mephentermine	11	naphazoline	2
leptazole	10	pangamic acid	2
acetophenetidin	9	sulfonamide (sic)	2
cocaine	9	acetophenazine	1
methocarbamol	9	amydricaine	1
phenylpropanolamine	8	berberine	1
salicylic acid	8	bromide	1
hyoscine	8	camphor (rectal swab)	1
amylocaine	7	capsaicine	1
brucine	7	chlorbutanol	1
quinine	7	cinchonine	1
thozalinone	7	cincophen	1
apomorphine	6	danthron	1
alcohol	6	dapsone	1

Table 11. Categories of Medication

1. Medication To Win
   - Acute: short-acting stimulants, such as amphetamine, cocaine, narcotics
   - Chronic: repeated dosing for weeks, such as with vitamins or anabolic steroids
   - "Washy" Horses: administration of a very small dose of a depressant or tranquilizer to "take the edge off" of an excitable horse
   - Always illegal and usually considered an "inside job"
2. Medication To Lose
   - Depressants: large doses of a tranquilizer, sedative or depressant
   - Always illegal and usually considered an "outside job"
3. Medication To Restore Normal Performance
   - Nonsteroidal anti-inflammatory drugs, such as phenylbutazone and its congeners. Often permitted under controlled medication rules
   - Corticosteroids: sometimes administered intra-articularly to control joint pain; occasionally permissible
   - Local anesthesia: nerve or joint blocks; always illegal
4. Accidental, Inadvertent or Technical Doping
   - The accidental occurrence of a positive, such as procaine from procaine penicillin, caffeine from cocoa husks in food pellets, or methocarbamol from glyceryl guaiacolate
5. Medication To "Mask" Other Drugs
   - Administration of dipyrone and thiamin thought to interfere with the detection of illegal medication
6. Medication To Dilute Other Drugs
   - Diuretics such as furosemide, ethacrynic acid, bumetanide
7. Miscellaneous Mechanisms
   - "Blood doping"
   - "Bicarbonate doping"

ability to run.[4] The advantage claimed for this form of medication is that it enables horses to run "up to form" through a long and arduous racing season and, therefore, allows for full fields at race meets. The disadvantage is that allowing unsound horses to run through the use of phenylbutazone and similar drugs increases the likelihood of breakdowns and serious injury or death to both horse and jockey. Further, there is the likelihood of influencing the gene pool of animals bred, which, according to some, would "alter the breed" in undesirable ways.

Other types of medication may be used to interfere with drug detection. Furosemide and other diuretics dilute some drugs in urine and, under certain circumstances, may interfere with medication control. For example, at one time California, Illinois and other states had rules that set a maximum permissible concentration of phenylbutazone in equine urine. This rule, however, could be circumvented simply by administering furosemide shortly before a race. This can reduce urinary concentrations of phenylbutazone up to 50-fold and considerably reduces the possibility of an illegal concentration of phenylbutazone in the urine.

"Masking" is the popular name for the use of one drug to interfere with or reduce the probability of detecting another. Dipyrone and thiamin were once popularly considered masking agents and are still occasionally found in large concentrations in equine urine samples. Similarly, legitimate therapeutic agents such as phenylbutazone may, if present in sufficient concentration, interfere with the detection of other drugs.

Other miscellaneous forms of doping include "blood doping" and "bicarbonate doping." In blood doping, a volume of blood is withdrawn from an animal and the RBC are separated and

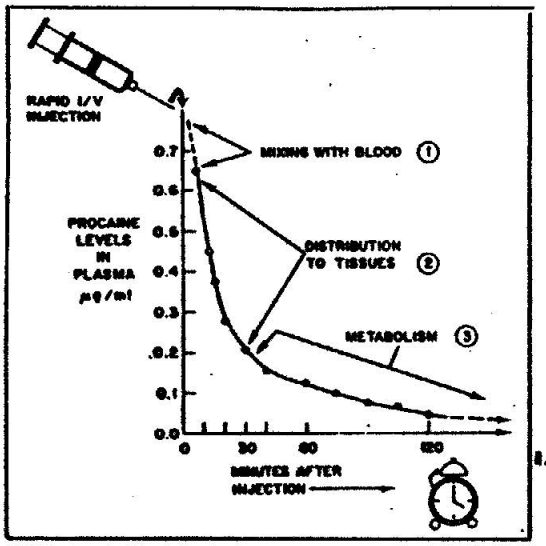

Fig 2. The 3 phases of drug distribution. Decline in plasma levels of procaine after rapid iv injection at 2.5 mg/kg.

refrigerated. The RBC are injected back into the horse shortly before a race to raise the PCV and supposedly improve performance for the critical event. This form of doping has also been performed on human athletes, especially marathon runners.

The objective of bicarbonate doping is to increase the base reserves of the animal and thus its resistance to fatigue. While there is evidence as to the value of this maneuver under some circumstances, it is not clear if this mechanism has even been studied in horses.

The incidence of the use of illegal medication is unknown. The violations of medication rules are reportedly about one "positive" per 500-1000 samples in postrace testing and perhaps somewhat less in prerace testing. Strangely enough, these rates have remained relatively constant over the years despite huge changes in the types of drug available, the patterns of drug use, and improvements in analytic methodology. The list of drugs detected over the years is tremendous and has for many years been headed by procaine (Table 10). However, many of these procaine positives are attributable to the therapeutic use of procaine penicillin, which highlights one of the critical problems for practitioners.

Drug Clearance Time

The problem of "clearance time" (time required for a drug to be undetectible after administration) is, for a veterinarian, the central problem in racing chemistry. In the final analysis, what the veterinarian wants to know is how close to race time a prohibited drug can be administered and still "clear" the urine so the analyst does not declare a medication violation. Unfortunately, there is no simple answer to this question for several reasons. First, drugs take a very long time to be eliminated from a horse's body. Depending on a particular drug's half-life, the amount of the drug is reduced by 50% over a certain period, then again by 50% after that same period, until the drug is finally eliminated (Figs 2, 3). For example, the average dose of phenylbutazone for a horse contains about 6×10^{21} molecules of drug. About 90% of this dose is eliminated in the first 24 hours, and a further 9% the next day. However, it takes about 21 days for the entire dose to be eliminated. A good analyst can find phenylbutazone in plasma or urine for up to 9 days after the last dose was given.

