376 part 1

CORRUPT HORSERACING PRACTICES

HEARINGS

BEFORE THE

SUBCOMMITTEE ON CRIMINAL JUSTICE

OF THE

COMMITTEE ON THE JUDICIARY

HOUSE OF REPRESENTATIVES

NINETY-EIGHTH CONGRESS

FIRST SESSION

ON

CORRUPT HORSERACING PRACTICES

APRIL 27 AND MAY 18, 1983

Serial No. 156

Printed for the use of the Committee on the Judiciary

U.S. GOVERNMENT PRINTING OFFICE

43-815 O WASHINGTON :

CORRUPT HORSERACING PRACTICES

HEARINGS

SUBCOMMITTEE ON CRIMINAL JUSTICE

COMMITTEE ON THE JUDICIARY
HOUSE OF REPRESENTATIVES

Serial No. 156

COMMITTEE ON THE JUDICIARY

PETER W. RODINO, JR., New Jersey, *Chairman*

JACK BROOKS, Texas
ROBERT W. KASTENMEIER, Wisconsin
DON EDWARDS, California
JOHN CONYERS, JR., Michigan
JOHN F. SEIBERLING, Ohio
ROMANO L. MAZZOLI, Kentucky
WILLIAM J. HUGHES, New Jersey
SAM B. HALL, JR., Texas
MIKE SYNAR, Oklahoma
PATRICIA SCHROEDER, Colorado
DAN GLICKMAN, Kansas
HAROLD WASHINGTON, Illinois
BARNEY FRANK, Massachusetts
GEO. W. CROCKETT, JR., Michigan
CHARLES E. SCHUMER, New York
BRUCE A. MORRISON, Connecticut
EDWARD F. FEIGHAN, Ohio
LAWRENCE J. SMITH, Florida
HOWARD L. BERMAN, California

HAMILTON FISH, JR., New York
CARLOS J. MOORHEAD, California
HENRY J. HYDE, Illinois
THOMAS N. KINDNESS, Ohio
HAROLD S. SAWYER, Michigan
DAN LUNGREN, California
F. JAMES SENSENBRENNER, JR., Wisconsin
BILL McCOLLUM, Florida
E. CLAY SHAW, JR., Florida
GEORGE W. GEKAS, Pennsylvania
MICHAEL DEWINE, Ohio

ALAN A. PARKER, *General Counsel*
GARNER J. CLINE, *Staff Director*
FRANKLIN G. POLK, *Associate Counsel*

SUBCOMMITTEE ON CRIMINAL JUSTICE

JOHN CONYERS, JR., Michigan, *Chairman*

DON EDWARDS, California
JOHN F. SEIBERLING, Ohio
HOWARD L. BERMAN, California

GEORGE W. GEKAS, Pennsylvania
BILL McCOLLUM, Florida
MICHAEL DEWINE, OHIO

THOMAS W. HUTCHISON, *Counsel*
MICHAEL E. WARD, *Assistant Counsel*
GAIL E. BOWMAN, *Assistant Counsel*
RONALD A. STROMAN, *Assistant Counsel*
RAYMOND V. SMIETANKA, *Associate Counsel*
BENNIE B. WILLIAMS, *Clerk*
ANNE I. WEST, *Clerk*
CHERYL REYNOLDS, *Clerk*

CONTENTS

HEARINGS HELD

WITNESSES

ADDITIONAL MATERIAL

Delegate Paul Weisengoff says it's tough to get everybody in the industry to agree on a reform package.

DEL. PAUL WEISENGOFF: "You've got really three partners in the horse racing industry...you've got the horsemen, the tracks, owners and the state. The horsemen feel they should get a bigger share. The track owners say they're not getting enough."

SEN. JULIAN LAPIDES: "There's not an unselfish person in the entire industry. They're all out for the buck."

State senator Julian Lapides says the racing industry doesn't have many friends in the legislature because of its history of greed and corruption.

SEN. JULIAN LAPIDES: "I'm not really sympathetic to the racing industry in Maryland. I guess I remember '72, the scandal that lead to the downfall of Governor Mandel. I remember that too well...If this is the sport of kings...I think royalty has ended in Maryland."

Horse racing is an important part of Maryland's heritage and economy. People we talked with on the farms, the backstretch, on the tracks itself say the state needs to make a commitment to the industry to help it get back on its feet. To help make Maryland racing a winner once again.

This January a number of racing reform bills are expected to be introduced in the legislature. Some will deal with the abuses the I-Team documented, ranging from drug use to conflicts of interest.

In the months to come, we'll keep you posted on what happens to that legislation.

We will have a recess within a recess, so the gentleman can remove the apparatus from the table, and then we will go on with the hearing. So please don't anyone move.

[Recess.]

Mr. GEKAS. We call to the table the next scheduled witness, or group of witnesses. First of all, Caldwell Butler, former Member of the House of Representatives; David Vienna; and Dr. Thomas Tobin.

We understand that former Congressman Butler, who is now acting as special counsel to the Horsemen's Benevolent & Protective Association, is heading this team that will be presenting testimony to us; while David Vienna is president of David Vienna & Associates, a local group representing the horsemen's association with whom Representative Butler is associated. And from what we have in front of us, we note Mr. Vienna has worked as a reporter, as a professional staff member of the U.S. Senate Subcommittee on Executive Organization, and an investigator for the Senate Permanent Subcommittee on Investigation.

Dr. Tobin is a professor of Veterinary Science and Toxicology at the University of Kentucky. Dr. Tobin has the most imposing list of publications to his credit, and is engaged in extensive consulting in equine forensic pharmacology and toxicology.

Gentlemen, welcome. You may proceed at your discretion, with whichever one wants to take the lead to so proceed.

TESTIMONY OF CALDWELL BUTLER, SPECIAL COUNSEL, HORSEMEN'S BENEVOLENT & PROTECTIVE ASSOCIATION, DAVID VIENNA, PRESIDENT, VIENNA & ASSOCIATES, AND DR. THOMAS TOBIN, PROFESSOR OF VETERINARY SCIENCE AND TOXICOLOGY, UNIVERSITY OF KENTUCKY

Mr. BUTLER. Thank you, Mr. Chairman. If you don't mind, I will go first.

Mr. GEKAS. Proceed.

Mr. BUTLER. I am a practicing attorney, as you mentioned. I have recently been employed as the special counsel for the Horsemen's Benevolent & Protective Association. There is earlier testimony in the record on behalf of this association, on September 30, 1982. This organization is comprised of 50,000 owners and 5,000 trainers of thoroughbred racing horses. They are the persons upon whom this legislation could impact most directly.

You correctly identified Dr. Tobin as being here, and his credentials in the field. I want to emphasize that Dr. Tobin is appearing at his own expense, he is receiving no remuneration for his time or expenses from the Horsemen's Benevolent & Protective Association, and he is here at the invitation of the subcommittee.

Mr. Vienna is the Washington representative of the Horsemen's Benevolent & Protective Association, and you correctly identify his other credentials.

We would like to say at the outset that we appreciate the fact that the chairman is having these hearings. It would be nice if he could be here. They are serving a very useful purpose in providing the industry with a reason for careful self-examination and the impetus for the various associations and individuals who are part of this industry to work together and to resolve their differences. We are also particularly grateful that the chairman has afforded this association another opportunity to present its views. We appreciate the continuing interest in having a full and complete record.

In preparation for this hearing, we undertook to review the testimony earlier adduced to see what we could consider appropriate to provide a balanced record. It's apparent that the proponents of this legislation believe that the drugging and numbing of racehorses is rampant in the country; that States are either unable or unwilling to control these practices; and that additional Federal legislation is indicated now.

However, we are inclined to the view, from our examination of the record of the hearings, that no additional Federal legislation is indicated at this time. The testimony and the evidence we will present today will be directed toward establishing that the alleged scientific information and the information regarding medication-induced effects which have been provided to this subcommittee is out of date, contradicted by reputable scientists as well as Government agencies; that the testimony with regard to the conduct of State regulatory agencies is not altogether accurate; and we would endeavor to supply for the hearing record additional evidence of the circumstances surrounding the death of a jockey at Pimlico in 1978 so that your record in this regard will be more balanced.

We will share with you our perception of the cost of this legislation to the Department of Justice and the impact that this will

have on its budget. We will also share with you the evidence which we have been able to find concerning the racehorse medication, detection and enforcement programs in the five States of California, Florida, Michigan, Ohio, and Pennsylvania, being the jurisdictions from which the members of this subcommittee come.

And finally, we will present our views on the impact of this program on our criminal justice system generally.

Please understand, it is not our purpose to impeach the integrity of any witness or to impugn their motives, but we do think it important that the record be complete and accurate to the extent possible. We view it as our responsibility to assist this subcommittee in forming its judgment by endeavoring to provide a complete and objective record.

There are several questions which must be answered in the affirmative before the subcommittee can in good conscience recommend H.R. 1694 to the full committee:

First, is the administration of stimulants, depressants and narcotics for the purpose of influencing the outcome of horseraces going undetected and unpunished in such an amount or to such a degree that additional Federal legislation is required now?

Does there exist sufficient agreement among experts as to the detrimental effects of therapeutic medications on the racing animal as to justify criminal prosecutions for their use?

And even if the answers to the above questions are in the affirmative, does this justify the diversion of expenditures of $61 million annually and the diversion of more than 150 narcotics agents from the primary missions of the Drug Enforcement Administration?

As will appear from the earlier hearing record, this association believes that it is wrong to administer medication to a horse for the purpose of influencing the outcome of a race, but it believes that it is most appropriate and humane to administer medication to animals for their own therapy.

At this point, Mr. Chairman, we would like to give the subcommittee an opportunity to hear from Mr. Vienna and Dr. Tobin.

Mr. Vienna has provided us with an extensive analysis of the testimony which is already in the record. This is the basis of the bound volume marked "Testimony on H.R. 1694" which is now before you. This is the bound volume. We ask that it be printed as a part of the record of this hearing. May we have that, Mr. Chairman? May this be printed as a part of the record of this hearing?

Mr. GEKAS. Without objection.

[The information referred to follows:]

Testimony on H.R. 1694

of the

HORSEMEN'S BENEVOLENT

&

PROTECTIVE ASSOCIATION

before the

HOUSE CRIMINAL JUSTICE SUBCOMMITTEE

May 18, 1983

ANALYSES OF THE TESTIMONY OF FOUR PROPONENTS
OF THE CORRUPT HORSE RACING PRACTICES ACT
BEFORE THE SUBCOMMITTEE ON CRIMINAL JUSTICE

In response to the Chairman's request that documentary evidence of facts that would mitigate or contradict the testimony previously provided to the Subcommittee be included with any statement, the Horsemen's Benevolent & Protective Association has analyzed the testimony of four of the major witnesses in favor of the Corrupt Horse Racing Practices Act. These witnesses were selected because their testimony contained the most compelling statements in support of the legislation. Exhibits with documentary evidence are cited and are contained within the tabs that immediately follow the text of the four analyses.

The following is the table of contents for the analyses:

ANALYSIS OF THE TESTIMONY OF ROBERT O. BAKER BEFORE THE HOUSE CRIMINAL JUSTICE SUBCOMMITTEE ON SEPTEMBER 30, 1982

Mr. Robert O. Baker, a field investigator for the Humane Society of the United States, testified on September 30, 1982 with regard to Phenylbutazone:

> The most flagrant abuse of drugs on the race tracks at the present time is their utilization to enable sore, injured or even lame horses to compete in spite of their infirmaties. The most commonly used drugs for this purpose are anti-inflammatory analgesic drugs such as Butazolidin...and the steroid cortisone type drugs. These classes of drugs provide symptomatic relief only. They suppress inflammation and heat of an injury, they reduce swelling and pain, but they do not cure the underlying pathological condition of that injury. Thus, these drugs should normally only be used in conjunction with rest.
>
> Unfortunately, these pain-killing drugs are permitted to be administered at race time by numerous racing jurisdictions throughout the country, and are being used to mask a horse's pain, thus enabling the horse to run full out on an injured leg. This practice nearly always aggravates injuries and often an injured leg shatters under the stress of racing, causing dangerous spills in which horses are crippled and must be destroyed. [Transcript pages 68 and 69]

ANALYSIS

Phenylbutazone is a member of the "non-steroidal anti-inflammatory" (NSAID) group of drugs, and is the member of this group most commonly used in horses. The principal drug of this group is aspirin. Aspirin is so readily and easily available that most people do not think of it as a drug. It is, in fact, the original member of the phenylbutazone group of drugs, and produces much the same effect in man as phenylbutazone does in the horse. If you wish to keep phenylbutazone in the horse in perspective, it is useful to think of it as being basically just horse aspirin.

Phenylbutazone is not a pain-killing drug. What it does affect, however, is pain associated with inflammatory responses. Chemicals called prostaglandins are synthesized in inflamed tissues and act to hypersensitize inflamed areas to pain. Anybody who has had anything as simple as a sunburn is familiar with the problem of hypersensitivity to pain in sunburned skin. What drugs like aspirin and phenylbutazone do is act to block the synthesis of prostanglandins and therefore reduce the hypersensitivity of the inflamed tissue to pain, eventually normalizing inflamed tissue. It is important to remember that these drugs have essentially no effect on anything other than inflamed tissues, any more than aspirin that you take for a headache will numb your hands or legs.

Phenylbutazone does not stimulate horses --- no more than aspirin stimulants man. While phenylbutazone will enable a horse with musculo-skeletal problems to perform up to his natural or "innate" ability, it is not considered to have any stimulant action or to enable a horse to "excel" himself. Phenylbutazone is no more capable of stimulating an exceptional or unusual performance in the horse than aspirin is in man.

Phenylbutazone does not "numb" horses. It is important to distinguish between the actions of numbing drugs such as local anesthetics and phenylbutazone. Local anesthetics are injected into a nerve or joint, and "numb" a specific area. Such treatment blocks both pain perception (numbing) and what is called "proprioception." Since proprioception is in fact the horse's awareness of where his leg is and what it is doing, a horse treated with a local anesthetic loses much more than simple pain perception. Because of the loss of proprioception with local anesthetics, the horse is not quite sure of where his leg is, and his risk of a misstep and breakdown is thought to be increased. It is important to remember that phenylbutazone does not affect normal pain or proprioception in an area, and its actions are therefore completely different from the local anesthetics.

Phenylbutazone has no narcotic actions. Phenylbutazone is quite different from the narcotic analgesics, which act to reduce pain perception in the brain. They do this by dissociating an individual emotionally from pain and also by inducing euphoria. Beyond this, the narcotic analgesics or morphine-like drugs also stimulate running activity in the horse. As one might expect, phenylbutazone - an aspirin-like drug - has none of these effects; phenylbutazone does not suppress pain perception in the brain or induce euphoria or running behavior.

Sources for this analysis are contained as Exhibit 1, in the like numbered tab section. Articles included are:

1. Goodman, et al. "The Pharmacological Basis of Therapeutics," in Drug Therapy of Inflammation, page 684 and others. McMillan Publishing Co., New York. 1980.

2. Equine Medicine and Surgery, Mansmann, McAllister & Pratt, Eds Anti-inflammatory Drugs, By F.W. Jones, page 156 and others. American Veterinary Publications, Drawer KK, Santa Barbara, CA. 1982.

3. Tobin, T. Drugs and the Performance Horse, Charles C. Thomas, Springfield, IL, 62717. 1981

4. Tobin, T. "Phenylbutazone: ITS Actions, Effects and Role in Equine Medicine," Modern Equine Medicine. Jan-Feb 1983.

Mr. Baker testified that after phenylbutazone was approved for use after 1975 in the State of West Virginia, the number of horses sustaining injuries serious enough to be destroyed increased 114 per cent at Waterford Park, according to a report by Caroline Gall, D.V.M. [Transcript page 69]

ANALYSIS

The data from Waterford Park is not consistent with studies from other states.

California legalized phenylbutazone at the end of 1970. Between 1970 and 1978, during which period phenylbutazone was legalized for use in racing horses, and during which period the percentage of horses racing on phenylbutazone steadily increased, the number of horses suffering breakdowns which required destruction of the horse remained steady at about 100 per year over most of the decade of the 1970s. In actual fact, one can calculate this data to show that as the usage of phenylbutazone went up, the incidence of breakdowns remained about the same. Far from the use of phenylbutazone increasing the incidence of breakdowns, there appears from the California data to be a negative correlation between phenylbutazone use and breakdowns.

This data from California is supported by studies from Colorado. These records were kept by Dr. Gene Bierhaus of the Colorado Racing Commission and they show that over nine years, the probability of a horse having an accident which required his destruction was not any greater if the horse was treated with phenylbutazone. Doctor Bierhaus concluded on the basis of his records that in his experience, use of phenylbutazone in Colorado was not associated with an increased incidence of breakdown.

In another study carried out by workers from the University of Pennsylvania at the Pennsylvania National Racetrack, it was found that the risk of breakdowns was about 1 per 206 horses at-risk. Reporting this data to the Jockey Club Round Table, it was pointed out that "this breakdown rate was not higher than the incidence of injuries occurring at tracks in New Jersey and New York, which have restrictions on the use of phenylbutazone."

Thus, three studies contrast with the reported study of Doctor Gall, which, to our knowledge, has never been published.

Exhibit 2 contains the California and Pennsylvania studies.

Mr. Baker testified [Transcript pages 69-71] that textbooks entitled Equine Medicine and Surgery, Lameness in Horses, Biomechanics of Lameness in Horses as well as the Merck Veterinary Manual and a report published in the Journal of American Veterinary Association say that horses should not be trained while being given anti-imflammatory drugs such as phenylbutazone. Specifically, he testified:

> The textbook Equine Medicine and Surgery, which is the principal textbook in equine medicine used by the veterinary schools in this country, states in the chapter on the musculo-skeletal system: 'The horse should not be trained while being given anti-inflammatory drugs because it will not protect the leg as it would if pain were experienced normally'

ANALYSIS

We believe Mr. Baker may have cited texts published in the early 1970's and thus based on experience gathered in the 1960's, before controlled medication programs became widespread. The second edition of Equine Medicine and Surgery, referenced by Mr. Baker, was published in 1972, with a single section on the musculo-skeletal system. The third edition, published in 1982, has a section on the musculo system and another on the skeletal system. In addition, the 1982 third edition has a section on non-steroidal anti-inflammatory drugs, which contains a statement that appears to contradict the section he quotes. In the new edition is the following statement:

> These products may also be used prophylactically since they do not impair healing or immunity. They are useful to control post-surgical pain and swelling, and may prevent soreness and injury associated with training. Phenylbutazone administered 23 hours before time trials has improved performance, perhaps

> due to the relief of subclinical lameness. In a controlled study, naproxen prevented training complications. Intermittent (2-3 times weekly) administration of similar products resulted in apparent clinical benefit.

In essence, the author states that these agents prevent soreness and injury associated with training.

In other work, Dr. D. Hamm has shown that when young Quarter Horses in training and racing were treated with Naproxen, another non-steroidal anti-imflammatory drug, there were less interruptions of training and racing in the NSAID-treated group.

For example, during the training season, the untreated group lost about 13% of their training time due to musculo-skeletal disorders, while the Naproxen-treated horses lost only about 3% of their time. More dramatically, when these horses went on to racing, the proportion of days lost to racing in the control group was 41%, much greated than the 6% of racing time lost in the Naproxen-treated group. In agreement with these observations, the horse treated continuously with Naproxen also raced more significantly that the untreated horses.

The author concluded that continuous treatment of horses with Naproxen during the first four months of training appears beneficial. The treated animals showed less musculoskeletal disease during early training and racing.