Each drug has its own individual half-life that is reasonably constant from horse to horse. Given a drug's half-life, it is easy to calculate the drug levels for any given time in an animal if the concentration at a specific time is known. Knowledge of a drug's pharmacology allows one to predict what the pharmacologic effects of the drug were or will be. This kind of data is also important in calculating blood levels of a drug after a given dose, and from this kind of information one can predict the probable pharmacologic effects from a given dose.[5]

The size of the dose administered may, under some circumstances, have very little effect on the clearance time for a drug. The clearance

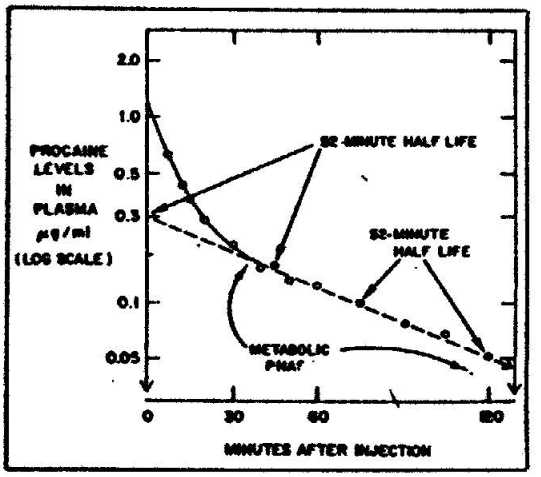

Fig 3. Semilog plot of distribution and elimination of procaine. The data from Figure 2 were replotted with the drug axis on a logarithmic scale. Note there is no zero on a log scale and the time taken for the dotted line portion of the curve to halve is constant.

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 that must elapse until the drug is not detectible. This period depends only on the sensitivity of the analytic method. If the analytic method used for the drug is very sensitive, dose size has very little effect on clearance time (Fig 4). Therefore, huge increases in dose size are required to produce very small changes in clearance time because of the logarithmic relationship between dose and time. Because most drugs are usually administered at well-defined dosages, the effect on clearance time of the usual range in therapeutic doses is trivial. It should be emphasized, however, that if the drug detection methods are only marginal, the effects of increased doses on clearance time can be quite marked (Fig 4).

A drug's clearance time depends primarily (but not absolutely) on the sensitivity of the analytic method used because drugs are extreted for long periods at lower than detectible levels. Therefore, if the analyst cannot detect the drug, the clearance time is zero and does not vary. However, if the analyst can detect the drug, the period of detection depends primarily on the sensitivity of the analytic method used and only secondarily on other factors.

Figure 5 illustrates the excretion curve for furosemide in horse urine and the effect of different detection methods on the apparent clearance time for the drug. If the analyst used a relatively insensitive method, the clearance time for furosemide would be about 18 hours. Use of more sensitive methods would extend the clearance time to 36 or 72 hours.[6] Use of a highly sensitive method, such as radioimmunoassay, might allow detection of the drug for 100 hours or longer. It is clear from this discussion that clearance time depends primarily on the sensitivity of the analytic method used.

Other factors that may influence clearance time include age, sex, build, amount of body fat, size of dose, route of administration, and urinary volume and pH. Analysts often argue that because of these variations, statements of "average" clearance times are impractical. This argument ignores the fact that veterinarians adjust the dosage to take these factors into account and that changes in dosage in general have only small effects on clearance time. With a few important exceptions in the area of the effects of urinary pH on the concentration of certain drugs in urine, these effects are likely to be small compared with the 10,000-fold range in drug concentrations demonstrated in the experiment of Figure 5.[7]

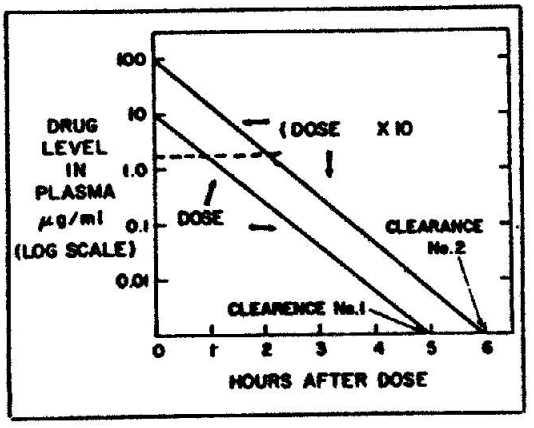

Fig 4. Relationship between dose and "clearance time." Dose "x" of a hypothetic drug generated the plasma levels in the lower curve. At least 10 times the dose ("x" x 10) must be given to raise plasma levels 10-fold and increase clearance time by 1 hour or 20%. The more sensitive the analytic method, the smaller the effect of dose on clearance time. However, if the analytic method is not sensitive (dashed line) differences in dose make large differences in clearance times.

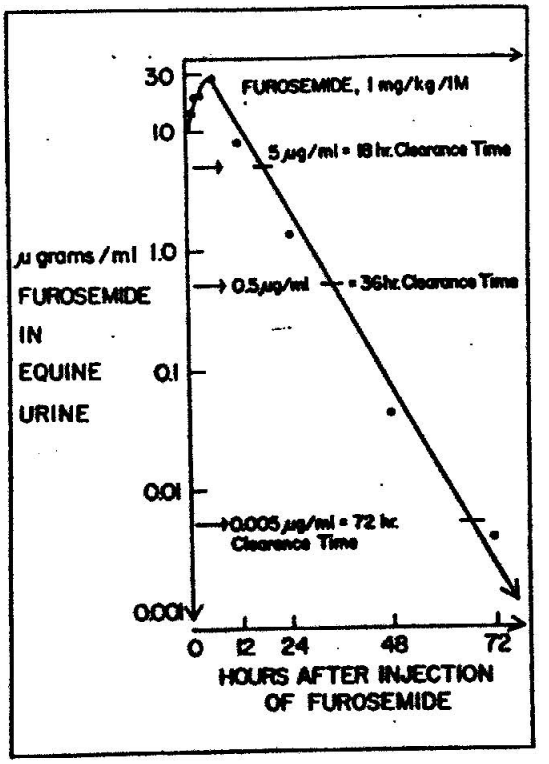

Fig 5. "Clearance times" for furosemide in urine. The solid circles and line show urinary concentrations of furosemide after an injection of furosemide at 1 mg/kg. The crosses on the line show effective clearance times given analytic methods of different sensitivity.

Biologic Fluids Used in Drug Testing

The biologic fluids available for drug testing include sweat, saliva, blood and urine. Depending on the medication being assayed and the analytic methodology available, one or more of these biologic fluids may be used.[6]

Sweat: Information on the excretion of drugs in sweat is limited. Sweat is usually not available before a race, which limits its use in prerace testing. A more fundamental problem, however, is that it is very difficult to refute a defense that drugs detected in sweat were surface contaminants and never actually present in the horse. Sweat is now almost never used in drug testing.

Saliva: Saliva is rapidly and easily collected by the introduction of pads moistened with dilute acetic acid into the horse's mouth. This procedure yields up to 15 ml of diluted saliva. Unfortunately, acidic, highly protein-bound drugs are not generally detected in saliva and entry of alkaline drugs into alkaline saliva is restricted.[6] Because of these problems, saliva is not, to my knowledge, used routinely as a biologic fluid in most drug testing programs in the US.[6]

Blood: Blood is the most easily collected biologic sample and is the only biologic sample used in prerace testing. When performed properly, blood collection does not harm the horse. Since a veterinarian does the sampling, additional information about the clinical condition of the horse is simultaneously obtained.

Drugs detected in blood are usually in an unchanged form, in contrast to urine, in which most of the drug is usually present as metabolites. Similarly, endogenous background levels of a drug are normally lower in blood than in urine. Because the drugs are actually found in the blood at the time of racing and because blood levels of drugs can usually be confidently translated into pharmacologic effects, the significance of a particular blood level of a drug is relatively definitive given an appropriate research base. Blood levels of drugs are also not affected by other drugs, such as diuretics, in contrast with the diluting effects observed in urine under some circumstances. Because most drugs are rapidly cleared from the blood, the likelihood of detecting traces of a drug in blood days after administration are much less than with urine.[6]

The analytic technics required in blood testing are somewhat more exacting than those required for urine because the volume of a blood sample is r[illegible]ely small (10-20 ml) and because drug[illegible] drug metabolites are often found in low[illegible]oncentrations in blood than in urine. However, the small size of blood samples is not a problem when sensitive analytic technics are used. A further problem is that some drugs or drug metabolites readily detected in urine may not be detectible in blood.[6]

Urine: Collection of a urine sample is slow, difficult and expensive compared with collection of blood. Collection is always by spontaneous voiding of urine, and some horses take up to 3 hours to produce a sample. While diuretics accelerate voiding of a urine sample, the increased volume of urine produced can dilute certain drugs or drug metabolites. Only a small number of horses from a given race are tested and the use of urine is virtually restricted to postrace testing because of the difficulty with collection of urine samples.[6]

Although collection of urine samples may be time-consuming, urine testing has a number of advantages over other testing procedures and is currently the backbone of most drug-testing programs. Because relatively large quantities of urine are available for testing (200-500 ml), the analytic methods required are often not as exacting as those required for blood testing. A further advantage is that many drugs or drug metabolites are found in much higher levels in urine than in blood. Another advantage is that a sufficient volume is available for split samples and confirmatory tests.[6]

The principal problem with urine testing is that urine volume and pH are highly variable. Because of this, one can only very rarely even estimate how the urinary level of a drug relates to plasma level and, therefore, how the urinary level of a drug might relate to the time of administration or pharmacologic effect. A further problem with urinary levels of drugs is that traces of a drug may show up in urine many days after the drug is no longer detectible in plasma.[6,7] For example, if the minimum detectible level of procaine in equine fluids is 4 ng/ml, procaine may be detectible for only about 3 days in plasma but is still detectible on the thirteenth day in urine. Similarly, furosemide is found in plasma after IM injection for not more than 12 hours but is detectible in urine for 72 hours.[6] More importantly for the veterinarian, the glucuronide metabolites of narcotics seem to take relatively long times to clear urine. Therefore, pentazocine and fentanyl cannot be detected in equine plasma for longer than 8 or 12 hours, respectively, but can

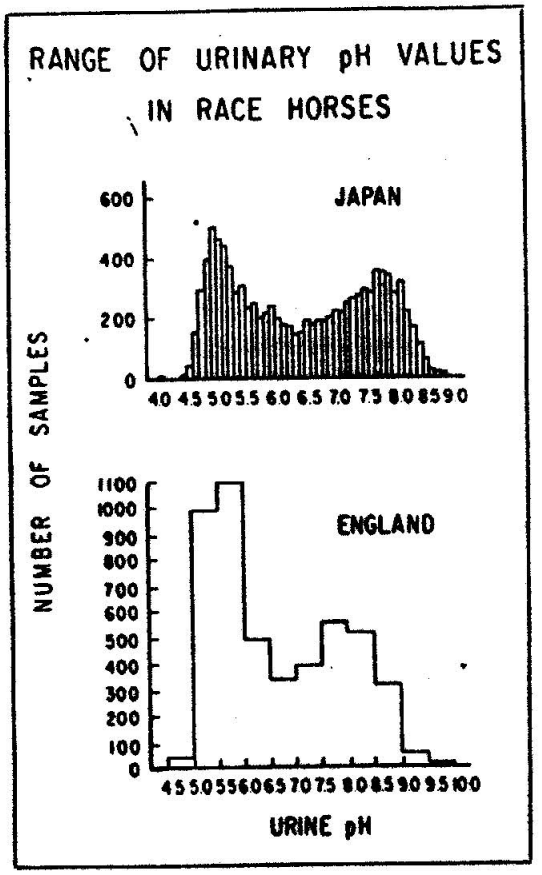

Fig 6. Range of urinary pH values in race horses. The upper graph shows the range of urinary pH values of about 10,000 postrace urine samples from Japan. The lower graph shows the range of pH values from official postrace urine samples in England in 1974.

be detected in urine for 4 days (fentanyl) and 5 days (pentazocine).