Other works referenced by Mr. Baker were published before new information was available. For example, Lameness in Horses was published in 1974 and Biomechanics of Lameness in Horses was published in 1969. An edition of the Merck Veterinary Manual was published in 1973. Some of these works have been updated in new editions. We were unable to confirm whether his testimony was based on these older editions.

Exhibit 3, contained in the like-numbered tab section attached to this analysis, contains sources used in the preparation of this comment. They include:

1. Jones EW: "Anti-inflammatory Drugs." Equine Medicine and Surgery, Vol 1, p 156, 1982.

2. Tobin T: "Pharmacology Review: The non-steroidal anti-inflammatory drugs. I. Phenylbutazone." J Eq Med Surg 3, 253-258, 1979.

3. Tobin T: "Pharmacology Review: The non-steroidal anti-inflammatory drugs. II. Equiproxen, meclofenamic acid, flunixin and others." J Eq Med Surg 3, 298-302, 1979.

4. Hamm D: "Continuous administration of naproxen to horses during training." J Eq Med Surg 2, 125-128, 1978.

Mr. Baker testified:

> There is also strong evidence to show that Butazolidin significantly interferes with the detection of many dangerous and illegal drugs which might show up in post race urine and blood tests. Recent studies by six of the leading State testing laboratories in the country have reported that Butazolidin is capable of interfering with the detection of all classes of drugs including stimulants, depressants and powerful synthetic type drugs which can prove extremely harmful to horses. [Transcript page 76].

ANALYSIS

We are unaware of the recent studies in six States. To our knowledge, there is no experimental evidence and reports suggesting that masking by phenylbutazone is a significant problem.

In 1977, the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners reported that "The usual doses of phenylbutazone, given on race day, may interfere with the detection of other medications, depending upon the analytical methods used."

It has been reported that "Phenylbutazone and its metaolites may interfere with analysis for unauthorized drugs in blood and urine, depending upon the concentration of phenylbutazone, the sensitivity and specificity of the analytical techniques, and the expected scope of drug coverage." 1/ The drug testing process, by its nature, separates acidic drugs such as phenylbutazone from basic drugs. The illegal medications are almost invariably basic, lipid soluble agents. Thus, the configurations of the standard testing processes are such that phenylbutazone or its metabolites have little potential to interfere with testing for stimulants, depressants, narcotics, local anesthetics and tranquilizers. 2 3/ Acidic drugs are, by their nature, generally readily tested for in blood, where masking of these agents is not a problem. 2/

The metabolite of phenylbutazone which has the potential to interfere with drug testing is oxyphenbutazone. Oxyphenbutazone levels in horse urine do not relate to the dose of phenylbutazone administered, but to the pH of the urine of the horse. 4/

Because the concentration of the potentially masking metabolite in horse urine is randomly affected by the metabolism of the horse and oxyphenbutazone has the potential to mask in a small percentage of horses, the probability of a horseman being able to get concentrations of oxyphenbutazone into his horse's urine is small, and well randomized. 4/

Natural constituents of urine provide a background against which all drug testing must be done. Concentrations of phenylbutazone and its metabolites associates with therapeutic medication do not, in general, add significantly to this natural background. 5/ General agreement exists among racing chemists that at phenylbutazone blood levels of 2 mg/ml, masking is not significant. 6/ Analysis of "hard drug" positive call rates from states that have banned the use of phenylbutazone or comparisons of call rates between states running with and without phenylbutazone shows no evidence that the use of phenylbutazone interferes with drug testing. 7/

The footnotes below are the sources for this analysis. Exhibit 4 contains the text of these sources and they appear in Tab 4 of this analysis.

1. Gabel AA, Tobin T., Ray RS, Maylin GA, "Phenylbutazone (Butazolidin) in Horses: A Review." J Eq Med Surg, 1: 221-225, 1977.

2. Tobin T, Drugs and the Performance Horse. Charles C. Thomas, Publ. 1981.

3. Tobin, T, "Pharmocology Review: Testing for Drugs in Horses." J Eq Med Surg, 2(12):518-524, 1978.

4. Houston T, Tobin T, Blake JW, "Effect of Urine pH on Urine Levels of Oxyphenbutazone in Racing Horses." J Drug Metab Dist, submitted for publication, 1983.

5. Tobin T, Preliminary Results from Post-Race Blood and Urine Samples from Latonia Race Course in February, 1983. Unpublished Data.

6. American Horse Counsel Phenylbutazone Study, 1983.

7. Positive Call Rates for "Hard Drug Positives" in Controlled Medication and No Medication Jurisdictions.

With regard to the use of Lasix, a diuretic used to control the rupture of blood vessels in the lungs, Mr. Baker testified:

> Unfortunately, just as trainers attempt to keep their musculo-skeletal cripples racing by the use of Butazoladin, they also strive to keep their pulmonary cripples racing through the use of Lasix. This practice is not only cruel, but also appear to be based on clinical misconception and untenable economics. The number of horses observed bleeding on the race tracks in California actually increased by 20 per cent after Lassix was permitted by the CHRD (California Horse Racing Board). From the above statistics, Lasix could hardly be considered a drug effective in the prevention of pulmonary hemorrhage nor a drug necessary for the economic survival of horse racing. [Transcript pages 74-75]

ANALYSIS

Controlled studies at the University of Kentucky and the Ohio State University have shown that furosemide (Lasix) has no effect on performance in racing horses 1/. Similarly, a retrospective study of track times at Louisville Downs showed no statistical difference in the speed of horses with and without furosemide. 2,3/ If indeed furosemide was a drug capable of keeping pulmonary cripples racing, one would expect some effect to improve performance in actual practice. No such effect has been observed. 4/

There is, further, no evidence whatsoever to suggest that this practice is cruel, or that it is based on clinical misconceptions.

The fact that the number of observed bleeders increased when the CHRB approved a medication for use in bleeders shows that horsemen reported bleeders and that they use therapeutic and beneficial medication in their horses when such medications are permitted them. The witness' conclusions appear not to be supported by scientific data or clinical experience.

The footnotes below are the sources for the analysis. Exhibit 5 contains the texts of these sources and they appear in Tab 5 of this analysis.

1. Tobin T: "Pharmacological Review: A review of recent research on furosemide in the horse." J Eq Med Surg 2, 314 (1979).

2. Gabel AA, Tobin Thomas, Ray Richard S and Maylin GA: "Furosemide in Horses: A Review." J Eq Med Surg 1, 215-218 (1977).

3. 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 Eq Med Surg 2, 216-226.

4. Tobin T. Drugs and the Performance Horse. Charles C Thomas, Springfield, Illinois (1981).

Mr. Baker testified:

> ...There has been no evidence to date that proves Lasix significantly prevents the occurrence of pulmonary hemorrhage nor is there one iota of evidence to substantiate that Lasix cures the pathological conditions in the lungs which causes this hemorrhaging. [Transcript page 75]

ANALYSIS

Approval of furosemide for use in racing horses depends on:

(1) Clinical experience of equine practitioners and horsemen, who hold that furosemide significantly reduces the incidence of epistaxis. 1/

(2) Its specific approval by the Food and Drug Administration and the manufacturer for use in the treatment of pulmonary congestion in horses. 2/ Pulmonary congestion is thought to be one of the factors which causes epistaxis in horses. 3/

(3) Clinical evidence shows that horses with atrial fibrillation and resulting pulmonary congestion have a high incidence of epistaxis. 3/.

(4) The need for a medication to control epistaxis and the danger to life and limb of horses and jockeys. 4/

There is, therefore, a clear cut pharmacological basis, a clear medical rationale, regulatory approval, and good clinical evidence, both from university hospital studies and from equine practice, to support the use of furosemide in the prophylaxis of epistaxis. 4/

Furosemide is the drug of choice of the veterinary profession for the treatment of epistaxis. The recommended dose is 0.5 mg/kg intravenously, four hours prior to post time. 4/

Inasmuch as it is possible for any drug to "cure" a disease, furosemide "cures" epistaxis. 4/

The footnotes appearing below are the sources for this analysis. Exhibit 6 contains the texts of these sources and they appear in Tab 6 of this analysis.

1. Gabel, A.A. et al., "Furosemide in Horses, a Review," J Eq Med Surg 1, 215, 1977.

2. Lasix (R) Box Label.

3. Fregin, G.F. & Deem ,D.A., "Epistasix in Horses with a Trial Fibrillation," Proc. Amer. Assoc. Eq. Pract. #26 Anaheim, Ca. 1980 p 431-433.

4. Tobin, T., Drugs and the Performance Horse, Charles C. Thomas, Springfield, Illinois, 1981.

ANALYSIS OF THE PREPARED STATEMENT OF JAMES M. KRAMON BEFORE THE HOUSE CRIMINAL JUSTICE SUBCOMMITTEE DECEMBER, 15, 1982

On December 15, 1982, James M. Kramon, a Baltimore attorney, testified about a case in which he represented the family of Robert Pineda, a jockey, who died from injuries sustained in a race at Pimlico on May 3, 1978. A horse named Easy Edith, running in front of Mr. Pineda's mount, broke down and fell. Mr. Pineda's horse tumbled into Easy Edith and Mr. Pineda was crushed.

Mr. Kramon established his "credentials" as an expert, saying:

> Because a number of prior negligence cases involving racetracks injuries and fatalities had been instituted without recovery, and because this case presented difficult questions involving equine locomotion and pharmacology and some rather complex theories of tort liability, I and several associates at my law firm spent hundreds and hundreds of hours learning much about the racing business. In the course of that work, I personally travelled to a number of other States and I spoke with experts in a variety of racetrack related subjects in many parts of the country. I personally retained two equine veterinarians for use in the case, one former racetrack veterinarian and the other perhaps the preeminent national authority on equine locomotion....I also had the opportunity to meet and/or speak with numerous individuals involved in racetrack work of different sorts, including veterinarians, trainers, jockeys, a variety of race track functionaries and others. [Statement, pages 1 and 2).

Thus, Mr. Kramon established his credibility as a witness.

Mr. Kramon said in his statement:

> ....I and my associates were able, after a considerable time and with much difficulty, to obtain what I believe to be reliable information concerning certain practices at racetracks. I must assume that the defendants and their attorneys also felt this to be the case because very late in the proceedings, the case was settled for a substantial -- but not public -- amount of money. (Statement, Page 3)

ANALYSIS

The deceased left a wife and two small children. Settlements in such cases frequently are "substantial."

On March 16, 1983, Walter Fialkewicz, our associate who is a former Special Agent for the Drug Enforcement Administration, interviewed one of the defendants, David Zipf, D.V.M., the Maryland Racing Commission veterianian. The interview took place at Pimlico. Dr. Zipf told Mr. Fialkewicz that the malpractice insurance companies for the two private veterinarians in the case paid $200,000 in settling the matter; the trainer and owner paid a total of $5,000; and the Maryland Racing Commission, in behalf of Dr. Zipf, paid approximately $10,000.

Mr. Kramon said in his statement:

> I can state without equivocation that drugs are certainly utilized in enormous quantities at least where they are permitted to be utilized. In my case, for example, no less than eleven different drugs were administered to the horse which caused the particular fatality and little, if any, attention was paid to the individual, cumulative and synergistic effects of so significant a number of drugs. [Statement, page 3).

ANALYSIS

Mr. Kramon appears to base his statement that drugs are "utilized in enormous quantities" upon his experience with Easy Edith, which was given eleven drugs. The accident took place on May 3, 1978. These drugs, according to the record, were administered between the end of March and April 8, 1978 -- three weeks before the race.

None of the "drugs," some of which were simply vitamins, were prohibited substances. It is unknown whether these medications were in Easy Edith's system at the time of the accident because there was neither a post mortem examination nor was a blood sample drawn at the time of the accident.

The veterinarians for the plaintiff felt strongly that Easy Edith's accident was drug-related. On the other hand, the veterinarians for the defendants felt equally strongly to the contrary. Exhibit 7 contains the reports of the Plaintiff's veterinarians, William R. Porter, D.V.M. and James R. Rooney, D.V.M., and the report of the Defendants' veterianarian, Albert A. Gabel, D.V.M., a professor of veterinary medicine at Ohio State University.

Thus, Mr. Kramon's views reflect those of the plaintiff's veterianians, which are contradicted by the veterinarian for the defense.

Mr. Kramon said in his statement:

> "The drug which was virtually certainly responsible for the fatality in the Pineda case was, contrary to much of the publicity which attended the case, not Butazolidin and not an analgesic. That drug was a hydrocortisone derivative, the purpose of which is to relieve pain and soreness by anti-inflammatory action.
>
> "The corticosteroid family of drugs, which are customarily administered by innoculation into previously evacuated joints, are particularly insidious at race courses. The reason for this is that the normal responses of race horses (or human beings, for that matter) to pain, which responses result in certain protective adjustments when left unhampered, are removed by the utilization of corticosteroid drugs. The race horse, therefore, runs as though he or she did not suffer from the symptoms of some form of physical defect and the result of such activity upon an extremely heavy and very exercised animal may be to allow intolerable pressure to be placed upon a joint or structure which, but for the corticosteroid, would cause the animal to compensate applying less pressure or running in a compensatory fashion. [Statement page 4)

ANALYSIS

The thrust of Mr. Kramon's contention was that treatment of Easy Edith's knee caused the horse to run on that joint, which led to a fracture of the cannon bone.

Dr. Gable, in response to the contention that a hydrocortisone derivative "was virtually certainly responsible for the fatality," said this is an "unbelievably incorrect conclusion." Dr. Gable says that "There is no correlation between the injection of cortisone in the knee and the fracture of the cannon bone" [both of which happened to Easy Edith]. See Dr. Gable's remarks contained in Exhibit 7.

Mr. Kramon said in his statement:

> The fall of the horse which caused the death of the decedent was, sadly, predictable. There were, in fact, many warnings of what might, and what ultimately did, transpire. The horse in question had been manifesting certain characteristics of lameness, such as headnodding, for some time before the accident. The horse had also been experiencing a rather sharp decline in her performance and, indeed, there are economic reasons why, one might hypothesize, the owners were particularly anxious to have her perform well at the juncture at which she failed. [Statement, page 5)

ANALYSIS

Although Mr. Kramon contends the breakdown of Easy Edith could have been forecast, Dr. Gable terms this

> an unbelievable conclusion based upon no real evidence [his emphasis]....This could not be predicted by scientific or empirical methods. Besides, it was not the knee but the cannon bone, one joint and three to four inches of cannon bone away, from the 'puffy knee joint'. [Exhibit 7]

ANALYSIS OF THE TESTIMONY OF TED COCHRAN
BEFORE THE HOUSE CRIMINAL JUSTICE SUBCOMMITTEE
SEPTEMBER 30, 1982

The only chemist to testify at the hearings in 1982 was Ted Cochran, a former official racing chemist for the States of Pennsylvania, New Mexico and Arizona. Mr. Cochran's testimony focused on two areas: medications and the adequacy of State regulatory enforcement.

With regard to the integrity of State enforcement, he testified:

> I can come to you after eight years of experience and tell you that the system doesn't work. The system is unable to control instances of fraudulent practices using permissive medication, and it is most unable to control the usage of strictly illegal drugs whose only purpose on the race track is to fraudulently influence the outcome of a horse race. [Transcript, page 14].

ANALYSIS

Mr. Cochran's eight years of experience in racing chemistry establishes his credentials for offering testimony on the effectiveness of the State enforcement process.

Mr. Cochran said:

> The problem is that one study that is frequently quoted by the horsemen found as many as 90 per cent of the horse population afflicted [with bleeding]. Data from track vets and bleeders lists in other States seriously dispute this study and most recently a more thorough study has been published on horses at an urban Philadelphia track which places the number of horses afflicted in even the most mild way at less than half. Further, this study shows that Lassix is not particularly effective in most of these cases and in those cases where some benefit was noted, the beneficial effect may have been as much from rest as from the chemical. It is interesting to note that neither the manufacturer nor the FDA recommends the drug for this problem.
>
> Of more interest is the fact that where Lasix is allowed, many non bleeders opt to run on it and in fact do perform better with the drug than without it. [Statement pages 7, 8].

ANALYSIS

The highest percentage of bleeders reported is 75.4 per cent by Raphel and Soma. 1,2,3/ No studies suggesting a 90 per cent incidence have been reported.

The drug label (Exhibit 6) approved by both the Food and Drug Administration and the manufacturer states that Lassix is approved for treatment of pulmonary congestion, which is considered one of the causes of epistaxis. 4/

There is no evidence that the performance of non-bleeders is improved by furosemide. 5,6,7/

The footnotes refer to the following materials, which are included in Exhibit 8.

1. Pascoe, JR, Ferraro GL, Cannon JH, Arthur RM, Wheat JD: "Exercise-induced pulmonary hemmorrhage in racing Thoroughbreds: a preliminary study." Am J Vet Res, 42(5); 703-707, 1981.

2. Raphel, CF: "Endoscopic findings in the upper respiratory tract of 479 horses." JAVMA, 181(5); 470-473, 1981.

3. Raphel CF, Soma LR: "Exercise-induced pulmonary hemorrhage in Thoroughbreds after racing and breezing." AM J Vet Res, 43(7); 1123-1127, 1982.

4. Fregin GF, Deem DA: "Epistaxis in horses with atrial fibrillation." Proc Am Assoc Equine Pract, 26, 431-433, 1980.

5. Tobin T: "Pharmacology review: a review of recent research on furosemide in the horse. J. Eq Med Surg, 2:314, 320-321, 1978.

6. Gabel AA, Tobin T, Ray RS, Maylin GA: "Furosemide (Lasix) in horses: a review." J Eq Med Surg, 1:215-218, 1977.

7. 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 Eq Med Surg, 2:216-226, 1978.

Mr. Cochran testified:

> ...Racing chemists unanimously agree that the diuretic effect of Lasix -- the dilution of drugs in the urine -- makes their detection more difficult. While some would argue that more care and better tests would overcome the problem, a panel of noted chemists have asserted that their research shows that Lasix renders some narcotic stimulants undetectable. Basic research into the fundamental mechanism of this phenomenon is being conducted but on a budget estimated to be less than $10,000." [Statement, page 8]

ANALYSIS

Furosemide (Lasix (R)) does not interfere with the detection of any drug in blood. Lasix does not "flush" drugs out of horses. 1/

Furosemide (Lasix (R)) does not affect the concentration or the detection of basic lipid soluable drugs in equine urine. In tests at the University of Kentucky, Lasix did not significantly affect the detection of procaine or ritalin in equine urine. 1/

Furosemide (Lasix (R)) can dilute out and make the detection of certain water soluble drugs and drug metabolites more difficult in equine urine. 1/

This effect is transient, for the diuretic effects of furosemide are quite transient. 2/ 3/

Based on research at the University of Kentucky and elsewhere, if the dose of furosemide is 0.5 mg/kg, and if the dose is administered four hours prior to post time, then there is no interference with drug detection. 4/ Under these conditions, the detection of some drugs such as narcotic analgesics or Schedule II drugs is actually enhanced after treatment with furosemide. 5/

The budget for research on furosemide at the University of Kentucky alone has been about $80,000 and has resulted in the publication of at least eight research papers and reviews on furosemide. Assuming that at least half this sum has been spent at other institutions, the budget for basic research on furosemide would be well over $100,000.

The sources for these comments are noted below and appear in Exhibit 9.

1/ Tobin, T. et al, "The Pharmacology of Furosemide in the Horse I, Effects on the Disposition of Procaine Methylphenidate, Phenylbutazone and Pentazocine," J. Eq Med. Surg. Vol. 1, 402, 1977.

2/ Roberts et al, "The Pharmacology of Furosemide in the Horse II, Its Detection, Pharmacokinetics and Clearance from Urine," J. Eq. Med. Surg. 2, 185, 1978.

3/ Tobin et al, "The Pharmacology of Furosemide in the Horse III, Dose and Time Response Relationships, Effects of Repeated Dosing, and Performance Effects," J. Eq. Med. Surg. 2, 216, 1978.