A further problem with many drugs is that their concentration in urine depends on urinary pH. Urine samples from horses at English and Japanese race tracks had a pH range from 4.5, which is relatively acidic, to 10.0, which is quite basic (Fig 6).[10,11] In each case, the frequency distribution curve was biphasic, with the greatest number of samples with an acid pH of about 5.0; another peak on the basic side showed a large proportion of equine urine samples with a pH of about 8.0. Both distributions, however, showed that the range of urinary pH values varied 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 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 a drug such as procaine, one can calculate the possible range of procaine concentrations in equine urine using the Henderson-Hasselbach equation.[7] However, because of the urinary pH factor, there is a rather mind-boggling 9000-fold possible range in urinary procaine concentrations given a single, fixed plasma level of the drug (Table 12). This huge range of possible procaine concentrations in horse urine leads this author to the conclusion that estimation of the time of procaine administration (in the context of days) based upon the concentration of the drug in urine is nearly impossible. This same effect presumably holds to a greater or lesser degree for other highly ionizable lipid-soluble drugs in urine, which emphasizes the difficulty in judging dose or time of administration from urinary levels of drugs.[7]

The final problem with urine testing is that the concentration of some drugs in urine can be diluted by the diuretic actions of drugs such as furosemide. For example, the concentration of phenylbutazone in urine can be reduced up to 50-fold within an hour of IV administration of furosemide. This effect also probably occurs with other nonsteroidal anti-inflammatory drugs. Similarly, the diuretic effect of furosemide dilutes urinary concentrations of the glucuronide metabolite of pentazocine up to 50-fold over a similar period; such dilution can transiently interfere with detection of these drugs in equine urine.[12]

Prerace Testing

In prerace testing a blood sample is withdrawn from all horses within 2 hours of the race and analyzed at a trackside laboratory. In the event a forbidden substance is found, extra samples may be drawn and the horse scratched. Prerace blood testing is always performed in conjunction with postrace urine testing. The analytic methods used in prerace testing are generally similar to those used in postrace testing within the time constraints allowed by the prerace testing situation.[8]

Prerace testing is the only mechanism to directly prevent the racing of illegally medicated horses. Testing coverage is equitable because all horses entered are tested. Animals with positive tests are readily available for resampling, further testing or observation. Since illegally medicated horses are prevented from running and winning, there is no incentive to legally challenge the testing process as occurs

Table 12. Accuracy of Different Analytic Methods[12]

Method	Range	Uncertainty (±)	Distinguishable Regions*
Thin-Layer Chromatography	5 cm	0.5 cm	5
Ultraviolet Spectrophotometry	140 nm	5 nm	14
Fluorescence Emission	440 nm	5 nm	44
Fluorescence Excitation	400 nm	5 nm	40
Liquid Chromatography	24 ml	0.4 ml	30
Gas Chromatography	3.2 min	0.16 min	10
Selected Ion Monitoring	3.2 min	0.16 min	10
Mass Spectrometry	360 amu/esu	0.5 amu/esu	360
Infrared Spectrophotometry	3350/cm	10/cm	168
Color Test (Marquis)	350 nm	25 nm	1-7
Crystal Test	see text	—	81

* Distinguishable Regions = Range/ [2 × Uncertainty]

when a large purse is at stake. Finally, prerace testing acts as a highly visible and immediate deterrent to illegal medication and, as such, creates an atmosphere of confidence in the legitimacy of racing.

The principal problems with prerace testing are the technical problems imposed by the use of blood as a test medium. In addition, the short period of 2 hours or less available before a race imposes certain technical limitations on the testing process. Finally, neither the time nor equipment for unequivocal confirmation of the presence of drugs is usually available trackside, although this situation will improve as mass spectrometry technology improves.

Postrace Testing

Postrace testing is usually performed on urine samples collected after a race and optimally on blood and urine samples drawn after a race. The first- and second-place horses are usually sampled, as are commonly beaten favorites or other horses the stewards select. Blood and urine are collected in a postrace holding facility and usually cooled or frozen and shipped to a distant laboratory for analysis.

The same general analytic procedures used in prerace testing are used in postrace testing, as outlined below. The principal difference between pre- and postrace testing is that the time constraints are less compelling and therefore the range of technics available is greater in postrace testing. Adequate time is available for replication of analytic findings and postrace testing laboratories almost always have mass spectrometers for confirmation of positives.

The major advantages of postrace testing are those associated with the relatively large (compared with prerace testing) amounts of time and sample volumes available. The principal problem with postrace testing is the tendency of drugs, such as caffeine, fentanyl, procaine, pemoline, pentazocine and others, to appear in equine urine for many days after administration and long after any pharmacologic effect has dissipated.

Drug Testing Methods

Although testing for drugs in horses has been performed for nearly 70 years, most veterinarians know relatively little about the

methods used and how a chemist concludes a sample is "positive."[1] Because of this, most veterinarians cannot evaluate the evidence analysts develop or advise a client about the quality of such evidence. In this section, the process of drug testing is outlined to help veterinarians assess the evidence on which an analyst may "call a positive."

A positive is called when an analyst reports the presence of a drug that violates a medication rule. An analyst makes such a report when there is sufficient information to unequivocally identify a specific drug or foreign chemical. Partial data, which do not satisfy the analyst that a specific drug is present, are considered "suspicious." A suspicious sample may be upgraded to a positive at any time by the accumulation of more data, depending on the medication rules of the jurisdiction.

It should be clearly understood that analytic methods deemed acceptable in research investigations may be of very limited value in forensic work.[13] Relatively simple analytic methods are often adequate in experimental circumstances in which a known drug is administered, often repeatedly, to a horse under well-controlled conditions. In a forensic situation, however, no control (*ie*, pre- or postadministration) samples are available and samples are drawn from large numbers of horses under widely differing conditions. Horses tested will have been treated with many different drugs, feed additives and domestic remedies, and will have been exposed to many different varieties of plants and chemicals.

Because of the strict qualifying conditions in which forensic testing is performed, scrupulous care must be taken to ensure accuracy, quite apart from the consideration of the personal and professional reputations and livelihoods involved. Because of these considerations, positives should only be called on the strongest scientific grounds.[1,14] One of the purposes of this section is to help the veterinarian differentiate between excellent and perhaps marginal analytic results.

The requirement for accuracy in forensic testing is far more stringent than is generally appreciated by laymen and even some professionals. It is relatively easy to have no "false-positives" in tests on 20 samples using a simple analytic method. Although this suggests the test is accurate at least 20 of 21 times, or about 95% of the time, such an assumption is incorrect. The reason for this is that the incidence of illegal drug use is usually very low. As a general rule, rates of drug detection are much less than 1%; therefore, simply evaluating an analytic method at the 5% level is not very helpful since, if the incidence of drug use is a relatively high 1%, the research base creates the possibility that 4 out of 5 or 80% of the positives were false-positives. Since more usual rates of illegal drug detection are in the area of 1 in 300 to 1 in 1000 of all samples tested, evaluation for accuracy at the 1 in 20 level is worthless as a validating procedure for a forensic method. Because of this problem and the huge number of possible entities with which any agent may be confused, forensic testing has traditionally relied on only the highest quality analytic procedures and has emphasized the use of independent confirmatory tests. In a nutshell, evidence for the presence of a drug in a horse has traditionally been that of unequivocal identification of the drug.

Given satisfactory testing technics, the next problem is that of security of the sample, the so-called "chain of evidence." Biologic samples should be obtained before witnesses and the samples sealed with evidence tape. Samples are then placed in a cooled, secure container and transported to the laboratory as rapidly as possible. Receipt by the laboratory should be recorded in a bound laboratory log on the page dated for that day. The log should record the numbers of the samples received, their approximate volume(s) and any comments on their appearance. Once the sample is opened, the pH of urine samples should be noted and recorded, as pH can be important in determining the amounts of some drugs found in urine.

In addition, the laboratory should have an up-to-date, loose-leaf manual on the testing procedures in use on that particular day since methods in all laboratories change with time, sometimes quite rapidly. As part of this manual, the laboratory should have clear-cut, written criteria set up in advance to differentiate between data points.[13]

If the procedure of the International Olympic Committee is followed, as by many European laboratories, split samples are obtained and sent to the laboratory in bottles labeled "A" and "B." The analysis is started on the A sample and the B sample is frozen. In the event of a "positive," the B sample is available for confirmatory analysis, either in the presence of a referee or by an independent laboratory. This procedure safeguards the interests of the owners; in many European laboratories, analysis is not started in the absence of a referee's sam-

ple. This system has proven its worth in Europe because at least one "positive" called in 1978 was not confirmed by an unquestionably competent refereeing laboratory in another country. Unfortunately, many jurisdictions in the US do not require a referee sample and it is very easy to dispose of or allow to deteriorate the remains of a test sample, and with it the only possibility of checking the analytic work on which the "positive" was called.[1]

Analytic Methods

Different testing procedures provide varying kinds of evidence about the presence of a drug. The first sign of the presence of a drug indicates a "suspicious sample." As more evidence is accumulated, the analyst must ask himself how good or useful the evidence is and at what point a "positive" should be called. The general procedure in drug analysis is outlined in Figure 7.

Ultraviolet Spectrometry: In ultraviolet (UV) spectrometry, light of shorter and shorter wave lengths is directed through the drug solution. If a drug is present in sufficient concentration and absorbs UV light, the instrument plots a graph of the drug's absorbance of light at each wavelength. A typical UV absorbance spectrum for flunixin in urine is shown in Figure 8. From the shape of the curve and the wavelengths at which the peaks occur, the analyst may suspect the presence of a certain drug. The pH of the system is then changed and specific changes in the shape of the absorbance curve tentatively identify a particular drug.[14]

There are 2 principal problems with UV data as a basis for drug identification. The first is that many compounds share broadly similar UV absorption spectra, so the test cannot distinguish barbiturates from other compounds. Since not all barbiturates are pharmacologically active, the method cannot reliably identify barbiturates. Similarly, amphetamine, atropine and fentanyl have broadly similar UV absorption curves despite their very different structures and pharmacologic action.

The second problem is that the portion of the UV spectrum used by analysts is only about 200 nm wide, which means there are at most about 200 different values for UV absorption peaks. If only about 20% of the compounds absorb in the UV, there are about 4000 different compounds for each UV peak. The problem is further complicated by the fact that extracts of horse urine contain unknown compounds that also absorb UV light. Because no drug-free sample is available, it is not possible to run matched controls. For these reasons, UV data may suggest the presence of a compound but cannot positively identify it. The method is therefore considered a screening technic by most forensic experts.[14]

Thin-Layer Chromatography: Thin-layer chromatography (TLC) is a useful screening technic that derives its name from the fact that the experiment is performed in a thin layer of silica gel or other absorbent coated on a glass or metal plate. The drug extract is spotted about 1 cm from the edge of a plate stood on edge in a solvent (Fig 9). As the solvent migrates up the plate, the compounds in the spot move along the plate at varying speeds, depending on their affinity for either the solvent or the gel.