4/ Combie, Jr. et al, The Pharmacology of Furosemide in the Horse V, the Duration of Reduction of Urinary Concentrations of Drugs, J. Eq. Vet. Sci. 1, p. 203, 1981.

5/ Combie et al, "Furosemide, Patella Vulgata, Glucuronidase and Drug Analysis: Conditions for Enhancement of the TLC Detection of Apomorphine, Butorphanol, Hydromorphone Nalbuphine, Oxymorphone and Pentazocine in Equine Urine," Res. Commun, Chem. Path. Pharmacol 35, p. 27, 1982.

6/ Tobin, T., Drugs and the Performance Horse, Charles S. Thomas, Springfield, Illinois, 1981.

Mr. Cochran testified:

> In the past ten years, however, there has been more impetus put on this (exchange of information among racing chemists) and we have gotten some really fine analytical minds in the field. Many of the advances are due to these relative newcomers to the field. But there is a certain rivalry between the established chemists, those of the AORC (Association of Official Racing Chemists) and those who have been doing this work for as long as 30 years, and the new chemist. The new chemists feel the AORC chemists have not kept up, have not advanced the state-of-the-art, have not worked on their own initiative to protect the integrity. [Transcript, page 16]

ANALYSIS

Jennifer V. Smith, Ph.D., the Director of the New Mexico and Arizona Racing Commissions' laboratory (AnaCor), who was a colleague of Mr. Cochran, said in a May 12, 1983 letter to David Vienna:

> Progress in racing chemistry as in other scientific fields has been largely made by individuals associated with university or government facilities supported by grants....
>
> I am a relatively recent associate member of the AORC, however, my experience in communication with other racing chemists has been very positive. I can call any racing chemist and have called them to discuss problems and questions. In fact, I have called Dr. Maylin concerning a new drug that was appearing in the horse racing circuit. Dr. Maylin supplied me with a standard of the drug and the method used in his laboratory to detect this drug.

Dr. Smith's letter is Exhibit 10.

Mr. Cochran testified:

> In all my experience in the scientific field, I found the field of racing chemistry is one of the most difficult of all analytical chemical disciplines. A chemist who tries to detect a potent narcotic stimulant like fentanyl is truly looking for a needle in a hay stack, because the analogy is most apt. The levels at which fentanyl is used on the track and then excreted into the urine would be comparable to looking for that single needle in a hundred tons of hay. We cannot, as racing chemists, scientists and citizens, allow any impediment to the doing of this important work. [Transcript, pages 14, 15]

ANALYSIS

The implication is that it is difficult to test for fentanyl.

In a letter to David Vienna, Dr. Smith said:

> At the present time, there is a radioimmunoassy kit available to the racing chemist for detecting fentanyl. This has enabled the analytical racing chemist to easily screen for fentanyl. To go back to Mr. Cochran's analogy, if one is looking for a needle in almost 100 tons of hay, this task is easy when you use a strong magnet. i.e., the fentanyl radioimmunoassay kit. (Exhibit 10)

Mr. Cochran testified:

> There are three or four States in the country that do a real good job of this (enforcement).... But take a State with which I am familiar, like New Mexico. For the entire State of New Mexico, five tracks, boasting the world's richest horse race...New Mexico sees fit to have one investigator for the entire State. [Transcript, page 21]

ANALYSIS

Dr. Smith (Exhibit 10) wrote:

> How does Ted Cochran know that only three or four states of the racing states are doing a decent job? New Mexico has a total of five racing tracks, only three of these tracks race at any one time. Currently, the winner's urine and serum is analyzed for drugs and the serum from two other horses is specifically taken for quantitation of the phenylbutazone levels. There is one investigator full time, however, various state police are assigned to work part-time during the peak racing season, May through August. In addition, this year two state police were assigned to investigate the racing industry in New Mexico.

Mr. Cochran testified:

> In New Mexico it is quite literally easier for a horseman to get 'elephant juice' (M-99) illegally that it is for the chemist to legally obtain his standards. [Statement, pages 1, 2]
>
> Its use around the country, especially in the west and southwest is legend. [Statement, page 3]

ANALYSIS

Dr. Smith (Exhibit 10) said:

> How does Mr. Cochran know how easy it is for a horseman to get M-99, 'elephant juice'? According to one veterinarian, the current black market price for 'elephant juice' is between $8,000 and $16,000 for a vial or approximately $200/dose.

M-99 is manufactured by only one company. It is a Controlled Substance and unauthorized possession could subject an individual to a Federal felony prosecution.

Mr. Cochran testified:

> I had the same (Pennsylvania) racing commission give me in writing a memo telling me not to find, to not look for, certain drugs. It may not seem important, except they weren't really willing to handle the flack from the horsemen who wanted to use that drug, the drug DMSO. And it is a drug. The FDA Has said it is a drug in the horse. Despite the fact that I had presented to them evidence that one trainer who had been reported positive for the use of DMSO had, in all probability, used DMSO to cover up the use of Dilaudid, a powerful narcotic. Their response to that was 'We can't handle it. You won't find it.' [Transcript, page 24]

In addition, he said:

> Would I rock the boat when I was in Pennsylvania? No. I needed my job, too. The pressures can be unbelievable, Mr. Chairman. With one stroke of a pen, a contract which a comany depends upon can be gone. The next time the state employee goes in the hospital, maybe his wife has a baby, you wouldn't believe the snag that occurs in his Blue Cross benefits. [Transcript, page 27]

ANALYSIS

Clifford Woodward, Ph.D., the Director of the Pennsylvania Race Horse Testing Laboratories, wrote a letter (Exhibit 11) to David Vienna discussing the above statements. Dr. Woodward said:

> The allegations concerning DMSO contain the following elements of truth: DMSO is a drug approved for use by the FDA and we were instructed not to test for it; however, there is some information left out of his testimony which will make it obvious that this example is greatly distorted in its interpretation.
>
> DMSO (dimethylsulfoxide) is a naturally occurring substance in water sources, and in many plants and animals. The data on its presence in the horse has not been published to my

> knowledge; however...[there is] good evidence that DMSO is present in the natural, untreated horse....
>
> The rule under which we test Harness Racing Commission samples in Pennsylvania is the strictest in the nation. It specifies that:
>
> > ...'In a laboratory analysis of any specimen or sample of saliva, urine, or blood, a positive result is the presence of a substance foreign to the natural horse except where tolerances and standards for such substance is established by the State Harness Racing Commission.'
>
> ...As you may gather from the above rule, the Commission was perfectly within its mandate when it instructed us not to test for DMSO, a substance which is not foreign to the natural horse.....
>
> The second allegation pertaining to DMSO, that it was used to 'mask Dilaudid' is new to me. I have never heard of this before and I presided over the entire duration of Mr. Cochran's tenure in Pennsylvania. If anything, DMSO would be used to assist the entry of a drug into the body, thereby making its detection easier, not more difficult. I have not observed DMSO to interfere with our testing to date. It is possible to conjecture that it might interfere with a testing method in the future; however, regulators must deal with reality or a likely possibility, not wishful thinking.
>
> ...The allegations concerning employee health benefits could not have been pertaining to Pennsylvania. The Commissions or Departments do not enter into employee health benefit determinations at all once the employee is hired; this is totally between Blue Cross-Blue Shield or the HMO and the employee. There is simply no way that such a thing as Mr. Cochran describes could occur in Pennsylvania.

Mr. Cochran, immediately after relating his experiences in Pennsylvania, moved to discuss what befell him in New Mexico. He said in his statement.

> My experiences in private industry are not much better. Offered a position at a New Mexico firm in order to attract contracts for racing analysis, I found that profitability was the overriding motivation. While an admirable quality in private enterprise, this limitation seriously hampered testing efforts. The lab was marginally equipped and minimally staffed. [Statement, page 10]

Dr. Smith (Exhibit 10), who worked at the lab with Mr. Cochran, said in her letter:

> The staff of AnaChem was comprised of 2 Ph.D. chemists, 1 Ph.D. pharmacologist, 2 pre-med students, 1 person with a B.S. degree and a background in equine research at Cornell University and a vast experience in radio-immunoassays and Mr. Cochran. The equipment included 2 gas chromatographs, 1 DuPont gas mass-spectrometer, a model used by the racing chemists of Connecticut and Florida, liquid-liquid extraction equipment, and a liquid scintillation counter and much, much more.

Mr. Cochran said:

> When the racing contracts began to show reasonable profits, corporate management still refused to upgrade the sub-standard mass spectrometer, the instrument which is the cornerstone of the testing program. Because of the condition of this instrument, I was unable to confirm several urine samples containing levorphanol (a narcotic). When I confronted corporate management with the situation and threatened to stop signing reports and resign, they told me not to worry about notice and leave now. [Statement page 10].

ANALYSIS

Dr. Smith (Exhibit 10) wrote:

> One of the most important aspects of the mass-spectrometer is how large the library of mass spectrums is in its computer. As to Mr. Cochran's wanting to confirm that urine samples contained levorphanol, levorphanol had not been shown to be there by any other scientific method. Using the gas chromatograph-mass spectrometer to search for a drug is not a good scientific practice. A mass spectrometer can help you to see a 'needle in a haystack' but it cannot find it. Mr. Cochran, desiring the lab director's position, stated that if the position was not given to him he would resign. He tendered his resignation and it was accepted.

Mr. Cochran said:

> Soon thereafter (leaving the Albuquerque lab), the new lab director called two morphine positives which had to be dismissed by the commission for irregularities in the lab procedures. I had a chance to review the data along with another racing chemist of long experience. We both concluded that the data clearly showed the presence of Numorphan rather than morphine but the trainers went unpunished. [Statement, pages 10,11]

ANALYSIS

Dr. Smith (Exhibit 10) wrote:

> The two morphine positives were called based on positive results from a radioimmunoassay specific for morphine and confirmation of morphine on the mass-spectrometer. The commission dismissed the case for irregularities in the test barn procedures, for example, water buckets were hung on the fence and it could not be ruled out that morphine had not been dropped into these buckets....In New Mexico after a urine sample is collected from a horse, the sample is split. This is so the horseman may have the results of the urine test confirmed by a lab of his choice. In this case, the laboratory of the State of New Mexico analyzed one of the urines in question and found that it contained morphine. The other urine sample was sent to Bob Vessney of (Truesdail Laboratories, the racing chemist of) California and he too found that the split contained morphine.
>
> Further additional information from a veterinarian revealed that the trainers used Pantopan (Roche) a product that contains opium alkaloids including morphine and codeine, this formulation is possibly popular due to its low cost and ready availability. Regarding the numorphan: Mr. Cochran failed to pass the AORC exam (identification of ten unknown drugs in urine) which makes his opinion regarding drug identity appear rather weak. Such errors in Mr. Cochran's interpretation of the results and irregularities in his testimony are indicative of his strong personal biases in these matters.

ANALYSIS OF THE TESTIMONY OF MARC S. PAULHUS BEFORE THE HOUSE CRIMINAL JUSTICE SUBCOMMITTEE ON SEPTEMBER 30, 1982

Marc Paulhus, a field investigator for the Humane Societry of the United States, testified before the Subcommittee that illegal drugs can be used to influence horse races. He said:

> Tony Cuilla, a protected Federal witness who was apprehended in 1975, claimed he fixed more than 2,000 races in 17 States on 37 tracks over a ten-year period. Mr. Cuilla said he would administer Acepromozine to several horses in a race, if he could not get the jockeys to hold back the horses [Transcript, page 81].

ANALYSIS

Mr. Cuilla has been out of action for 8 years. In that time, testing by States has improved substantially. Acepromozine, which was allegedly used by Mr. Cuilla, can now be detected through testing.

According to reports of the Association of Official Racing Chemists, acepromazine positives have been called since 1978. Between 1978 and 1981, there were 21 such positives.

Mr. Paulhus testified:

> There are two ways that those in racing would be interested in using these substances. First -- and these are the drugs that are most frequently detected -- are those that would be used to stimulate an animal to win a race. The reason why they are the most frequently detected is not because they are most commonly used, but simply because the only samples that most States take are from the first place and sometimes the second place horse. A few States take a random sample, but only two States test every horse by pre-race testing, which is really the only way to control these problems. [p. 80 transcript].

ANALYSIS

For many years, the most commonly reported medication in horse racing has been procaine, most likely from procaine penicillin. The next most commonly called positive would depend upon the medications rules in each jurisdiction. If the State bans phenylbutazone, this class of drug is generally the most commonly called positive. If a State does not prohibit phenylbutazone, the type of drug most commonly called is likely to be a stimulant, but at about the rate of 1 in 1,000 to 1 in 10,000 tests.

All States have provisions for random sampling of horses at the stewards' request.

Because pre-race testing primarily picks up the acidic or permitted "soft" drugs, and not the basic or hard stimulant, depressant narcotics or local anesthetic or tranquilizer group of drugs, such testing is not as effective.

"An Evaluation of Pre- and Postrace Testing and Blood and Urine Drug Testing in Horses," written by Drs. Tobin, Maylin, Henion, Woodward, Ray, Johnston and Blake appears as Exhibit 12.

Mr. Paulhus testified:

> Another way in which drugs are used to affect the performance of race horses is to depress the animals, and generally several animals in a race, so they put on a poor performance. This is rarely detected by chemists because they simply don't get the samples to test." [pp 80-81 transcript].

ANALYSIS

This situation is known as an "outside job." A person, other than the owner or trainer, wants to stop a certain horse or group of horses. To this end, a depressant drug is administered to a horse or a group of horses in a race, so that the "doper" can bet with more certainty on the field."

This type of situation may be detected prior to the race, since the horse may be obviously depressed or showing penile protrusion. This horse would be scratched and drug-tested. Sometimes the horse starts but runs unaccountably badly. The stewards may nominate such a horse for a special test, as they have the right to do this at any time. Sometimes the owners will approach either the steward or the laboratory and request a test on a horse that has run unaccountably badly.

Sometimes, when someone has gotten to more than one horse in a field, the fact that a portion of the field has been stopped can be obvious to all. In such cases, the stewards are likely to order tests of all horses.

Medication in this way is almost always an "outside job," done by somebody outside the racing stable, and usually by organized gangs, to manipulate the betting payoff. These cases are detected in a variety of ways as outlined above.

Because of the technical difficulty of making this approach work successfully, it is a relatively rarely used strategy.

Mr. Paulhus testified:

> What we do know is that it (illegal drugging) is serious -- so serious that even with woeful policing and testing methods, I can cite the following examples of illegal drug scandals in the past 2 years. [Statement, page 6]

and he gave the following examples:

(a) The New York Post reported on February 15, 1980 that a new potent and undetectable analgesic named Acupan was being used by a small group of racing stables.

(b) June 25, 1980, Rampo was made to forfeit a first place purse won at Bowie in Maryland, when it tested positive for Diphenhydramine.

(c) September 27, 1980, 20 of 90 entries at Keystone Race Track in Pennsylvania were scratched by their trainers when officials announced they would begin testing that day for Banamine.

(d) In November, 1980, the New Mexico Governor's Organized Crime Prevention Committee reported on illegal drug use at the State's tracks.

(e) On April 14, 1981, a Maryland trainer was suspended after a vial of Ritalin, a stimulant, was found in his car.

(f) On April 22, 1981, a New Mexico horse owner disciplined by the State Commission 15 times since 1970 was fined $1,000 and suspended for 30 days after vials of amphetamines and pain killers were found in his trailer.

(g) On August 17, 1981, a winner of a race at Sarotoga was disqualified when the horse tested positive for Phenothiazine, a tranquilizer.

(h) On November 15, 1981, a Florida trainer began serving a six month suspension after running three horses under the influence of Sublimaze.

(i) It was reported on December 6, 1981, Tarberry, which placed second in a race at Aqueduct the week before, was disqualified when the animal tested positive for Procaine.

ANALYSIS

Mr. Paulhus offers no evidence of his own. Of the nine examples he cites, eight reflect the actions of State governments. These are the very agencies he characterizes as having "woeful policing and testing methods." Either the States are not doing their jobs or they are. It is less than fair to use the State's own enforcement actions as evidence of the "scandal" and therefore justification that the Federal government must intervene in this enforcement area. In the one example not based upon a State action, he cites a newspaper report, which is hearsay.

Mr. Paulhus testified:

> At one meeting I had with horsemen in West Virginia, several individuals laughingly admitted that it had been the policy of the state racing commission to allow suspensions to be served after the racing season was over. [Statement, page 15].

ANALYSIS

James Wise interviewed Roger R. Ramey, Chairman of the West Virginia Racing Commission, who asked that the Commission Executive Secretary, Alfred K. Hays, send a letter [Exhibit 13] to Mr. Wise. Mr. Hays wrote:

> The West Virginia Racing Commission does not have a policy allowing any owner, trainer, jockey, etc. to serve suspended racing days for medication or any other violation of W. Va. Rules when the subject race track is closed. Also, by reciprocal agreement, a suspended permit holder is prohibited from racing at any other tracks under the jurisdiction of the National Association of State Racing Commissioners.

In addition to this Commission's statement, the racing schedule in the State makes it near impossible for suspensions to be served when the racing season is over. There are two tracks in West Virginia. One has racing in all but two weeks in December and the other races year around.

Mr. Paulhus testifed:

> At this time there is not a single State in full compliance with the NASRC medication guidelines and participating in the Uniform Drug Testing and Quality Assurance Program. [Statement, page 12]

ANALYSIS

The statement is not entirely accurate. Pennsylvania harness racing is in full compliance with the NASRC guidelines and the State is participating in the quality assurance program.

For the balance of the States, the use of the conjunctive word "and" could make the statement misleading.

The NASRC guidelines do two things: they recommend trace levels of phenyolbutazone and prohibition of all other drugs as well as recommending the methodology of testing race horses. Specificially, the current recommendations of the NASRC require:

1. Trace levels of any type of nonsteroidal anti-inflammatory medication such as phenylbutazone, not to exceed 2 micrograms/milliliter in the blood.
2. No trace levels of any other medications.
3. Pre-race veterinary examination of every horse.
4. Post race blood tests of every horse, with urine testing of every horse understood.
5. Post-mortem examinations.
6. Freezing of unspecified samples for future testing.

There are at least nine jurisdictions that exceed these guidelines in that they ban the use of all drugs. They are: Arkansas, Delaware (harness), Idaho, Illinois, Maine (harness), Maryland (harness), Montana, New Mexico, and Ohio. However, these jurisdictions are not in absolute compliance with the NASRC guidelines because, although they conduct post race urine tests, they do not routinely conduct post-race blood tests as well. This information is contained in a survey conducted by the National Association of State Racing Commissioners which is an attachment to Caldwell Butler's testimony presented May 18, 1983, to the Subcommittee.

Experts say the urine test is far more sensitive to the detection of drugs. The higher cost of blood testing is the principle reason why most racing jurisdictions do not conduct both tests.

Most, but not all, jurisdictions meet the guideline for pre-race veterinary examinations.

If the NASRC adopts the new guideline recommendation proposed at the association's April, 1983 annual convention, every racing jurisdiction will meet the medication guidelines without changing their current rules.

The Uniform Testing and Quality Assurance Program, sponsored by the NASRC, involves 23 of the 29 racing jurisdictions. A total of 85 per cent of racing horses are tested in laboratories participating in the program. Several other States will begin participation when their Legislatures appropriate funds. Using the testing facilities at Ohio State University and Cornell as their base labs, the NASRC sends actual samples with instructions for techniques and methodology to each participating racing jurisdiction to demonstrate and learn new detection capabilities. In this way, uniform testing procedures are being implemented in the racing States.

Under the program, labs, suspecting the presence of a new drug in a sample but lacking the expertise or means of detecting it, send samples to other participating labs for help. This help may be in actual detection of the drug or in the development of a new detection procedure.