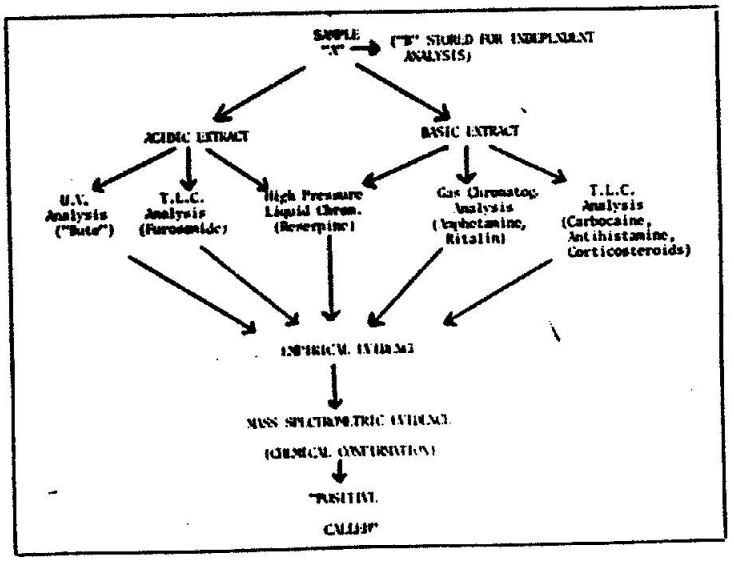

Fig 7. General flow-sheet for drug analysis.

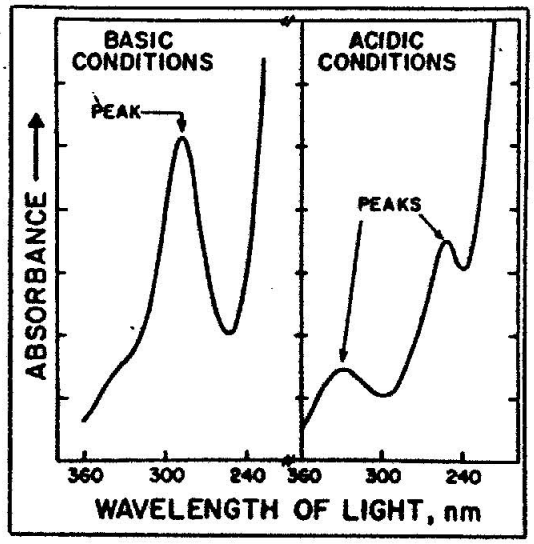

Fig 8. UV absorbance pattern of flunixin. The right-hand panel shows 2 absorbance peaks for flunixin at 250 nm and 330 nm under acidic conditions, while the left-hand panel shows a single peak at 290 nm under basic conditions.

When the presence of a drug is suspected, the analyst makes an educated guess as to which drug it might be. The experiment is then performed again with the identified control beside the unknown. If the spots clearly do not migrate the same distance, the control drug and the unknown are not the same substance. If the spots migrate together (within the somewhat elastic limits of experimental error), they may be but are not necessarily the same substance.

The TLC method cannot definitively identify a drug because of the large number of drugs that have the same migration patterns. The maximum number of spots one can separate physically on a TLC plate is about 20. With 4.2 million chemical candidates, one has about 210,000 possible chemicals for each spot. A 99% specific color or other marker reaction would reduce this number 100-fold and leave "only" 2000 possible candidates for each spot. "Specific" extraction conditions might reduce this number even further, but a considerable probability of overlap in TLC systems still exists. The TLC method simply cannot generate a specific identification. Similarly, high-performance TLC (HPTLC), which under optimal conditions is only about twice as accurate as standard TLC, is also unable to generate specific identification data.

Since most forensic chemists are aware of the lack of specificity of TLC, they usually compare the control drug and the unknown in a number of different TLC systems. If both spots again migrate the same distance, the chances that the analyst's guess is correct are improved. In an experimental test of this procedure, 138 drugs were tested. The investigators were unable to separate 25 of these drugs in 4 TLC solvent systems; in experiments with 7 TLC systems, overlaps were still found. A reasonable conclusion is that the number of solvent systems required to separate pure solutions of just the 4000 drugs in common use without risk of overlap is astronomic.

It is also important to remember that the 20% overlap rate in the above experiment was found with pure solutions of single drugs.[15] Under realistic, nonexperimental conditions, using multiple TLC systems for drug identification may result in "partial" false-positives. A partial false-positive occurs due to the coincidence of 2 different substances in a plasma sample, neither of which alone could give rise to a false-positive in the 4 TLC systems used. Therefore,

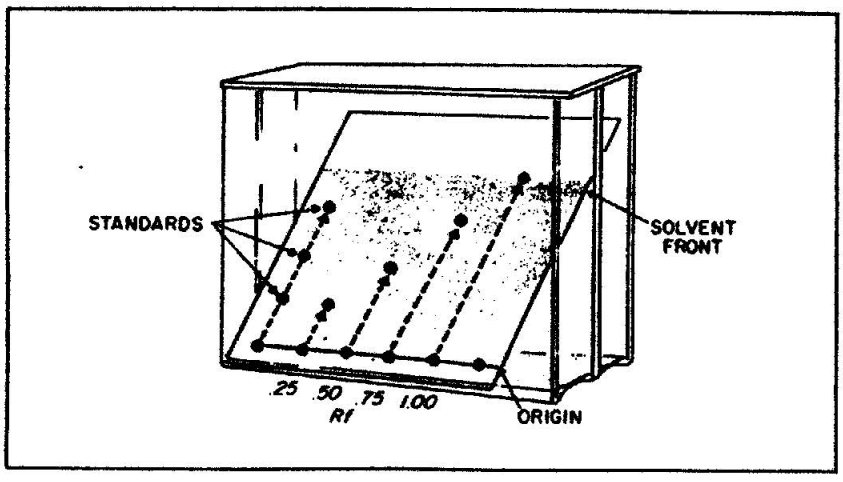

Fig 9. Thin-layer chromatography. Drug standards and unknowns are spotted at the origin and the plate stood on end in the solvent. The fraction of the distance migrated by the drug in comparison with the distance migrated by the solvent front is the Rf of the drug.

multiple TLC system analysis of biologic samples cannot be considered specific for even a subclass of chemicals run with the standard in 4 systems because there is no way to tell if the 4 test spots from a blood or urine sample are due to 1 or 4 substances. In its current form, TLC readily proves the absence of a drug but cannot, even with the use of multiple systems, prove its presence. It is consequently not considered a specific test by forensic authorities.