In testimony before the Subcommittee on April 27, 1983, Dr. Joseph O'Dea, representing the National Association of State Racing Commissioners, discussed the guidelines, their proposed modifications and the quality assurance program with the Chairman. The Chairman asked that Dr. O'Dea provide all updates and modifications to the Subcommittee for these programs and guidelines, therefore, these materials will not be duplicated here. (Transcript, pages 67-79).

Mr. Paulus testified:

> "Pre-race testing requiring the taking of blood samples from all horses reduced the likelihood that race fixers can successfully escape detection by tranquilizers or depressants to slow down a horse."

ANALYSIS

This statement is in error. Pre-race testing does not aid particularly in the detection of depressants. Most of the potent phenothiazine-type tranquilizers are not detectable in blood and are thus not detectable in a pre-race test. While reserpine is detectable in blood samples only, if post-race testing is carried out correctly (ie, with blood and urine sample testing), the testing is many times more efficient than simple pre-race testing.

The references for the above statement are listed below and contained in Exhibit 14.

1. Tobin, T. et al, "An Evaluation of Pre-and Post-Race Testing and Blood and Urine Drug Testing in Horses," J. Eq. Med. Surg 3; 85090 (1979)

2. Ballard, S. and Tobin, T., "Pharmacology, Pharmacokinetics, and Behavioral Effects of Acepromazine in the Horse," Fourth Int'l Conf. on the Control of the Use of Drugs in Racehorses, May, 1981, the Victoria Racing Club, Melbourne, Australia

3. Tobin, T., "Pharmacology Review: A Review of the Pharmacology of Reserpine in the Horse," J. Eq. Med. Surg 2: 433 (1978)

4. Tobin, T., Drugs and the Performance Horse, Charles C. Thomas, Springfield, Illinois 1981

Mr. Paulhus testified:

> "We have a situation where the Nevada casinos have simulcast races from all over the country. The important thing to consider here is this is from all different kinds of rules. If they telecast Kentucky races, 40 different substances are legal. They have got, in my opinion, a mediocre laboratory, and I don't think they have a very well-controlled program." (page 83 of the transcript of the September 29, 1982 hearing.)

ANALYSIS

In a letter to David Vienna dated May 12, 1983, J. W. Blake, PhD, Director of the Equine Drug Testing Laboratory of the University of Kentucky, responded to Mr. Paulhus' contention that Kentucky has a mediocre laboratory. Dr. Blake's letter is Exhibit 15. In his letter, Dr. Blake states that Kentucky is a member of the NASRC Quality Assurance Program and is affiliated with the Regional Laboratory at Ohio State University. Dr. Blake says of Kentucky's experience with the Quality Assurance Program:

> "During this time one set of unknown samples has been analyzed - a true measure of a laboratory's competence with respect to its peer laboratories. With regard to Kentucky's performance on this set, the comment of Ohio State's Quality Assurance Officer was 'Totally correct; excellent documentation; good job!'"

GOODMAN and GILMAN's The Pharmacological Basis of Therapeutics

SIXTH EDITION

MACMILLAN PUBLISHING CO., INC.
New York

COLLIER MACMILLAN CANADA, LTD.
Toronto

BAILLIÈRE TINDALL
London

The analgesic action of morphine occurs centrally, whereas the work of Lim and colleagues (1964) clearly shows that aspirin acts mainly peripherally. Thus, by preventing the synthesis and release of prostaglandins in inflammation, aspirin may avert the sensitization of pain receptors to mechanical stimulation or to other mediators. This hypothesis explains why aspirin is ineffective as an analgesic in noninflamed tissues, as shown by the Randall-Selitto test (1957). Most data are consistent with the presumption that aspirin is only effective as an analgesic in pathological conditions or experimental models in which prostaglandins are synthesized locally. This concept also explains why aspirin-like drugs are ineffective against sharp, "stabbing" pain, which is caused by direct stimulation of sensory nerves, but are effective in the dull, "throbbing" pain of inflammation, where prostaglandins apparently sensitize the nerve endings.

As mentioned previously, aspirin-like drugs do not affect the hyperalgesia or the pain caused by direct action of prostaglandins, consistent with the notion that it is their synthesis that is inhibited. A possible exception to this is the fenamates, which may have some antagonistic action against prostaglandins, as well as potent capability to inhibit their synthesis (*see* Ferreira, 1972; Ferreira *et al.*, 1973; Moncada *et al.*, 1975).

EQUINE MEDICINE AND SURGERY

THIRD EDITION

VOLUME ONE

Edited by

R.A. MANSMANN, VMD, PhD
Santa Barbara Equine Practice
Goleta, California

E.S. McALLISTER, VMD, MS
Diplomate, American College of
Veterinary Internal Medicine
Centre Equine Practice
Centre Hall, Pennsylvania

P.W. PRATT, VMD
Book Editor
American Veterinary Publications
Santa Barbara, California

AMERICAN VETERINARY PUBLICATIONS
DRAWER KK, SANTA BARBARA, CALIFORNIA
1982

These products may also be used prophylactically since they do not impair healing or immunity. They are useful to control postsurgical pain and swelling, and may prevent soreness and injury associated with training. Phenylbutazone administered 23 hours before time trials has improved performance, perhaps due to the relief of subclinical lameness. In a controlled study, naproxen prevented training complications. Intermittent (2–3 times weekly) administration of similar products resulted in apparent clinical benefit.

PHENYLBUTAZONE: ITS ACTIONS, EFFECTS, AND ROLE IN EQUINE MEDICINE

THOMAS TOBIN, PhD, MRCVS
Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture
University of Kentucky, Lexington, KY 40546

Phenylbutazone is a member of the "non-steroidal anti-inflammatory" group of drugs, and is the member of this group most commonly used in horses. The principal drug of this group with which you are familiar is aspirin. While aspirin is so readily and easily available that most people do not think of it as a drug, it is, in fact, the original member of the phenylbutazone group of drugs, and produces much the same effect in man as phenylbutazone does in the horse. If you wish to keep phenylbutazone in the horse in perspective, it is often useful to think of it as being basically just horse aspirin.

As a member of the aspirin-like group of drugs, phenylbutazone has essentially little effect on normal pain perception. What it does affect, however, is pain associated with inflammatory responses. It turns out that chemicals called prostaglandins are synthesized in inflamed tissues and act to hypersensitize inflamed areas to pain. Anybody who has had anything as simple as a sunburn is familiar with the problem of hypersensitivity to pain in sunburned skin. What drugs like aspirin and phenylbutazone do, is act to block the synthesis of prostaglandins and therefore reduce the hypersensitivity of the inflamed tissue to pain, eventually normalizing inflamed tissue. It is important to remember that these drugs have essentially no "numbing" effect on anything other than inflamed areas, any more than the aspirin that you take for a headache will numb your hands or legs.

Phenylbutazone is not thought to be stimulant in horses — no more than aspirin is stimulant in man. While phenylbutazone will enable a sore horse to perform up to his natural or "innate" ability, it is not thought of as having any stimulant action or enabling a horse to "excel" himself. Phenylbutazone is no more capable of stimulating an exceptional or unusual performance in the horse than aspirin is in man.

It is important to distinguish between the actions of phenylbutazone in the horse and the local anesthetics. Local anesthetics are injected into a nerve or joint, and "numb" a specific area. Such treatment blocks both pain perception and what is called "proprioception." Since proprioception is in fact the horse's awareness of where his leg is and what it is doing, a horse treated with a local anesthetic loses much more than simple pain perception. Because of the loss of proprioception with local anesthetics, the horse is not quite sure of where his leg is, and his risk of a misstep and breakdown is thought to be increased. It is important to remember that phenylbutazone does not affect normal pain or proprioception in an area, and its actions are therefore completely different from the local anesthetics.

Phenylbutazone is also quite different from the narcotic analgesics, which act to reduce pain perception in the brain. They do this by dissociating an individual emotionally from pain and also by inducing euphoria. Beyond this, the narcotic analgesics or morphine-like drugs also stimulate running activity in the horse. As one might expect, phenylbutazone — an aspirin-like drug — has none of these effects and does not suppress pain perception in the brain or induce euphoria or running behavior.

What phenylbutazone very simply does is suppress inflammatory responses and, at the same time, reduce the hypersensitivity to pain that goes with the inflammatory response. This has led to the argument that horses treated with phenylbutazone will run on injured joints or tendons and will therefore be more likely to break down. This is rather like suggesting that people who take aspirin are more likely to go out and break a leg. In general, the statistics of racetrack breakdowns do not support this argument. For example, the statistics from California show that the incidence of breakdowns requiring destruction of Thoroughbred racehorses stayed constant during the 1970's, during which period the use of phenylbutazone in racing horses was legalized and the percentages of horses running on phenylbutazone increased. All in all, the data does not appear to bear out the hypothesis that use of phenylbutazone leads to increased breakdowns and deaths in racing horses.

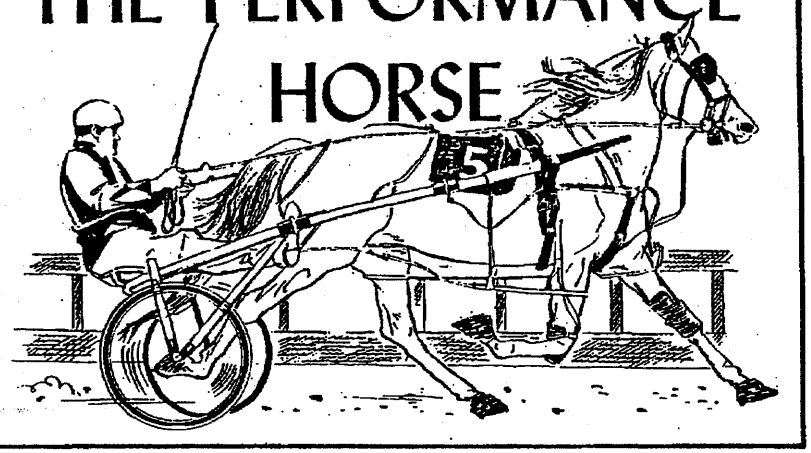
Another charge sometimes laid against phenylbutazone is that its presence in urine samples makes the detection of other illegal drugs more difficult. This ability of "bute" to make the detection of other drugs in urine more difficult is spoken of as the "masking" problem. By and large, masking is not a scientific issue, but rather a political or "pseudo-issue." In our hands, in Kentucky, the levels of phenylbutazone which we "see" in our post-race samples has caused no significant analytical problems. Kentucky, further, has one of the highest "positive" call rates for illegal drugs of any racing state.

In summary, phenylbutazone is a member of the aspirin-like group of anti-inflammatory agents, and phenylbutazone in the horse is the approximate equivalent of aspirin in man. It has no direct effects to numb tissues, affect proprioception, or reduce pain perception in the brain. Its action is simply to reduce the hypersensitivity to pain or normalize inflamed areas, and aids the horseman in fielding racing sound horses. Its use does not appear to give rise to an increased incidence of breakdowns, nor does it significantly interfere with testing for illegal drugs. Phenylbutazone has become controversial largely because it is misunderstood. Phenylbutazone is, by and large, the horse's equivalent of aspirin, and is about as likely to damage horses or stimulate unusual performances as aspirin in man.

Abstracted from DRUGS AND THE PERFORMANCE HORSE *by Thomas Tobin. Charles C Thomas, publisher. Springfield, IL 62717 $44.75 217-789-8980*

Next month PROFESSIONAL PLATFORM *will feature an article contrasting the viewpoint of Dr. Tobin.*

DRUGS AND THE PERFORMANCE HORSE



THOMAS TOBIN

M.V.B., M.Sc., Ph.D., M.R.C.V.S.

Kentucky Equine Drug Research Program
Department of Veterinary Science
University of Kentucky
Lexington, Kentucky

With Forewords by

H.R.H. The Prince Philip
Duke of Edinburgh

and

Ernst Jokl, M.D.

Honorary President, Research Committee
UNESCO, International Council of
Sport and Physical Education

"The great value of this book is that for the first time the facts about contemporary medications, drugs and analytical techniques are set out in plain unmistakable language by an acknowledged world authority. It is a book that will be welcomed by competitors, trainers, coaches, veterinarians and administrators."

–from the Foreword by H.R.H. The Prince Philip, Duke of Edinburgh

CHARLES C THOMAS • PUBLISHER • SPRINGFIELD • ILLINOIS

Chapter 6

PHENYLBUTAZONE AND ITS BROTHERS: THE NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

THE AVERAGE RACEHORSE starts about nine times a year and is breezed and galloped "flat out" countless more times than this. At full speed the average race horse hits speeds of over 35 miles per hour and places forces on the order of 2,000 foot-pounds, or about twice his body weight, on his lead foreleg with every stride. The forelegs of a racehorse are remarkably economical structures to carry such massive loads on the straightaway, and on turns the areas over which such loads are distributed are further reduced, thereby increasing the load/unit area and the likelihood of damage.

Further, some researchers have shown that bones have a honeycomblike microstructure and that the shocks of an extended gallop are absorbed by a micro-crushing of this structure. Then, at the end of a stressful bout of exercise the bone remodels, regenerates, and extra bone grows in the areas of greatest stress and micro-crushing. As the animal matures and the bone stiffens, more of the micro-crush load is carried by the cartilage, and if the injury is repeated, long term damage to the articular cartilages and surrounding surfaces is suffered.

Another event that occurs inescapably during exercise is that the bone becomes weakened as the micro-crushing process progresses. To break a normal cannon bone at the start of a race it takes about 16,000 foot-pounds of force, but the amount of micro-crushing that can take place in a race can reduce this force to about 9,000 pounds. The final result of this is that the stress of a fast race causes substantial changes in bones, joints, and ligaments in all horses, and it is not surprising that the horse, like any other athlete, is likely to turn up with tissue damage post-race. If the racing process is repeated, if the racing process has taken place on unusually hard surfaces, if the banking of the surface at the turns is less than optimal, or if the conformation of the horse makes it susceptible to particular types of trauma, then there is a substantial probability that the damage to the horse will increase to the point that the animal will come up lame. The lameness will be due to the pain and inflammation associated with the specific injury from which the animal suffers, and one of the time-honored approaches to this problem is to treat the animal with anti-inflammatory drugs.

The classic signs of inflammation were described by the Greeks: heat, redness, swelling, pain, and loss of function, all of which are quite familiar to anyone who has suffered anything as simple as a common sprain. The anti-inflammatory drugs used in equine medicine in the direct treatment of this problem fall into two principal groups, the corticosteroids, which we will be dealing with in the next chapter, and all the others. This other group of drugs is known collectively and rather logically as the non-steroidal anti-inflammatory drugs (NSAIDs).

The original member of the non-steroidal anti-inflammatory group of drugs is

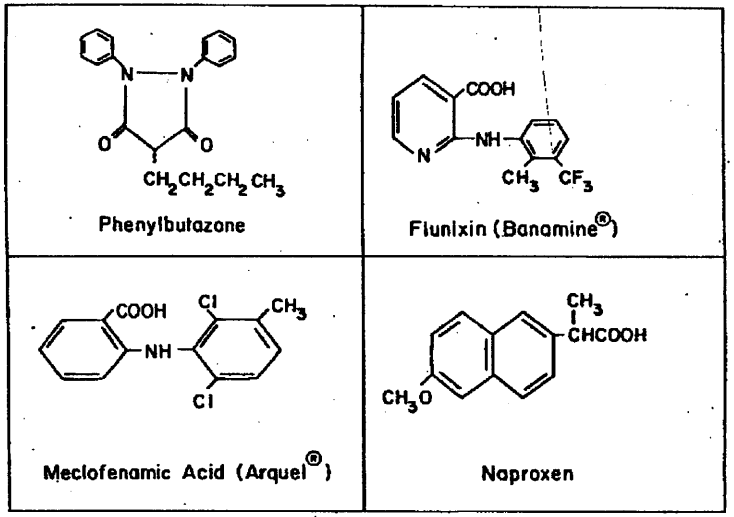
aspirin. The second oldest member of this group is phenylbutazone, which was introduced into human medicine around the time of World War II and shortly thereafter into veterinary medicine. Phenylbutazone was widely used in human medicine for a number of years, but it soon became apparent that its use was associated with a small incidence of specific and serious toxicities. Because phenylbutazone has caused deaths in people from aplastic anemia and agranulocytosis, its use in human patients is now restricted. With the realization of the therapeutic limitations of the corticosteroid derivatives and phenylbutazone, and the huge market for a good anti-inflammatory drug, drug companies have researched the non-steroidal anti-inflammatory field thoroughly in the last ten years or so. As a result, a large number of new non-steroidal anti-inflammatory drugs have been developed and recently become available in both human and equine medicine. A partial list of these agents is presented in Table 6-1.

The non-steroidal anti-inflammatory drugs all share a number of properties that seem to be required for their anti-inflammatory action (Fig. 6-1). All are acidic drugs, with a pKa of 4.5 or less. The acidic nature of these drugs means that they are all between 95 and 99 percent bound to plasma proteins. This plasma protein binding is important because it means that drugs of this group do not pass into saliva, and saliva testing is essentially useless for detection of this important group of drugs. Their acidic nature also seems to be important for their action and has led to suggestions that their acidity enables them to accumulate in inflamed tissues, which also tend to be acidic. This accumulation would then allow them to have more effect in inflamed areas than in normal tissue. However, these

TABLE 6-1
SOME NON-STEROIDAL ANTI-INFLAMMATORY DRUGS*

Generic Name	*Popular or Trade Name*
Phenylbutazone	Butazolidin
Oxyphenbutazone	Tandearil
Salicylates	Aspirin
Thiosalicylate	
Quinine	
Naproxen	Equiproxen
Meclofenamic Acid	Arquel
Indomethacin	Indocin
Ibroprofen	Motrin
Aminopyrine	
Flunixin	Banamine
Niflumic Acid	Nifluril
Flufenamic Acid	
Fenamic Acid	
Mefenamic Acid	Ponstel
Sulindac	Clinoril
Tolmetin	Tolectin
Fenoprofen Calcium	Nalfon
Ketoprofen	Orudis

* These drugs are all classified as non-steroidal anti-inflammatory drugs. They all appear to share the same basic mechanism of action as phenylbutazone and also the same general pattern of toxicity. Some, such as quinine and aspirin, are centuries old, but the clinical use of most is relatively recent.



e 6-1. Non-steroidal anti-inflammatory drugs (1) are highly acidic drugs, (2) are highly protein in plasma, (3) are difficult to detect in saliva, (4) act by inhibiting prostaglandin synthesis, (5) are to be diluted in urine by furosemide.

agents also tend to accumulate in the stomach, small intestine, and kidney, and we will see later that the toxicities produced by these agents are usually associated with these tissues (Fig. 6-2). Also, because prostaglandins are involved in the generation of fever, all these drugs are antifever, or antipyretic, agents (Fig. 6-2).

Among the non-steroidal anti-inflammatory agents, phenylbutazone remains the most popular and most widely used in equine medicine. After its introduction into equine medicine, phenylbutazone established itself as a very effective anti-inflammatory agent. It soon made its appearance on the racetrack, and pressure from horsemen for permission to use it during racing increased. In late 1959, Colorado approved phenylbutazone for use in racing, and the era of controlled medication was born.*

Phenylbutazone and the other non-steroidal anti-inflammatory drugs produce their anti-inflammatory effects by inhibiting the production of chemicals called prostaglandins. Prostaglandins are produced in large amounts in inflamed tissues and are involved in the blood vessel and tissue changes that cause the reddening, heat, swelling, pain, and loss of function that we associate with inflammation. They also have another peculiar action, in that they act to supersensitize pain receptors in the inflamed area to agents that cause pain. Anyone who has had anything as simple as a good sunburn is familiar with the great increase in sensitivity to even the lightest touch that occurs in sunburned tissue. Thus hypersensitivity to pain is a typical effect of certain prostaglandins. In fact, if

H. Bierhaus, Colorado Racing Commission veterinarian: personal communication, 1979.

Non-steroidal anti-inflammatory drugs –

(1) inhibit enzymes that form prostaglandins and therefore slowly reduce tissue prostaglandins

(2) Reduce pain hypersensitivity in inflamed tissues by reducing tissue prostaglandin levels

(3) Reduce fever (are antipyretic)

(4) Accumulate in stomach, kidney, and small intestine and tend to produce lesions in these tissues.