Gas Chromatography: Gas chromatography (GC) functions on the same principle as TLC but uses a gas rather than a liquid solvent. Because the substance to be chromatographed must be volatile, GC analysis is restricted to the approximately 400,000 volatile chemicals and perhaps another 400,000 that can be volatilized after appropriate treatment.

Gas chromatography is like TLC in that some drugs flow along with the gas and some stick to the column near the origin and never come out. However, if the appropriate column pack, gas, temperature and gas flow are used, drugs can be separated by GC.

As with TLC, comparison of the GC migration pattern of the unknown with that of a known standard aids identification of a drug. Because of the limited time available to most drug detection laboratories, especially prerace laboratories, short GC columns (1-2 meters) with short retention times are used. With these systems, one cannot separate more than about 100 compounds. Since there are about 800,000 compounds that can be volatilized, there are 8000 possible candidates for each peak. The usual procedure at this point is to further test the unknown by changing the column temperature once or twice to see if the material continues to adhere to the pattern of the standard. Then the pair (suspect and standard) are run on a different GC column at 3 different temperatures. If the unknown and standard continue to chromatograph together, many analysts conclude that they have identified a drug.

A helpful test, unfortunately rarely used, is to mix an equivalent amount of the suspect and authentic material, and chromatograph them together. If they are the same substance, the suspect peak doubles in size, remains symmetric, and has no tendency to "split" regardless of the chromatographic conditions.

Because GC only produces a small number of data points at best indirectly related to overall chemical structure, one cannot conclude from GC that one has identified a drug. All that can be concluded is that the unknown and the authentic compound are indistinguishable in the systems in which they were compared. While GC is usually considered more accurate than TLC, just how accurate is not clear.

Gas Chromatography-Mass Spectrometry: In gas chromatography-mass spectrometry (GC-MS), the unknown materials forming the peaks in the gas chromatograph are fed directly into a mass spectrometer, which functions as a detector for the gas chromatograph. As well as simply detecting the peak, the mass spectrometer measures the substance's molecular weight and determines its fragmentation pattern. This produces what is sometimes called a molecular "fingerprint" for each drug and is considered among the best evidence available as to the identity of a drug. In addition, the mass spectrometer can detect nanogram quantities of drugs and as such is sufficiently sensitive for drug detection in body fluids of horses.

High-Performance Liquid Chromatography: High-performance liquid chromatography (HPLC) is similar to GC but uses a liquid under very high pressure. Because liquid solvents are available for most chemical compounds, the potential field of application of HPLC is much wider than that for GC, which requires volatile compounds. Further, the theoretic efficiency of HPLC is very much higher than that of GC, although HPLC columns do not appear to withstand the contamination problems of routine screening of biologic samples as well as those used in GC. However, HPLC is only beginning to be used in routine drug testing.

Radioimmunoassay: In radioimmunoassay (RIA), a specific antibody to a drug molecule binds both a radiolabeled drug and whatever unlabeled drug may be added to the unknown plasma or urine sample. If a significant amount of nonradioactive drug is added to the system, the measurable radioactivity in the system is reduced. Given a supply of the specific antibody, RIA is rapid, inexpensive, sensitive and sufficiently specific to make a good screening test. However, since only a poorly defined portion of the drug binds to and interacts with the antibody, RIA is not a specific chemical test.

Microcrystal Test: The microcrystal test is one of the oldest, simplest and most sensitive tests used in analytic and forensic toxicology. In this test, the suspected drug interacts with a reagent to yield crystals of a shape and color characteristic for that drug. If microcrystal evidence is used, more than one microcrystal test should be run and color photographs of

both the standard and unknown microcrystals made for use in court.

Identification of microcrystals is usually based on their shape and color. However, another simple test rarely performed is the melting-point test. Since every crystal has a specific melting point, the melting point of crystals of a standard, the unknown, and a mixture of the standard and the unknown adds further evidence of identity. However, for reasons which remain unclear, melting-point tests on crystals are rarely done.

"Calling a Positive"

The average analyst has about 200 standards with which he compares any suspect peaks or spots in test results. Because the 200 standards cover many of the drugs commonly used on race horses, standards are often successfully matched with suspect drugs. A GC-MS with a computerized library provides access to about 40,000 chemical spectra, which improves the scope of the search 200-fold.

At some point in this matching procedure, the analyst may decide to call a positive when certain analytic criteria are met for the presence of a prohibited drug. In some jurisdictions these criteria are explicitly stated. For example, to call a positive in Canadian racing, the analyst must present evidence of identity in 3 distinct analytic systems. The Association of Official Racing Chemists also has a policy that a positive should not be called without, in general, a minimum of 3 independent analytic methods and an absolute recommendation that 2 be the minimum number of independent tests employed to call a positive.

While some American drug testing laboratories have an unwritten policy of not calling a positive on fewer than 3 independent analytic methods, most US racing jurisdictions do not have any explicit statements on what the analytic requirements for positive identification of a drug are. In these jurisdictions, the criteria on which the positives are called are the analyst's own. While this sytem allows the analyst considerable flexibility, it can also leave him open to pressures to call positives against his better judgement.

The analyst usually has various evidence generated by different methods, and the methods used and thus the quality of the evidence on which the identification is based varies from drug to drug. To accurately quantitate the information yielded from each analytic method, a process has been devised to produce a forensically defensible statement on the overall weight of the analytic data.[13] As illustrated by Table 3, GC-MS provides the most convincing data and is the method usually considered specific by most drug testing laboratories.

A very real problem arises in calling positives when GC-MS data are not available for economic or technical reasons. If GC-MS data are not available, positives called on data from TLC, GC or other empiric methods have a distinct probability of error. Data from the Cornell University Drug Testing Program, which has the most experience with GC-MS confirmation of analytic data, suggest that up to 10% of drug identifications obtained with TLC and GC are incorrect when tested by GC-MS. Analysts who call positives in the absence of GC-MS data should keep such estimated error levels in mind and only call positives on the very best of empiric evidence.