Figure 6-2

minute amounts of prostaglandins are injected into a normal joint, that joint soon becomes excruciatingly painful to move. This sensitizing action of the prostaglandins is represented schematically in Figure 6-3. It is clear from this mechanism of action that if the concentration of prostaglandins in inflamed tissues can be reduced, the signs of inflammation, swelling, and particularly the perception of pain in that area will also be reduced. It is important to remember, however, that this is a reduction in a hypersensitivity to pain, and that normal pain perception will be left in the area.

When phenylbutazone is injected intravenously, it takes about thirty minutes to distribute throughout the horse and begin to block formation of the prostaglandins. There are, however, unusually large amounts of prostaglandins already present in an inflamed tissue. Therefore, even though the formation of new prostaglandins is blocked, phenylbutazone will not appear to take effect until these high prostaglandin levels have been reduced. This process usually takes about three to four hours, and once the excess prostaglandin has dissipated, the supersensitivity to pain reduces and the swelling and heat in the area begin to decline. Loss of these signs of inflammation will require a further period of time, so it can take up to twelve hours or more for the full pharmacological action of phenylbutazone to become apparent.

Since most of the non-steroidal anti-inflammatory agents produce their effects in more or less the same way, they take a period of at least several hours to begin to produce effects, even when they are given by intravenous injection. On the other hand, when blood levels of these drugs decline, the concentration of the prostaglandins in the inflamed tissues build back up again, and the pain and other signs of inflammation begin to return. It is then time for another dose of phenylbutazone. It is clear from this mechanism of action that the therapeutic effects of phenylbutazone are directly related to its effects to reduce inflammation, and that any analgesic action it may have is secondary to and dependent on the anti-inflammatory effect. Phenylbutazone has no anesthetic actions and is not an anesthetic. It is simply a very effective anti-inflammatory drug and as such blocks or prevents hypersensitivity to pain, which is one of the cardinal signs of inflammation.

In an average-sized horse (1,000 lb), a dose of 2 to 3 grams per day of phenylbutazone intravenously, or about 4 grams orally, should produce optimal anti-inflammatory effect. Two grams daily should then maintain the effect. As the dose of the drug is increased, the blood levels increase and the plasma half-life of the drug is also increased (Fig. 6-4). If the drug is given orally instead of intravenously, it takes about five hours for peak blood levels to be attained and the time to attain peak pharmacological effect will be further delayed (Fig. 6-5). Urinary levels of phenylbutazone are usually higher than plasma levels of the drug, and urinary levels are detectable for at least twenty-four hours after a 2 gm/1000 lb dose IV (Fig. 6-6). Since the anti-inflammatory effect is not good for much more than about twenty-four hours, daily dosing with phenylbutazone is required.

For a long time the only way to assess the anti-inflammatory and lameness-alleviating efficacy of phenylbutazone was simply by observing a treated horse. This led to some confusion about how effective the drug really was, how long it took to act, and for how long a period it was effective. Recently, however, Professor Pratt and his coworkers at the Massachusetts Institute of Technology have developed an instrument called a "force" plate, which can provide an

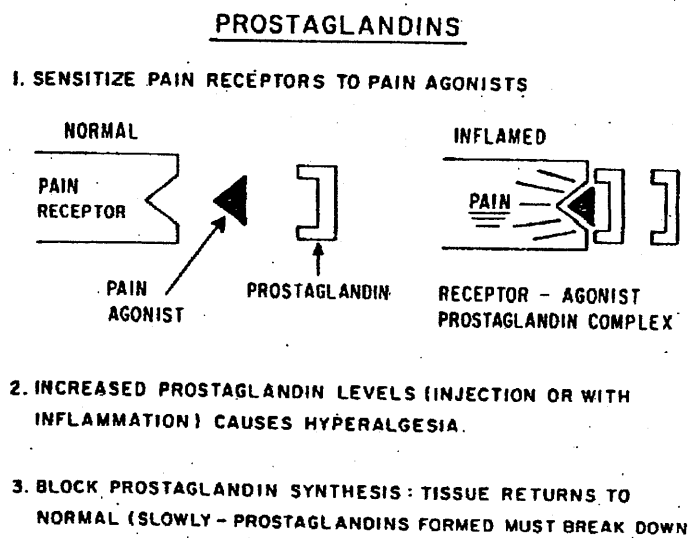


Figure 6-3. Schematic representation of pain-sensitizing action of the prostaglandins. Pain is mediated by certain chemicals which interact with pain receptors. The prostaglandins appear to act by increasing the sensitivity of pain receptors to pain agonists, here represented as a locking of the pain agonists onto the pain receptors. In an inflamed tissue the increased levels of prostaglandin cause the hypersensitivity characteristic of inflamed tissues. Once prostaglandin formation is blocked, the high tissue levels of prostaglandin have to decay over a number of hours before the tissue comes back to normal. This is the reason many NSAIDs often take several hours to act. Reproduced with permission from Tobin, *J Equine Med Surg*, 3:253, 1979.

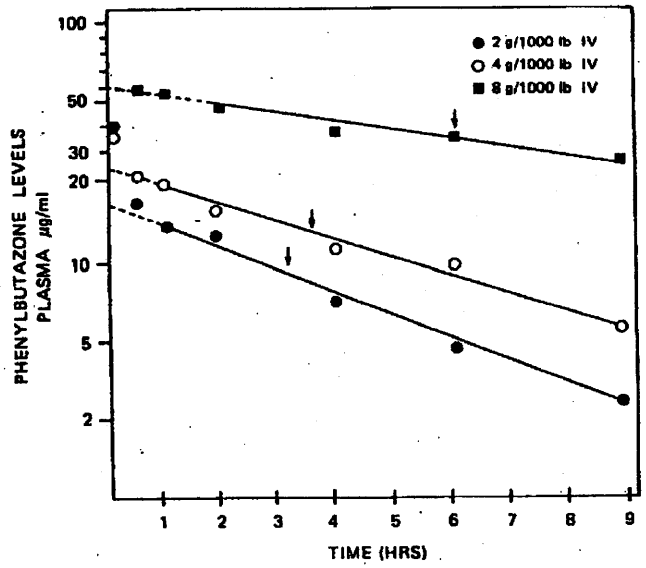


Figure 6-4. Plasma levels of phenylbutazone after administration of different doses. The symbols show plasma levels of phenylbutazone after administration of 2, 4, and 8 gm of phenylbutazone/1000 lb. to horses. At the lower dose, the half-life of phenylbutazone was approximately three hours, while at the higher dose, and half-life was six hours. This is an unusual response and is likely due to oxyphenbutazone, which appears to block the metabolism of phenylbutazone. If high dose levels of phenylbutazone are continued, the blood levels of phenylbutazone increase after every dose (cumulate), the metabolism of phenylbutazone essentially stops, and phenylbutazone toxicity and death may ensue. From Piperno et al. (1968).

objective measurement of lameness and the effects of drugs on lameness. The instrument that does this measures small variations in the load on a horse's leg. It turns out that a horse is very unsteady on a lame leg and that this unsteadiness, in the form of constant small readjustments of weight on this leg, can be measured by means of the "force plate." Then, after phenylbutazone is administered, the horse becomes much more comfortable and thus steadier on his sore leg, which begins to be comparable with his good leg (Fig. 6-7). Using this instrument, Pratt showed that 3 grams of phenylbutazone takes several hours to act after oral administration and its effects are largely over by twenty-four hours. If the dose is given intravenously the onset of action is a little faster, but probably not much faster, because the reduction of tissue prostaglandins by phenylbutazone and other NSAIDs takes some time to develop.

Other ways of measuring the anti-inflammatory actions of the NSAIDs include inducing lameness either chemically or surgically and then measuring the effects of drugs on the lameness. While lameness may be estimated by an experienced clinician, who then scores the animal on a numerical system based on the severity of the lameness, these methods are highly subject to observer bias and are

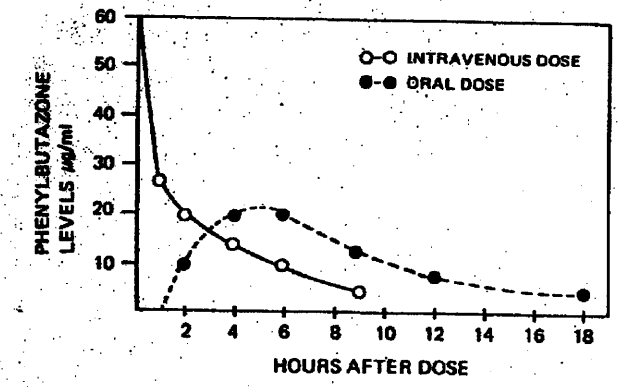


Figure 6-5. Plasma levels of phenylbutazone after oral and IV administration. The open circles (o—o) show plasma levels of phenylbutazone after intravenous administration of 4 gm/1000 lb., while the solid circles (●—●) show plasma levels after oral administration of the same dose. After oral administration, blood levels peak later and lower than after intravenous administration, and blood levels also remain higher longer. From Piperno et al. (1968).

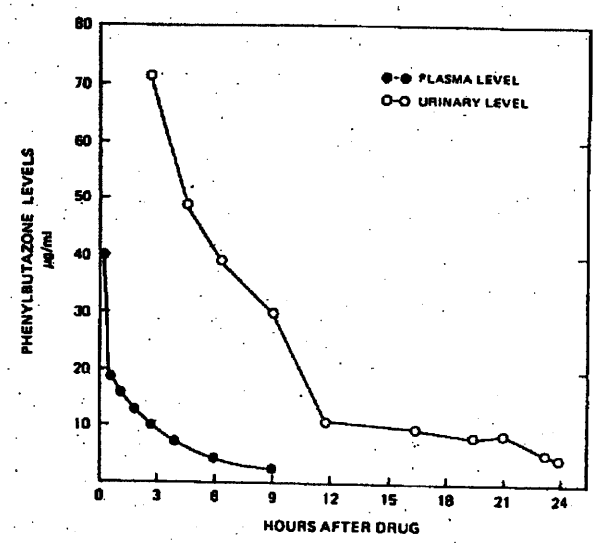


Figure 6-6. Urinary excretion of phenylbutazone. The solid circles (●—●) show plasma levels of phenylbutazone after 2 gm/1000 lb. IV, while the open circles (o—o) show urinary levels of the phenylbutazone in the same horse. From Piperno et al. (1968).

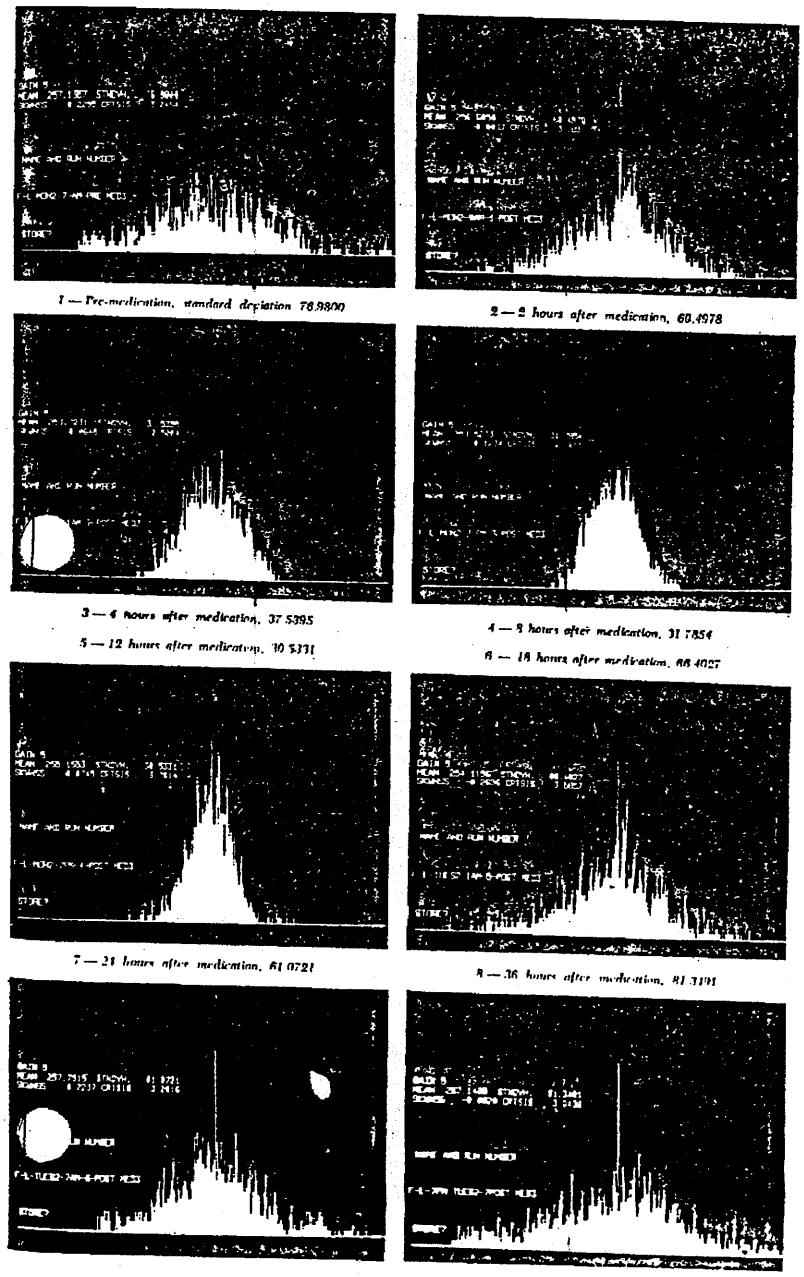
sometimes referred to as the win, place, and show method of evaluating pharmacological effects. Recently, however, stride length has been shown to be very sensitive to the action of these drugs and is now being used to quantitate the effects of drugs on lameness. Stride length may be accurately measured by trotting horses over a freshly raked sanded area and measuring the changes in stride length by the distance between the footprints. Other methods include simple tape measuring of the diameter of an inflamed joint and recording the effects of different drugs on the diameter.

The therapeutic effects that may be expected from phenylbutazone in a horse are perhaps most clearly characterized by the DANCER'S IMAGE case. DANCER'S IMAGE, the champion Candian two-year-old, finished first in the 1968 Kentucky Derby. After the race, however, Kenny Smith, the Kentucky State Racing Commission chemist, reported to the stewards that the urine sample taken from DANCER'S IMAGE contained a medication known as phenylbutazone, and/or a derivative thereof. This finding precipitated a court battle which lasted for years and during which the Kentucky State Racing Commission produced a statement of findings of fact. These findings constitute a classic example of the type of therapeutic response that may be expected from phenylbutazone and other members of the non-steroidal anti-inflammatory group of drugs; it reads as follows:

> DANCER'S IMAGE had prominent ankles before he began his racing career, that is, ankles prone or predisposed to trouble under the strain and stress of racing. They were carefully watched and treated from the inception of his racing career by Louis C. Cavalaris, his trainer. The right ankle had begun swelling in early 1968 at Bowie Race Course in Maryland. It was X-rayed at the time of the Wood Memorial in New York by Dr. Girard and prior to and after the Kentucky Derby by Dr. Harthill. It was swollen on Saturday, April 27, 1968, and Sunday, April 28, 1968, and successfully treated by Dr. Harthill with the concurrence of Mr. Cavalaris upon the latter date with a four-gram dose of Butazolidin[3] (the trade name for phenylbutazone). There was dramatic improvement on April 29, 1968, and the horse was in racing condition and worked out well. However, the condition of the right ankle had again deteriorated by Thursday morning, May 2, 1968, so much so that it was the worst it ever had been up to that time. The ankle was swollen and engorged, a condition known as a red-hot osselet. Dr. Scanlan, the track veterinarian at Churchill Downs, observed DANCER'S IMAGE that day and noticed the horse was so lame and sore that had his condition been the same on Saturday, May 4, 1968, Dr. Scanlan would have recommended to the Stewards that DANCER'S IMAGE be scratched. The ankle began dramatic improvement Thursday afternoon, was in good condition on Friday, May 3, 1968, and on Saturday morning and at the time of the race was in excellent condition and racing sound. The ankle remained sound after the race and through Sunday and Monday. However, by Tuesday, May 7, 1968, the ankle had reverted to its condition of the previous Thursday.

→

Figure 6-7. Measurement of the time course of action of phenylbutazone by force plate. A lame horse constantly readjusts his weight on his sore leg, and the magnitude and frequency of these readjustments are thought to be a measure of the levels of discomfort that the horse is experiencing. The "spread" of distribution of these readjustments by the sore leg in a lame horse is presented in illustration 1 and has a quantitative value (standard error) of about 77. This horse was then dosed with 3 gm of phenylbutazone orally and the spread of the readjustments measured. By twelve hours after dosing with phenylbutazone (illustration 5), the spread of the readjustments was reduced to 30.5, and inspection of the data shows the shape of the distribution curve to be much more compact. Similarly, illustrations 2 through 8 show the development and decay of the antilameness effect of phenylbutazone. The data suggest that the action of phenylbutazone peaks at between eight and twelve hours after an oral dose of the drug and is over between twenty-four hours and thirty-six hours after dosing. Courtesy of Professor Pratt, Massachusetts Institute of Technology.



Effective use of phenylbutazone will dramatically improve the condition and health of a horse with a phenylbutazone treatable condition. It affects the speed of a horse to the extent that it enables him to race when he would not otherwise be able to do so. It enables him to perform to his full potential when otherwise he would not be able to do so. Prohibition of the use of phenylbutazone is necessary in order to preserve honesty and integrity in racing because the indiscriminate use and withdrawal of the medication with many horses would vary their performance in racing. The rules of racing are designed to prevent use of medications and other devices for such a purpose.

Regardless of the amount of phenylbutazone and/or derivative thereof in the urine of DANCER'S IMAGE on Saturday, May 4, 1968, its presence in any amount shows that the administration affected the health and speed of the horse by enabling him to run racing sound.

The basic description of the condition of DANCER'S IMAGE and its improvement is what one may expect with drugs of the phenylbutazone type. DANCER'S IMAGE was apparently prone to musculoskeletal problems in his ankles, and this condition responded well to phenylbutazone. The ankles were X-rayed to check for skeletal problems, and the X-rays were apparently negative. Phenylbutazone was capable of rendering DANCER'S IMAGE racing sound and is capable of enabling horses with phenylbutazone-treatable conditions to perform to their full potential.

In addition to osselets (such as were involved in the DANCER'S IMAGE case), other conditions that Professor Gabel of the Ohio State University considers to respond well to phenylbutazone include sore feet (pedal osteitis), cunean tendon bursitis (jacks), spavins, minor sprains and muscle soreness, splints, navicular disease, and ringbones. Further, it is common practice for many horsemen to administer phenylbutazone after a race, as this will often prevent a horse from "cooling out sore." Under all of these circumstances, the use of phenylbutazone appears beneficial and, as pointed out by the Kentucky State Racing Commission, allows a horse to run up to his best form.

It is important to differentiate between the actions of phenylbutazone and those of other classes of drugs that might also be used in lieu of it. As pointed out earlier, the primary action of phenylbutazone is to block the inflammatory response and the hypersensitivity to pain that accompanies inflammation. Once this has been accomplished, the treated area is essentially normal with normal pain perception and also normal proprioception, which is the awareness that the leg is in a given position and carrying a certain amount of weight. This normal feedback from the affected area is important because it is what enables the horse to run properly on the treated leg.

On the other hand, if a horse is treated with a local anesthetic block, as outlined in Chapter 16, there is no perception of pain whatsoever, either normal or abnormal, from the joint. While this in itself is not a problem, there is also no proprioception, or sense of place, position, or presence from the limb. If a horse runs on a "numbed" leg, it is generally considered that his chances of making a misstep and thus causing a breakdown are greatly increased. However, it should be quite clearly understood that neither phenylbutazone nor any of the other non-steroidal anti-inflammatory drugs block either normal pain perception or proprioception in an area, and a horse therefore races in an essentially normal way when it is treated with phenylbutazone or other non-steroidal drugs.

Another mechanism of reducing the perception of pain in an area is to administer a centrally acting narcotic such as morphine. What morphine and the other

narcotic analgesics do is reduce the perception of pain in the brain. The narcotics therefore do not reduce the formation of pain impulses at the site of the lesion as does phenylbutazone, and neither do they block the transmission of pain along a nerve, as do the local anesthetics. What the narcotic analgesics do is reduce the sensitivity of the central nervous system to pain, which is a true analgesic effect. So far as is known, phenylbutazone and the other non-steroidal anti-inflammatory drugs have no significant effects on pain perception in the brain and thus no narcotic-like effects. Another and entirely distinct problem with the narcotic analgesics, however, is that they are powerful stimulants, as we will see later.

Although the pharmacological action of phenylbutazone is over within twenty-four hours, the time for the animal to "eliminate" phenylbutazone is much longer, as it is with any drug given to a horse. Since phenylbutazone is relatively easy to detect, it can be detected for a very long period indeed if the analyst so desires. Figure 6-8 shows some of the best data on plasma levels of phenylbutazone in the horse that have come to my attention. In this experiment, horses were given varying doses of phenylbutazone for up to three days, and plasma and urinary levels of the drug followed. Plasma levels started at about 20 to 30 µg/ml, declined with a half-life of about one day, and were still detectable in plasma eight days after dosing and in some urine samples on the ninth day after dosing. This experiment, when taken in conjunction with the previous data, emphasizes the

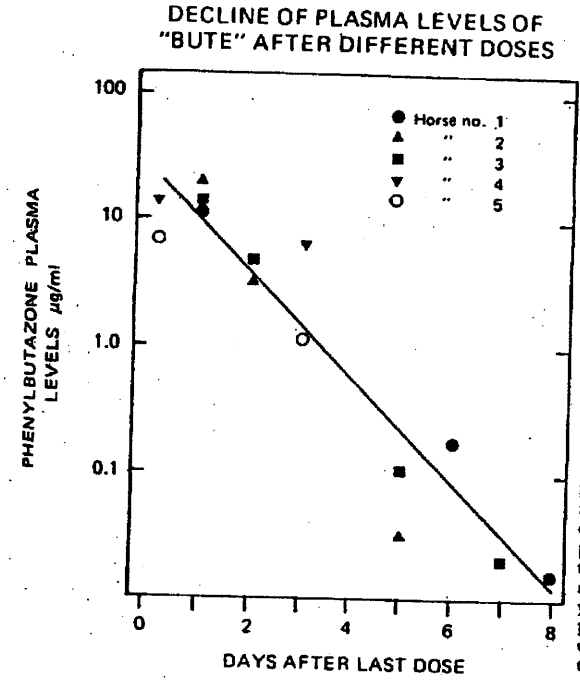


Figure 6-8. The symbols show plasma levels of phenylbutazone in horses that had been dosed with 2.4, and 6 gm phenylbutazone for up to three days before measurement of plasma levels. Phenylbutazone was detectable in plasma for eight days and in urine for up to nine days. After Norheim et al. (1978).

point that while the pharmacological actions of phenylbutazone may be over within twenty-four hours, detection of phenylbutazone in plasma and urine after treatment with this drug is possible for very long periods indeed.

Although the "clearance time" for phenylbutazone in plasma and urine obviously depends on the methods that the analyst elects to use, it is usually safe to assume that plasma and urine are clear by forty-eight to sixty hours after the last dose. Judging from the data of Figure 6-8 and what we know about the pharmacology of phenylbutazone, there appears to be little need for a phenylbutazone positive to be called when plasma or urinary levels of the drug are less than 1 µg/ml.

At the other end of the scale, some racing authorities have rulings designed to discourage race-day medication of horses with phenylbutazone. These rulings state that if certain levels, often 165 µg/ml, of phenylbutazone and/or metabolites are found in post-race urine, this level indicates race-day medication and that a "no medication on race day" rule has been infringed. In the absence of published data, the scientific status of these claims is somewhat uncertain, and rulings based on this information should be treated with caution.

As far as the horse is concerned, phenylbutazone, when used in the recommended doses and for the recommended periods of time, is a very safe drug. Over the years the number of doses administered to horses must be astronomic, while the incidence of reported side effects is small. The principal dangers with phenylbutazone are associated with its improper injection. If phenylbutazone is injected around the jugular vein by mistake, it may cause severe inflammation, abscessation, and eventual loss (sloughing) of the vein. This, however, can occur with many drugs and is a problem with injection technique rather than with phenylbutazone. Phenylbutazone may also be injected directly into the carotid artery in the neck by accident. If this happens the horse immediately becomes excited, falls prostrate, and may die. Some veterinarians and trainers report that phenylbutazone will occasionally depress a horse, especially with large doses in Standardbred horses. However, to judge from the number of horses running on phenylbutazone at some tracks, this effect must either be very rare or else horses get over it rapidly.

Reviewing the data available on phenylbutazone toxicity up to March 1977, the Veterinary Chemist's Advisory Committee to the National Association of State Racing Commissioners concluded that the side effects and toxicities of phenylbutazone at *usually used* clinical doses are minimal. While this statement still stands, data has recently become available on adverse reactions after higher doses of this drug over longer periods of use than recommended by the manufacturer. Because of the widespread use of this drug, it is appropriate for us to review this data so that veterinarians and horsemen may become aware of the circumstances under which these toxicities appear and the signs of toxicity when they occur.

The published and in-press reports of phenylbutazone toxicity both occurred when researchers elected to give higher than usual doses of phenylbutazone to horses to study its effects on liver metabolism of drugs. Working in Scotland, Snow gave 10 mg/kg (about 4.5 gm/1,000 lb) orally for from seven to fourteen days to ten ponies, while Gerber, in Switzerland, gave about 3.4 gm/1000 lb IV to

three horses for eight days. Clinical signs of toxicity were seen in most of these horses, and a number of deaths were recorded. These data emphasize the need for careful monitoring of horses maintained on high therapeutic doses of phenylbutazone.

In Snow's studies, the ponies stopped taking phenylbutazone orally by the sixth day and the drug had to be administered IV. Of the ten ponies in Snow's studies, only two showed no clinically recognizable side effects, while two showed a slight decrease in appetite. For four ponies, treatment was stopped because of the severity of the clinical signs, while three ponies died during the course of therapy. On postmortem, all of these animals had ulcerations in the mouth, peritonitis, and edema of the bowel, while one had a small stomach ulcer.

Clinical signs seen in these animals included dullness, listlessness, and inappetence, with diarrhea developing later. If the treatment was stopped, the ponies gradually returned to normal.

Blood tests showed that most of these horses showed a decrease in plasma protein concentration and increased plasma urea. Marked depressions in plasma calcium and potassium were also seen. These changes were interpreted by Snow as indicating marked water and sodium retention, which is a common side effect of phenylbutazone therapy in man. Snow felt that the critical event leading to death was the development of submucosal edema in the large intestine, leading to shock and then to death.

An essentially similar but less extensive study by Gerber produced broadly equivalent results. Gerber was also studying drug metabolism in horses, but he elected to give his horses 7.5 mg/kg of phenylbutazone IV. In two of three horses, ew signs of extreme depression, anorexia, fever, and impaired liver function. ile one horse recovered, one had to be put down, as peritonitis developed following a liver biopsy. As in Snow's experiments, one horse did not develop any significant clinical signs throughout the experiment.

In conclusion, it appears clear that high doses of phenylbutazone, maintained for more than five days, caused toxicity problems in approximately two out of three horses. While the precise basis of this effect is not clear, it is self-limiting if the drug is given orally, as the horse will refuse to eat and the drug will clear the horse. If the drug is given parenterally, it should not be given at these doses beyond five days and the horse should be carefully watched for signs of toxicity. While there are suggestions in the experimental work that the effect may be due to drug cumulation, horsemen are well advised to cut back or discontinue high doses of phenylbutazone at the first signs of inappetence, fever, edema, or leucopenia.

The pattern of minimal toxicity due to *therapeutic* doses of phenylbutazone in the horse contrasts sharply with the serious toxicities seen in man. In man, some type of side-effect is seen in from 1 to 40 percent of patients; these include peptic ulcer, liver and kidney problems, and, most seriously, inability to form red or white cells in the blood. A number of phenylbutazone-dependent deaths have been recorded, and for this reason its use is restricted in man and it is best used only in short-term therapy.

Because a dose of phenylbutazone persists a long time in the blood of man (half-life about 3 days) compared with its relatively short plasma half-life in the

horse (6 to 8 hours), it has been suggested that its lack of toxicity in the horse is due to its rapid metabolism by the horse. This theory ignores observations that drug toxicities are very often due to drug metabolites rather than to the drug itself, which metabolites are, of course, formed at a much greater rate in the horse than in man.

The question about the effect of phenylbutazone on the performance of horses has often been raised. Reviewing the subject of phenylbutazone and Lasix® in racing horses, 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 him to race nearer to his maximum capacity. Studying the effects of phenylbutazone in time trials in horses, Sanford and his colleagues found that phenylbutazone administered intramuscularly twenty-three hours before "time" trials improved performance in their horses. These workers were apparently rather surprised by this result and concluded that phenylbutazone had acted to relieve subclinical lameness rather than to stimulate the horses. This interpretation is well supported by some experiments from our laboratory, which show no effect of phenylbutazone on fentanyl-stimulated trotting in horses, minimizing suggestions that phenylbutazone either stimulates or depresses horses at the usually used clinical doses.

Another important question with phenylbutazone is whether or not it interferes with the detection of other drugs, the popularly called "masking" effect. While there is no doubt that the presence of any drug must make the detection and unequivocal identification of another drug somewhat more difficult, the consensus of the Veterinary Chemists Advisory Committee was that the usual doses of phenylbutazone given on race day may or may not interfere with the detection of other medications, depending on the analytical methods used.

As pointed out earlier in this chapter, the drug industry has been vigorously researching the area of non-steroidal anti-inflammatory drugs and in recent years has developed a number of new non-steroidal anti-inflammatory drugs. Pharmacologically, these are all closely related to phenylbutazone. Because of their recent introduction they are not as well characterized clinically as phenylbutazone, but it appears at this point that their basic pharmacology and actions in the horse are broadly similar to those of phenylbutazone.

*Equiproxen (Naproxen®)**

Equiproxen is another non-steroidal anti-inflammatory agent with analgesic and antipyretic actions. It is recommended for the relief of pain, inflammation, and lameness associated with "tying up" and soft tissue disease of the horse. Its therapeutic effects in the horse have been studied in some detail by Wynn-Jones and coworkers. These workers induced "tying up" in horses by injecting lactic acid into the back muscles of horses and then studied the effects of equiproxen on length of stride, pain, lameness, and tissue swelling in these horses.

Length of stride in the horse is a sensitive measure of lameness, and Kilian and Wynn-Jones were able to show that equiproxen greatly reduced the lameness

* Naproxen®, Diamond Laboratories, Des Moines, IA

caused by the lactic acid injections. In the untreated horses lameness peaked two days after the injury was induced and persisted for up to fourteen days, while in equiproxen-treated horses the lameness was greatly reduced and virtually eliminated within three days. Comparison of the areas under the curves for the treated and nontreated horses suggests that equiproxen treatment was at least 80 percent effective in reducing lameness. Similarly, equiproxen treatment had marked effects to reduce the amount of swelling, and other data showed that it also reduced pain equivalently. These data constitute the best and most clear-cut experimental work demonstrating the therapeutic effects of a non-steroidal anti-inflammatory drug that I am aware of, and clearly delineate the therapeutic efficacy of equiproxen.

Based on this experimental model, a study in which phenylbutazone and Naproxen® were directly compared led Jones and Hamm to conclude that, in the equine myositis model, equiproxen was superior to phenylbutazone for the more rapid relief of inflammatory swelling and associated lameness.

In other work, the response to equiproxen obtained in these studies was compared with the response in naturally occurring "tying up" disease. In the natural disease the average time for remission was found to be about five days and a favorable response was observed in more than 90 percent of the animals.

Hamm also evaluated the effects of continuous administrations of equiproxen during training to fifty yearling Quarterhorse colts. These colts were assigned to two equal groups at the beginning of a four-month training season, the yearlings assigned to the untreated (control) groups receiving standard medication for musculoskeletal disease as required. The test horses received about 5 gm daily of equiproxen in the feed during the training session. Hamm observed a marked and highly significant reduction in time lost during training and in the racing season in the horses on equiproxen, who lost only 3 percent of their training time, compared with a 13 percent loss in training time in the control group. The appearance of musculoskeletal problems was delayed in the equiproxen-treated group, and when musculoskeletal problems did appear their duration was reduced compared with the control group. The horses treated with equiproxen raced significantly more often than the control horses, and the overall frequency of musculoskeletal injuries was dramatically reduced, by fourfold during the training phase and by thirtyfold during the racing phase, for the horses on continuous equiproxen. These are very provocative data and, if independently confirmed, make a clear case for the benefits of anti-inflammatory medication of young horses in training and in racing.

After oral administration of equiproxen the drug is about 50 percent absorbed. The recommended dose is 10 mg/kg or about 5 mg/lb. After oral administration, plasma levels of the drug peak at about 25 μg/ml between two and three hours after dosing and then decline to less than 1 μg/ml between two and three hours after dosing and then decline to less than 1 μg/ml at twenty-four hours. If the drug is given twice a day, as recommended, one would then get a second peak of drug, which should decline to less than 5 μg/ml by twenty-four hours. Equiproxen thus apears likely to have little tendency to accumulate in the horse (Fig. 6-9).

Equiproxen is a very easy drug to detect in the horse. It is easily detectable by

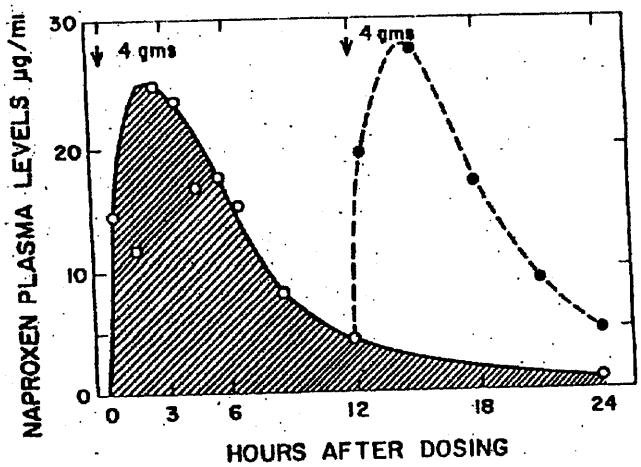


Figure 6-9. Plasma levels of naproxen after its oral administration. The open circles (o—o) show plasma levels of naproxen after dosing with 4 gm orally. The solid circles (●—●) show the calculated blood levels of naproxen if this dose is repeated at twelve hours.

ultraviolet spectrophotometry, either as the parent drug or as its major metabolite, 2-(6-hydroxynapthyl) propionic acid, both of which are excreted in high concentrations in the urine. The approximate half-life of these compounds in equine urine is about six hours, and it appears that at least sixty hours should be allowed for equiproxen to clear the urine of the horse.

Equiproxen appears to be a relatively safe drug in the horse in that three times the recommended dose can be given for up to forty-two days with no lesions attributable to equiproxen. In mares, equiproxen was administered in late pregnancy without apparent effect on the mare or the foal, and these mares were subsequently rebred and conceived normally.

Flunixin Meglumine (Banamine®)*

Flunixin is another member of the NSAID family that has recently been approved for use in equine practice. It appears to be more potent than other members of this group in that a dose of about 1.0 mg/kg produces good clinical effects. The pharmacology and pharmacokinetics of flunixin in the horse have been reported by Houdeshell and Hennessey. These workers reported that an intravenous dose of flunixin improved lameness by 55 percent and swelling by 34 percent at thirty hours postinjection, compared with a 52 percent and 23 percent improvement due to phenylbutazone. While these results might seem to suggest that flunixin is more effective than phenylbutazone, the apparent comparison of these drugs at thirty hours postdosing may be misleading, as phenylbutazone was only effective for twenty-four hours in these particular studies. A more appropri-

* Banamine®, Schering Corporation, Kenilworth, NJ

ate period for evaluating the relative merits of phenylbutazone and flunixin would be the times of peak drug effect, or after the horses had been on both drugs for a period. Because of the uncertainty of the times at which the actions of phenylbutazone and flunixin were compared in these studies, it is difficult to evaluate the relative effectiveness of these drugs. Flunixin produced approximately the same response whether it was given orally, intravenously, or intramuscularly.

Field trials with flunixin for a typical clinical spectrum of musculoskeletal disorders showed that remission of clinical signs occurred after two to three days of therapy. Overall, 40 percent of the horses were rated as having an excellent response, 34 percent as having a good response, 14 percent as fair, and 12 percent as poor. Unfortunately, no control data or positive control information with a well-characterized drug such as phenylbutazone were presented for comparative purposes.

It is characteristic of the NSAIDs that individual members of this group are more effective in some conditions rather than others. Both the literature and clinical experience suggest that flunixin is particularly useful in the treatment of colic and rapidly alleviates pain and distress associated with this condition. In studies on the use of this drug in animals with predominantly spastic and flatulent colics, Vernimb and Hennessey found an excellent or good response in 93 percent atulent colics, in 72 percent of spastic colics, 52 percent of statis colics, and 60 cent of other types. Fifteen percent of horses showed a fair response, and another 25 percent showed no response. The onset of the response to flunixin was also remarkably fast; about 40 percent of horses showed improvement in 15 minutes, and some evaluators reported favorable changes in four to eight minutes. These are remarkably rapid changes to be induced by an antiprostaglandin agent, which, as pointed out previously, generally must reduce existing tissue prostaglandin levels to normal before acting. In fact, a response within four minutes, which is not more than two circulation times for the horse, must be accounted a very rapid response indeed. The duration of relief after flunixin was more in keeping with the known time course of action of this drug and averaged six to eight hours after a single dose.

While flunixin is effective at 1.0 mg/kg, and 4.4 mg/kg of phenylbutazone is needed to produce an equivalent effect, this potency of flunixin should not in any way be taken as a particular advantage for flunixin. As far as the clinician and horseman are concerned, potency is primarily an academic concept. What counts in the clinical setting is the quality of the clinical response and the ratio between the dose producing a good clinical response and that which produces a toxic effect. The actual number of milligrams of a chemical required to produce the clinical effect is of little practical significance as long as its use in effective doses is not associated with side effects or toxic effects. The greater potency of flunixin may, however, have regulatory importance. Because flunixin is more potent than phenylbutazone, it is found in blood and urine samples of much lower concentrations than phenylbutazone or its metabolites. Because of this, flunixin may, under some circumstances, be more acceptable in medication programs than less potent ugs such as phenylbutazone because of its lesser potential for "masking."