Drugs and Performance

The question of the effect of stimulants on performance in horses remains unanswered. A clear, unequivocal and statistically sound demonstration of an action of central-stimulating drugs to improve performance in horses under racing conditions has yet to be published.[2,16,17] This has been largely due to the small number of animals used in these studies, which, combined with the day-to-day variability inherent to these studies, renders their successful conclusion difficult. A study of the actions of amphetamine in horses galloped 900-1600 meters revealed improved speed in each of 5 trials.[17] The mean improvement in speed was only 2.5%, which was not considered statistically significant. It is worth remembering, however, that a 1% improvement in performance is a tremendous improvement for an athlete, and athletes train for months to improve their performance by only a 1% margin.

In other studies, methamphetamine increased speed at all paces tested; however, in gallop tests the effect was variable.[16,18] Similarly, the stimulant actions of caffeine have been studied.[16,18,19] Injection of 2.5-5 g (5-10 mg/kg) caffeine and sodium benzoate enhanced the running performance of all horses, especially at a canter.[19] Caffeine given at 3-8 mg/kg increased speed significantly in gallop tests.[18] However, short gallop tests were used in the latter study to demonstrate the stimulant effects of these drugs; the significance of this effect under racing conditions remains unclear.

The stimulants that have recently drawn most attention are the narcotic analgesic, fentanyl (Sublimaze: McNeil), and the dopaminergic stimulant, apomorphine. Both of these drugs are potent locomotor stimulants in horses. The effect of fentanyl and apomorphine on locomotor activity in horses in box stalls was studied.[3] The number of steps taken in a 2 minute period was counted. After saline injection, animals averaged about 4 steps/2 minutes. Fentanyl injection resulted in a 25-fold increase to about 100 steps/2 minutes; apomorphine injection increased activity up to 160 steps/2 minutes. These experiments clearly show that fentanyl and apomorphine are potent, rapid-acting locomotor stimulants in horses.[3]

Irrespective of the effect of these drugs on the racing performance of horses, they all stimulate a running response at low doses, but cause incoordination at high doses. Because of the stimulation and apparent apprehension observed in animals given these drugs, which may make them more difficult to control, the use of these drugs in racing animals is not easily justified.[3]

Another area of controversy concerns the actions of nonsteroid anti-inflammatory drugs that supposedly restore normal performance.[4] These drugs do not generally improve performance in horses. In one series of experiments, phenylbutazone appeared to improve performance by relieving subclinical lameness rather than stimulating the horses.[18,20] The Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners concluded that phenylbutazone does not change the innate ability of a horse to race but, by relieving inflammation, may enable the animal to race nearer its maximum capability.[4] Recent experiments from our laboratory revealed no effect of phenylbutazone on fentanyl-stimulated trotting. These results refute suggestions that phenylbutazone in usual doses stimulates or depresses horses.[23]

Another question about phenylbutazone is its effect on the incidence of breakdowns. The Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners concluded that, on the basis of data gathered in California and Colorado, there is no evidence that the percentage of horses seriously injured or requiring destruction is any greater for horses racing on phenylbutazone than for those not given the drug. Further, the Committee was careful to point out that the incidence of serious injuries to horses racing in those states has not increased since the use of phenylbutazone was permitted, and emphasized that careful prerace clinical examinations and good control of medication are the most important parts of a successful controlled medication program.[4]

Regarding the short-term toxicity of phenylbutazone, the Veterinary Chemists Advisory Committee concluded there are minimal toxicity problems with phenylbutazone when it is used in usual doses.[4] However, some researchers have presented evidence that some horses given large doses of phenylbutazone for long periods develop serious and potentially fatal toxicities.[21,23,24]

In preliminary studies on the toxicity of phenylbutazone, severe side-effects occurred when the drug was given IV at 7.5 mg/kg daily for 8 days in a study to test for enzyme induction.[21] Clinical signs included complete anorexia, depression, moderate fever and greatly impaired liver function. Of the 2 horses that showed signs of toxicity, one developed peritonitis subsequent to a liver biopsy and was euthanized, and the other recovered after the drug was withdrawn. A third horse apparently had no problems. All horses in this study had parallel hematologic changes, the total WBC count dropping after the third day of drug administration; 2 horses became markedly leukopenic. The plasma half-life of phenylbutazone more than doubled and excretion of the drug was reduced to the extent that phenylbutazone was found in the urine of a healthy horse 14 days after the experiment.

Reviewing these results, the investigators concluded that the toxicity from long-term treatment with phenylbutazone may have been underestimated in the past. They note that if phenylbutazone is given in the food, the resulting anorexia automatically restricts intake of the drug. However, if the drug is given parenterally, it continues to accumulate and exert its toxic effects. They recommend, therefore, that horses on long-term phenylbutazone therapy be monitored carefully for signs of phenylbutazone toxicity, manifested as a febrile enteritis, leukopenia and anorexia, and that treatment be stopped at the onset of such signs.[21]

Another drug commonly permitted in controlled medication programs is furosemide. The justification for the use of furosemide is that it reportedly prevents epistaxis when used before a race.[25] Epistaxis is classically defined as bleeding from the nostrils. Although the in-

cidence of postrace epistaxis is not more than 2%, recent research has shown that 40% of horses have blood in the tracheobronchial tree postrace. The 2% incidence of epistaxis therefore represents the tip of an iceberg of an approximate 40% incidence of postrace tracheobronchial hemorrhage.[25]

In some racing jurisdictions, up to 80% of all horses running are given furosemide. One possible reason for this widespread use of furosemide is the belief that the drug improves performance. However, research results do not support this belief. Time trials on horses given and not given furosemide showed no improved performance from furosemide administration.[26] Similarly, a retrospective analysis of the times to pace one mile of horses racing with and without furosemide at the Louisville Downs meet in the summer of 1977 showed no improvement after furosemide use.[26]

Considering these facts, the conclusion at this time is that neither furosemide nor phenylbutazone, the 2 most widely used drugs in controlled medication schemes, improves the performance of horses under racing conditions.

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