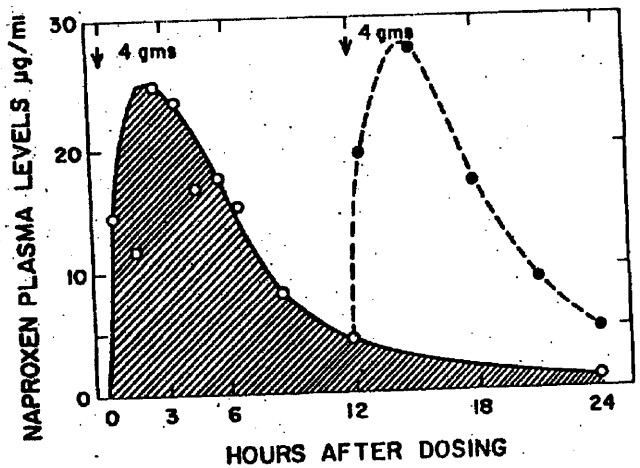


Figure 6-9. Plasma levels of naproxen after its oral administration. The open circles (o—o) show plasma levels of naproxen after dosing with 4 gm orally. The solid circles (●—●) show the calculated blood levels of naproxen if this dose is repeated at twelve hours.

ultraviolet spectrophotometry, either as the parent drug or as its major metabolite, 2-(6-hydroxynapthyl) propionic acid, both of which are excreted in high concentrations in the urine. The approximate half-life of these compounds in equine urine is about six hours, and it appears that at least sixty hours should be allowed for equiproxen to clear the urine of the horse.

Equiproxen appears to be a relatively safe drug in the horse in that three times the recommended dose can be given for up to forty-two days with no lesions attributable to equiproxen. In mares, equiproxen was administered in late pregnancy without apparent effect on the mare or the foal, and these mares were subsequently rebred and conceived normally.

Flunixin Meglumine (Banamine®)*

Flunixin is another member of the NSAID family that has recently been approved for use in equine practice. It appears to be more potent than other members of this group in that a dose of about 1.0 mg/kg produces good clinical effects. The pharmacology and pharmacokinetics of flunixin in the horse have been reported by Houdeshell and Hennessey. These workers reported that an intravenous dose of flunixin improved lameness by 55 percent and swelling by 34 percent at thirty hours postinjection, compared with a 52 percent and 23 percent improvement due to phenylbutazone. While these results might seem to suggest that flunixin is more effective than phenylbutazone, the apparent comparison of these drugs at thirty hours postdosing may be misleading, as phenylbutazone was only effective for twenty-four hours in these particular studies. A more appropri-

* Banamine®, Schering Corporation, Kenilworth, NJ

ate period for evaluating the relative merits of phenylbutazone and flunixin would be the times of peak drug effect, or after the horses had been on both drugs for a period. Because of the uncertainty of the times at which the actions of phenylbutazone and flunixin were compared in these studies, it is difficult to evaluate the relative effectiveness of these drugs. Flunixin produced approximately the same response whether it was given orally, intravenously, or intramuscularly.

Field trials with flunixin for a typical clinical spectrum of musculoskeletal disorders showed that remission of clinical signs occurred after two to three days of therapy. Overall, 40 percent of the horses were rated as having an excellent response, 34 percent as having a good response, 14 percent as fair, and 12 percent as poor. Unfortunately, no control data or positive control information with a well-characterized drug such as phenylbutazone were presented for comparative purposes.

It is characteristic of the NSAIDs that individual members of this group are more effective in some conditions rather than others. Both the literature and clinical experience suggest that flunixin is particularly useful in the treatment of colic and rapidly alleviates pain and distress associated with this condition. In studies on the use of this drug in animals with predominantly spastic and flatulent colics, Vernimb and Hennessey found an excellent or good response in 93 percent atulent colics, in 72 percent of spastic colics, 52 percent of statis colics, and 60 cent of other types. Fifteen percent of horses showed a fair response, and another 25 percent showed no response. The onset of the response to flunixin was also remarkably fast; about 40 percent of horses showed improvement in 15 minutes, and some evaluators reported favorable changes in four to eight minutes. These are remarkably rapid changes to be induced by an antiprostaglandin agent, which, as pointed out previously, generally must reduce existing tissue prostaglandin levels to normal before acting. In fact, a response within four minutes, which is not more than two circulation times for the horse, must be accounted a very rapid response indeed. The duration of relief after flunixin was more in keeping with the known time course of action of this drug and averaged six to eight hours after a single dose.

While flunixin is effective at 1.0 mg/kg, and 4.4 mg/kg of phenylbutazone is needed to produce an equivalent effect, this potency of flunixin should not in any way be taken as a particular advantage for flunixin. As far as the clinician and horseman are concerned, potency is primarily an academic concept. What counts in the clinical setting is the quality of the clinical response and the ratio between the dose producing a good clinical response and that which produces a toxic effect. The actual number of milligrams of a chemical required to produce the clinical effect is of little practical significance as long as its use in effective doses is not associated with side effects or toxic effects. The greater potency of flunixin may, however, have regulatory importance. Because flunixin is more potent than phenylbutazone, it is found in blood and urine samples of much lower concentrations than phenylbutazone or its metabolites. Because of this, flunixin may, under some circumstances, be more acceptable in medication programs than less potent ugs such as phenylbutazone because of its lesser potential for "masking."

If meclofenamic acid is fed orally for five days, urinary concentrations of the drug run at about 25 µg/ml throughout the dosing period. After the last dose of meclofenamic acid, the drug is reportedly detectable in urine for ninety-six hours, although the levels are very low from forty-eight hours on (Fig. 6-13). Again, since meclofenamic acid does not tend to accumulate in the body, the same rate of decline for meclofenamic acid in urine should be followed after single or multiple dose regimens. About 10 to 24 percent of the drug administered is eliminated in the urine, and it is thought that a good proportion of the drug is eliminated via the bile and feces. No data is available on the metabolism of meclofenamic acid in the horse.

In clinical trials on 304 horses suffering from osteoarthritis, navicular disease, laminitis, and soft or bony tissue conditions, it was found that 78 percent of the cases with navicular disease improved, 76 percent with laminitis improved, and 61 percent of the cases with osteoarthritis improved. Since all these cases were carefully screened to exclude horses that might improve with stall rest, these improvements are difficult to compare with reported improvements on other drugs. These workers also reported that it was not possible to predict which horses would improve on meclofenamic acid and which would not.

Signs of toxicity to meclofenamic acid appear at high dose levels (6 to 8 mg/lb) and include mouth ulcers, loss of appetite, depression, edema, and loss of weight. If the dosage rate is kept low (1 mg/lb), however, meclofenamic acid is a safe drug and useful in the treatment of musculoskeletal disease in the horse.

Aspirin

The oldest and best known of the non-steroidal anti-inflammatory group of drugs is aspirin, or acetylsalicylic acid. The fact that aspirin is a household remedy and very readily available should not delude one into thinking that it is anything but a very effective drug. Aspirin has long been the drug of choice in the

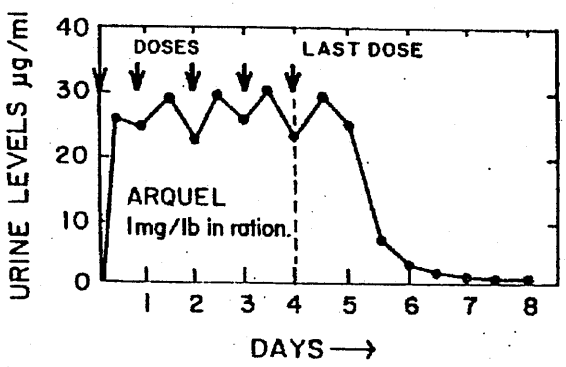


Figure 6-13. Urinary "clearance times" for meclofenamic acid. The solid circles (● —●) show urinary clearance times for meclofenamic acid in four horses after daily oral dosing with 1 mg/lb of Arquel®. Arquel® was still detectable in urine four days after the last dose. After Gannon et al., *AAEP Proceedings*, 1973.

treatment of human arthritis, but it is unfortunately not nearly as effective a drug in the horse.

Where aspirin is concerned, nature has played a number of tricks on horsemen. The first of these is that the urine of horses and other herbivores normally contains small quantities of salicylate. This salicylate either comes from the grass and hay that the horse ingests or is a product of the horse's metabolism. Whatever its origin, however, the result is that it is difficult for a chemist to call a salicylate "positive" because salicylate is a natural constituent of horse urine.

While salicylate might thus seem to be an answer to the horseman's prayer, the drug is, unfortunately very rapidly excreted by the horse. It turns out that as an acidic drug, salicylic acid is rapidly eliminated in the basic urine of the horse. The half-life of salicylate in the blood of horse with basic urine is less than one hour, so it is very difficult to maintain plasma levels of the drug. Thus, if you want to use salicylate effectively in the horse, you must give large doses of the drug and give them relatively close to the time at which you want the drug to take effect.

It would help considerably with the effects of salicylate if one could give aspirin intravenously or intramuscularly and in this way obtain a high blood level of the drug. However, since salicylate itself is not available as an injectable, this approach cannot be taken. On the other hand, a close relative of acetylsalicylic acid, thiosalicylate, is available and can be administered by injection.

Thiosalicylate is chemically a slightly different molecule from salicylic acid, in which an oxygen atom has been replaced with a sulfur atom. This chemical change results in this particular salicylate being available in an injectable form, which means that higher blood levels and thus effective concentrations of the drug are readily attained. No information is available on the half-life of thiosalicylate in the horse. However, it is presumably much like that of salicylate itself. Thiosalicylate was once much used in some racing jurisdictions as a substitute for phenylbutazone, particularly in those states that have upper limits on post-race urinary concentrations of phenylbutazone. Unfortunately, because of the presence of the sulfur atom, it is possible to distinguish this drug from normal salicylate, and a number of thiosalicylate "positives" have been called. Even though it makes little pharmacological sense to allow phenylbutazone and aspirin and ban thiosalicylate, this apparently is the status of thiosalicylate in racing chemistry at the moment.

Dimethyl Sulfoxide (DMSO)

Dimethyl sulfoxide (DMSO) is a colorless liquid with a weak odor, which leaves a bitter taste in the mouth shortly after its application to the body by any route. Chemically, DMSO is unusual in that it mixes well with both water and lipid-soluble compounds, and these chemical characteristics enable it to penetrate rapidly through cell membranes. DMSO also accelerates the movement of other substances through cell membranes and is thus useful in increasing the transcutaneous absorption of drugs. It is approved for use by the FDA in the dog and horse, but it should be used only in the medical or pure form, usually at between 80% and 90% strength.

As well as aiding the transcutaneous penetration of drugs, DMSO has been

reported to have many other pharmacological effects. Among these effects are anti-inflammatory actions, local anesthetic properties, antibacterial effects, and effects on diuresis and vasodilation. DMSO is also hygroscopic, absorbing up to 70 percent of its weight of water from the air. Similarly, when it is added to water, heat is released, and this effect may be produced locally when high concentrations of DMSO are applied to the skin. Most of the clear-cut effects of DMSO appear to require high concentrations of this agent, which means that its most marked pharmacological effects are associated with its local application.

In human medicine, DMSO has been reported by some investigators to be very effective in musculoskeletal disorders. Local treatment with DMSO resulted in marked improvement in about 50 percent of patients with chronic osteoarthritis, bursitis, periostitis, and tendinitis, while partial improvement was obtained in about 35 percent. The duration of treatment was rarely longer than three weeks, the response to DMSO was considered to compare favorably with that of intra-articular corticoids, and treatment with DMSO was associated with a reduction in areas of calcification in some patients.

In the horse, Teigland and Saurino have studied the action of DMSO on an inflammatory model and in clinical practice. In their experimental studies with induced inflammatory responses, the DMSO-treated sites never became as swollen and indurated as the untreated sites. Further, in the DMSO-treated sites the inflammation and edema dissipated more rapidly and the horses appeared to experience less pain and discomfort from the DMSO-treated lesions.

In their clinical studies, Teigland and Saurino reported on the use of DMSO on open wounds with subcutaneous edema and henforrhage. When DMSO was painted onto these lesions twice a day, the wounds were apparently less painful, the edema reduced, and the limb returned to normal more rapidly. Similarly, bursitis and synovitis responded well to these drugs, and osteoarthritic lesions also responded well. DMSO was also useful in the treatment of bucked shins, acting to reduce the discomfort of the animal markedly, but it did not, of course, change the requirement for rest in this condition. Because the lower limbs of the horse are very prone to edema and swelling, DMSO appears to be particularly useful in limb injuries in this species.

Along with its own pharmacological properties, DMSO markedly accelerates the absorption of other drugs, in some cases up to 100-fold. It is therefore often combined with steroids, where it aids their penetration into tissues, or with antibiotics, whose penetration into bacteria and thus antibacterial action it appears to accelerate. DMSO is also occasionally combined with steroids and furacin as a topical wound dressing.

When used in small amounts, the toxicity of DMSO appears to be minimal. Not more than 100 ml per day is the recommended topical dose for a horse, and the drug should not be used in horses treated with cholinesterase-inhibiting anthelmintics. While prolonged use of DMSO has been associated with changes in the lens of the eye in dogs and other mammals, there appears to be little likelihood of this occurring in the horse at the recommended dose rates.

DMSO is one of the few stubstances that an analyst can detect in a urine sample the moment that he opens the urine jar. This is because DMSO is transformed in the body to metabolites that yield a garlic odor, and this odor may be readily detectable in urine samples.

Although DMSO is not difficult to test for, it requires special testing techniques, and since it is a topical medication, most racing jurisdictions do not test specifically for it. Horsemen should be aware, however, that because of the ability of DMSO to accelerate the transcutaneous absorption of other drugs, its topical use in conjunction with agents such as benzocaine or corticosteroids may lead to increased blood or urine levels of these drugs and thus to inadvertent positives for these agents.

Orgotein (Palosein®)*

Orgotein is the generic name adopted for drug versions of a copper- and zinc-containing metalloprotein called superoxide dismutase. In 1964 it was discovered to be a potent anti-inflammatory agent, and it was soon shown to be an enzyme and was named superoxide dismutase. Beyond this, little was understood about the mechanism of its anti-inflammatory action, other than that it was different from most of the other anti-inflammatory drugs then in current use.

Superoxide dismutase has recently been shown to be an intracellular enzyme that is part of the body's defense against the highly toxic O_2^- of superoxide radical. Blood cells such as neutrophils and macrophages generate significant amounts of the superoxide radical as a killing agent in their attacks on bacteria, but these superoxides can also kill the phagocytes themselves. When administered as a drug, superoxide dismutase apparently scavenges this excess superoxide radical and prolongs phagocyte life.

peroxide dismutase also mobilizes polymorphonuclear leukocytes and in-ses their numbers at the site of inflammation. When administered by intra-articular injection it preserves or restores the viscosity of synovial fluid and does not interfere with the activity of host defense cells. In spite of the fact that it is a foreign protein, it appears to be very poorly antigenic, and it is essentially nontoxic. It may be administered by any route, but the safest and most economical route would appear to be intra-articular.

Clinical trials in humans have reportedly shown that orgotein is a safe, effective nonanalgesic anti-inflammatory drug. Its effects apparently take from two to six weeks to develop and persist for up to one month after termination of treatment. Orgotein may be administered systemically or, when the pathology is localized, injected into and around a site of a chronic inflammation.

In Sweden, Ahlengard and coworkers studied the efficacy of intra-articular orgotein in a series of cases of noninfective traumatic arthritis in horses. These workers concluded that intra-articular orgotein produced beneficial results, the effects being most marked (94% recovery) in horses that had shown lameness for less than two months, and less marked (49%) in horses that had been lame for more than two months before treatment.

Decker and coworkers also reported on the treatment of seventy horses with local and/or intra-articular injection of Palosein®. These workers considered the response obtained excellent, with fifty-three of the horses returning to racing

* Palosein®, Diagnostic Data, Inc., California

within a few days and some winning their races. Of twenty horses with fetlo problems, sixteen responded to treatment and most of these after only a sin dose of the drug.

As a naturally occurring metalloprotein of high molecular weight, Palosein" not likely to enter equine urine in significant amounts. Even if it did; it would l difficult to recover, detect, and prove to be of exogenous origin by currently us drug testing techniques.

Hyaluronic Acid

Hyaluronic acid is an essential component of synovial fluid and also of articula cartilage, which has recently been introduced as an intra-articular therapy fo joint problems. High molecular weight hyaluronic acid, which is the form injecte into joints, inhibits lymphocyte migration and phagocytosis and also reduces th permeability of the synovial membrane. Usually between 20 and 40 mg* of th sodium salt of hyaluronic acid (10 mg/ml) is injected into the joint, with a equivalent volume of synovia being removed. Reporting on a study of the effect of hyaluronic acid treatment in racing horses, Asheim and Lindblad saw "fr quently very good effects" with hyaluronic acid. These good effects were seen i horses that had previously been point fired, blistered, and sometimes treated wit intra-articular corticosteroids. These authors considered it remarkable that i most cases injection of 1 to 2 ml of hyaluronic acid was sufficient to cure th lameness. The only factors that appeared to predictably interfere with therap were pronounced bony changes in the joint or prior treatment with corticoster oids.

The mode of action of hyaluronic acid is not clear, but it appears to persist in th joint for days after an injection. One theory is that hyaluronic acid has good surface-protecting properties. Because of its high molecular weight and the large number of electrical charges carried by hyaluronic acid, diffusion of hyaluronic acid out from an intra-articular injection site and its detection in urine by curren routine screening methods is highly unlikely.

In summary, reviewing the properties of the non-steroidal anti-inflammator drugs, it is apparent that phenylbutazone is still the standard against which othe drugs are compared. All these drugs share a number of broadly similar character istics in that dosages are approximately equivalent from drug to drug, their time course of action is broadly similar, and their detection in blood or urine is not particularly difficult. There are, however, subtle differences in their clinical effectiveness against certain conditions, which makes certain agents the drugs of choice for specific conditions. In this way flunixin is apparently more effective against colic than other members of this group and is the drug of choice in this area. Similarly, Naproxen* appears to be the drug of choice for muscle soreness and is reported to be particularly effective in young horses with minor muscle problems. Meclofenamic acid has acquired a reputation for being useful in foot and hoof problems, and other specific strengths of individual members of this group will doubtless become apparent as clinical experience with these drugs

* More recent work suggests that smaller doses may be used.

increases. Therefore, despite the basically similar mechanism of action of the NSAIDs, they are all far from clinically equivalent, and it is a good idea to try different NSAIDs on clinical conditions that might be expected to respond to these drugs if the response to one member of this group is poor.

SELECTED REFERENCES

Ahlengard, S., Tufuesson, G., Petterson, H., and Andersson, T.: "Treatment of traumatic arthritis in the horse with intra-articular orgotein (Palosein®)." *Eq Vet J, 10*:122-124, 1978.

Ashelm, A., and Lindblad, G.: "Intra-articular treatment of arthritis in race horses with sodium hyaluronate." *Acta Vet Scand, 17*:379-394, 1976.

Clofalo, V. B., Latranyl, M. G., Patel, J. B., and Taber, R. I.: "Flunixin meglumine: a non-narcotic analgesic." *J Pharm Exp Ther, 200*:501-507, 1977.

Conner, G. H., Riley, W. F., Beck, C. C., and Coppock, R. W.: Arquel (Cl-583), "A new non-steroidal anti-inflammatory drug for horses." *Proc AAEP, 19*:81-90, 1973.

Davis, L. E., and Westfall, B. A.: Species differences in biotransformation and excretion of salicylate." *Am J Vet Res, 33*:1253-1262, 1972.

Decker, W. E., Edmonson, A. H., Hill, H. E., Holmes, R. A., Padmore, C. L., Warren, H. H., and Wood, W. C.: "Local administration of orgotein in horses," *MVP, 9*:773-774.

Ferreira, S. H., and Vane, J. R.: "New aspects of the mode of action of nonsteroid anti-inflammatory drugs." *Ann Rev Pharmacol, 14*:57-73, 1974.

Gabel, A. A., Tobin, T., Ray, R. S., and Mayling, G. A.: "Phenylbutazone in horses." A review. *J Eq Med Surg, 1*:221-225, 1977.

Gabriel, K. L., and Martin, J. E.: "Phenylbutazone short term versus long term administration to Thoroughbred and Standardbred horses." *JAVMA, 140*:337-341, 1962.

Goodman, L. S., and Gilman, A.: *The Pharmacological Basis of Therapeutics*, 5th ed., Macmillan Publishing Co., New York (1975).

Hamm, D.: "Continuous administration of naproxen to the horse during training." *J Eq Med Surg, 2*:125-128, 1978.

Poudeshell, J. W., and Hennessey, P. W.: "A new nonsteroidal anti-inflammatory analgesic for horses." *J Eq Med Surg, 1*:57-63, 1977.

[illegible]es, E. W., and Hamm, D.: "Comparative efficacy of phenylbutazone and naproxen in induced equine myositis." *J Eq Med Surg, 2*:341-347, 1978.

Kilian, J. G., Jones, E. W., Hamm, D., Riley, W. F., and Averkin, E.: "The efficacy of equiproxen (Naproxen) in a unique equine myositis model." *Proc AAEP, 20*:201-215, 1974.

Linton, J. A. M.: "The use of orgotein in the treatment of soft tissue injuries of the horse." *Irish Vet J, 30*:53-46, 1976.

Maylin, G. A.: "The disposition of phenylbutazone in the horse." *Proc AAEP, 20*:243-248, 1974.

Norheim, G., Hoie, R., Froslie, A., and Bergsjo, T. H.: "Gas chromatographic determination of small amounts of phenylbutazone and oxyphenbutazone in horse plasma and urine." *Fresenius Z Anal Chem, 289*:287-288, 1978.

Piperno, E., Ellis, D. J., Getty, S. M., and Brody, T. M.: "Plasma and urinary levels of phenylbutazone in the horse." *JAVMA, 153*:195-198, 1968.

Pratt, G.: "Breakthrough on understanding lameness." *The Blood-Horse, 103*:3758-3760, 1977.

Riley, W. F., Romane, W. M., Ellis, D. J., and Haury, K. W.: "Preliminary report on a new nonsteroidal anti-inflammatory agent in the horse." *Proc AAEP, 17*:293-300, 1971.

Salin, M. L., and McCord, J. M.: "Free radicals and inflammation protection of phagocytosing leukocytes by superoxide dismutase." *J Clin Invest, 56*:1319-1323, 1975.

Sanford, J.: "Effects of drugs on performance of the horse." In *Symposium on Large Animal Therapeutics*, University of Surrey, Guildford, Surrey, 1978.

Snow, D., and Bogan, J. A.: "Phenylbutazone toxicity in ponies," *Vet. Rec., 105*:26-30, 1979.

Teigland, M. G., and Saurino, V. R.: Clinical evaluation of dimethyl sulfoxide in equine applications. *Ann NY Acad Sci, 141*:471-477, 1967.

Tobin, T.: "Pharmacology Review: The nonsteroidal anti-inflammatory drugs. I. Phenylbutazone." *J Eq Med Surg, 6*:253-258, 1979.

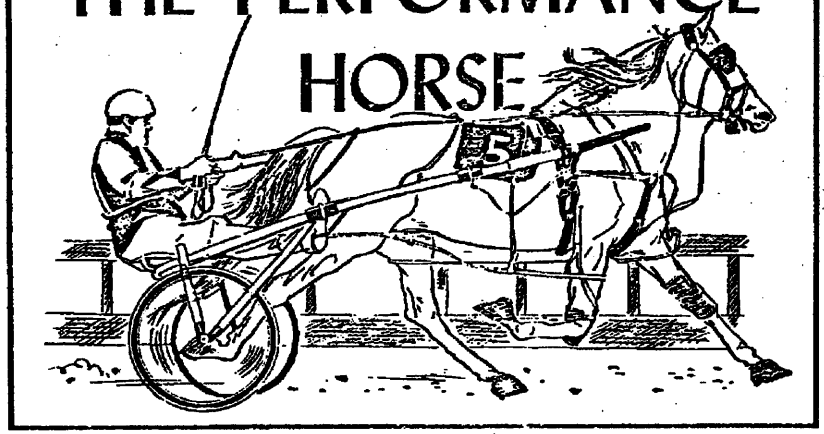
Tobin, T.: "Review: The nonsteroidal anti-inflammatory drugs. II. Equiproxen, meclofenamic acid and others." *J Eq Med Surg, 7*:298-302, 1979.

Vernimb, G. D., and Hennessey, P. W.: "Clinical studies on flunixin meglumine in the treatment of equine colic." *J Eq Med Surg, 1*:111-116, 1977.

Wanner, F., Rollinghoff, W., Gerber, H., and Preiny, R.: "A preliminary report; demethylations, hydroxylation and acetylation in the horse. Side effects of repeated phenylbutazone medication." Proceedings of the Third International Symposium on Equine Medication Control, Tobin, T., Blake, J. W. and Woods, W. E., Eds. Dept. of Veterinary Science, University of Kentucky, 1979.

Wigren, A., Falk, J., and Wik, O.: "The healing of cartilage injuries under the influence of joint immobilization and repeated hyaluronic acid injections." [illegible]

DRUGS AND THE PERFORMANCE HORSE



THOMAS TOBIN

M.V.B., M.Sc., Ph.D., M.R.C.V.S.

Kentucky Equine Drug Research Program
Department of Veterinary Science
University of Kentucky
Lexington, Kentucky

With Forewords by

H.R.H. The Prince Philip
Duke of Edinburgh
and
Ernst Jokl, M.D.

Honorary President, Research Committee
UNESCO, International Council of
Sport and Physical Education

"The great value of this book is that for the first time the facts about contemporary medications, drugs and analytical techniques are set out in plain unmistakable language by an acknowledged world authority. It is a book that will be welcomed by competitors, trainers, coaches, veterinarians and administrators."

–from the Foreword by H.R.H. The Prince Philip, Duke of Edinburgh

CHARLES C THOMAS • PUBLISHER • SPRINGFIELD • ILLINOIS

use is permitted is also not supported by the data. Figure 23-4 shows the blood levels of phenylbutazone found after various dosage schedules and drawn from the data of a number of different workers. The data show that blood levels of phenylbutazone in horses at about twelve hours after dosing (i.e. at optimal drug effect) are about 10 μg/ml. Similarly, if one studies plasma levels of drugs in a population of horses racing under a controlled medication program, one finds (Fig. 23-5) that the mean blood level of phenylbutazone in these horses is also about 9 to 10 μg/ml, with a fairly tight range around the mean. These data suggest no evidence for abuse or overusage of the drug and do not support widespread claims that phenylbutazone is abused under controlled medication programs.

The last and most emotional charge against phenylbutazone is that its use results in an increased number of breakdowns. In the layman's mind this charge has a certain plausibility, since phenylbutazone is thought of as being a powerful painkiller that enables injured horses to run "sound." Inspection of the available data, however, is not consistent with this hypothesis and is more consistent with

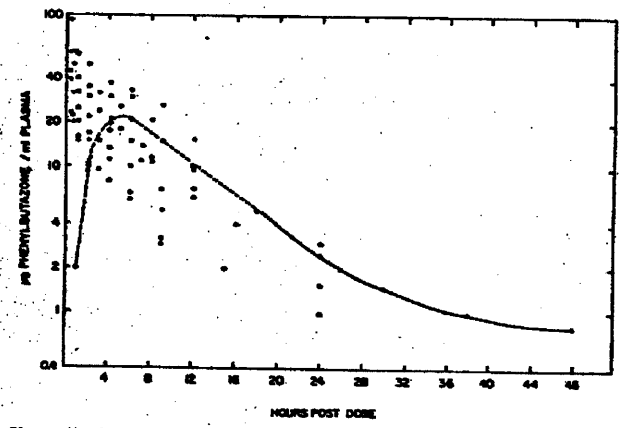


Figure 23-4. Plasma levels of phenylbutazone after different dosage schedules. The symbols show the plasma levels of phenylbutazone observed after administration of different dosage schedules of this drug to horses by different workers, as indicated below. The data show that therapeutic dosage schedules of phenylbutazone are associated with blood levels of this drug of about 10 μg/ml at the time of optimal therapeutic effect, which occurs at about twelve hours after dosing. The key presented below gives the data source, dose of phenylbutazone, and method of dosing.

- o Tobin, unpublished data 1/80; n=2, 4 mg/kg IV, GC method.
- • Tobin, *Am J Vet Res, 38*:123, 1977; n = 4, 6.6 mg/kg IV, spectrophoto. method.
- □ Tobin, *Am J Vet Res, 38*:123, 1977; n=4, 6.6 mg/kg IV, GC method.
- ▪ Maylin, *Proc 20th Meeting AAEP*, 1974, pp. 243-48; n=3, 4.4 mg/kg IV, GC method.
- ▲ Maylin, *Proc 20th Meeting AAEP*, 1974; same experiment, multiple doses (0 hr, 24 hr, 48 hr, 72 hr); data from 72 hr injection.
- ▲ Piperno et al, *J Am Vet Med Assoc, 153*:195, 1968; n=6, 4.4 mg/kg IV, spectrophoto. method.
- ▼ Piperno et al, *J Am Vet Med Assoc, 153*:195, 1968; n=1, 8.8 mg/kg IV, spectrophoto. method.
- ▼ Piperno et al, *J Am Vet Med Assoc, 153*:195, 1968; n=1, 17.6 mg/kg IV, spectrophoto. method.
- ◊ Piperno et al, *J Am Vet Med Assoc, 153*:195, 1968; n=1, 4.4 mg/kg IV, spectrophoto. method.
- ♦ Tobin et al, *J Equine Med Surg, 1*:402, 1977; n=7, 6.6 mg/kg IV, GC method.

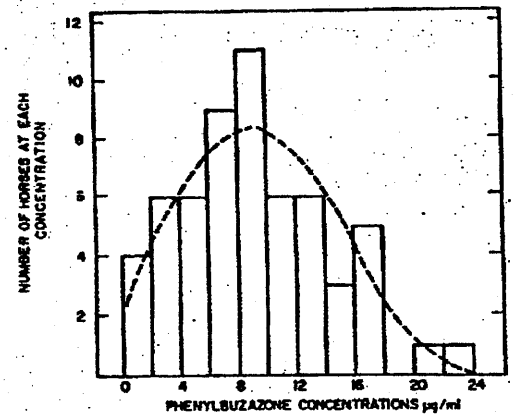
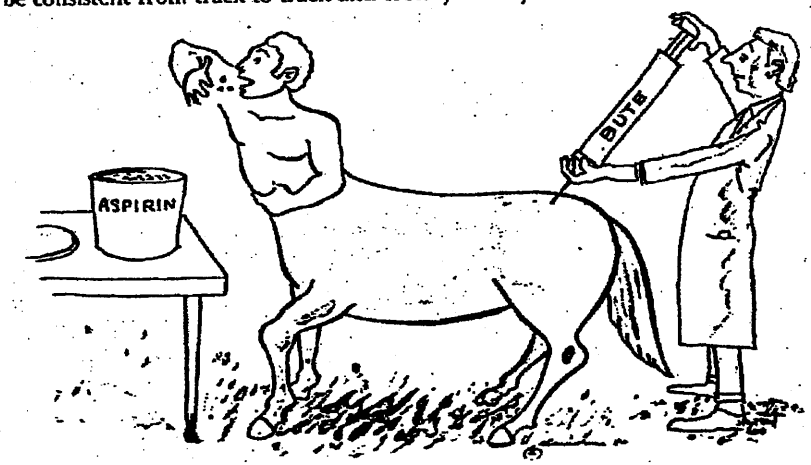


Figure 23-5 Phenylbutazone concentrations in the blood of fifty-eight racing horses. In a study in a midwestern racing state, blood levels of phenylbutazone in fifty-eight racing horses were measured. The vertical bars show the numbers of horses with blood levels of phenylbutazone in the indicated ranges. The dotted line represents an approximate distribution curve drawn by eye. The data show that the median blood level of phenylbutazone in the blood of these horses was about 9 µg/ml, with no blood levels above 24 µg/ml. The blood levels of phenylbutazone in these horses, therefore, cluster around the level to be expected in the blood of horses after therapeutic doses of this drug.

what is known of the pharmacology of phenylbutazone in the horse and, to bring the point home to the layman, of its human equivalent, aspirin.

The first point that has to be borne in mind when analyzing breakdown data is that the term "breakdown" has never been defined. Because of this, one man's definition of breakdown may be an injury severe enough to require destruction of a horse, while another man's breakdown may be the horse pulling up lame. Because there is no agreed operational definition as to what constitutes a breakdown, a more reasonable approach is to use the figures of accidents either fatal or leading to horses being "put down" on the track. This is because the circumstances under which horses are put down are fairly clearly defined and thus are likely to be consistent from track to track and from year to year.



As a drug, phenylbutazone in the horse is the approximate equivalent of aspirin in man.

As of this writing, three sets of "statistical" data on the effects of phenylbutazone on the incidence of breakdowns are available. The most clear-cut data are those from California racing, which are presented in Figure 23-6. As shown in this illustration, the rate at which horses broke down or had to be destroyed was not changed by the advent of racing on phenylbutazone in California. The data on horses destroyed on the track over this period are particularly convincing, since the indications for destruction are relatively unambiguous and not likely to vary much between different veterinarians or different tracks. On the other hand, "breakdown" is a poorly defined term, and the types of conditions described by this term are more likely to vary from track to track and from veterinarian to veterinarian. The relative stability of the number of horses destroyed in California racing, which has held steady at about 100 per year or about one in 500 starters over the decade of the 1970s, speaks very strongly against an increase in the injury rate of horses racing on phenylbutazone.

Further, comparison of the figures for the percent of starters on phenylbutazone against the percent of winners on phenylbutazone shows that the percentages of horses in both categories are approximately equal (Table 23-III). Horses running on phenylbutazone, therefore, do not appear to have any significant advantage over untreated horses, and these data, of course, suggest that they are not any less likely to finish (i.e. to break down) than untreated horses.

These data and conclusions from California Thoroughbred racing are well supported by data from Colorado Thoroughbred racing. According to Doctor

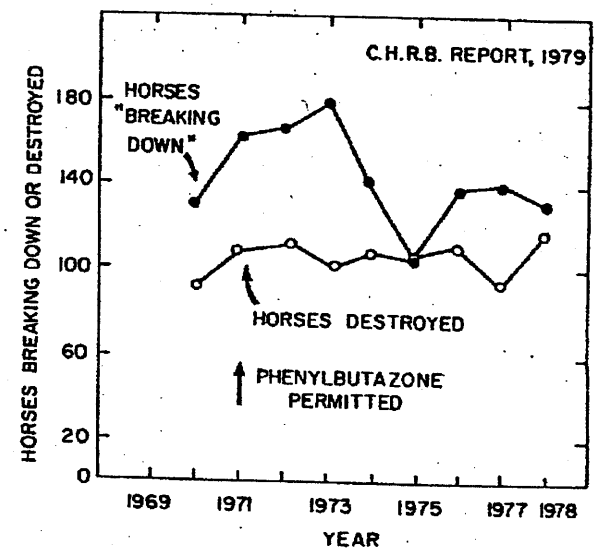


Figure 23-6. Breakdowns and horses destroyed in California racing. The open circles (o—o) show the number of horses destroyed in California racing, corrected for the number of starters, from 1970 to 1978, while the solid circles (●—●) show the number of breakdowns occurring over the same period.

TABLE 23-III
CONTROLLED MEDICATION STATISTICS
CALIFORNIA

Year	Starters on "Bute" %	Winners on "Bute" %
1971	47	48
1972	50	49
1973	52	50
1974	55	56
1975	61	62
1976	65	66
Six-Year Average:	55	54.33

Gene Bierhaus of the Colorado Racing Commission, the probability of a horse having an accident that requires his destruction is independent of whether or not the horse is on phenylbutazone. Further, the overall destruction rate of horses running in Colorado during the 1970s was a relatively low (1/1020), even though about 60 per cent of these horses were on phenylbutazone (Table 23-IV). This rate was less than half the rate at which breakdowns requiring destruction occurred prior to the advent of medication and has led Doctor Bierhaus to conclude that in his experience the incidence of breakdowns requiring destruction has not been increased by the advent of controlled medication programs.

These data from California and Colorado Thoroughbred racing are well sup-

TABLE 23-IV
COLORADO THOROUGHBRED RACING BREAKDOWNS
1954-1979

Year	Days of Racing	# Destroyed (Butazolidin was not available)
1954	45	12

In 1954 all of the racing was during the summer and track conditions were better, compared to 1971-77. 1/444 starters had to be destroyed.

Year	Days of Racing	Destroyed On "bute"	Destroyed No "bute"	Total
1971	60	4	2	6
1972	60	2	3	5
1973	67	5	4	9
1974	92	6	3	9
1975	84	3	2	5
1976	88	4	3	7
1977	99	5	2	7
1978	96	5	2	7
1979	44 (completed)	1	0	1
Total		35	21	56
% Breakdowns		62.5%	37.5%	100%

% of horses running on "bute" varies between 60 and 65%.

Sources: Doctor Gene Bierhaus, Chief Commission Veterinarian, Colorado, personal communication, 1979; Poppell, Carolyn F. (Assistant Science Advisor): *Issue Brief: Phenylbutazone and Furosemide in Race Horses in Maryland.* Maryland General Assembly, 1979.

ported by a careful pilot study carried out by workers from the University of Pennsylvania at Pennsylvania National Racetrack and reported at the Jockey Club Round Table conference, 1979. This study extended over a period of four months and covered forty-five days of observation. In this study, a breakdown was defined as an injury severe enough to prevent a horse finishing a race. Out of 3,910 horses at risk in this meet, they found 19 breakdowns, or a rate of about 1 in 206. These workers were very careful to point out that this breakdown rate was *"not higher than the incidence of injuries occurring at tracks in New Jersey and New York, which have restrictions on the use of phenylbutazone."* These observations would appear to deny claims that tracks allow the use of phenylbutazone become dumping grounds for unsound horses that can only run on "bute." Whatever the circumstances that brought horsemen and their horses to Pennsylvania National Racetrack, and whether or not they ran on phenylbutazone, these horses were not any more likely to break down than supposedly "sounder" horses racing without the benefit of phenylbutazone at neighboring tracks.

It appears, therefore, that at this point the weight of the evidence available suggests that the use of phenylbutazone is not associated with an increase in the breakdown rate. It must be emphasized, however, that the available data are far from complete and that the analyses performed on these data have, in general, been quite unsophisticated. The only detailed study in this area has been the University of Pennsylvania study, and more studies of this caliber would be very useful.

A number of other arguments have also been advanced against the use of phenylbutazone and furosemide in horses, which arguments have been repeated at the highest levels of government. It has been argued, for example, that the FDA has not approved furosemide for use in the treatment of epistaxis. The FDA however, has explicitly approved furosemide for use in the treatment of pulmonary congestion or lung edema in the horse. Since it is this action against pulmonary congestion which appears to be the basis of its action against epistaxis, a good case can be made that this approval covers its use in "bleeders."

The argument that these drugs are being put to uses for which they were not "intended" betrays ignorance of the whole human experience with drugs. This argument appears to suppose that somewhere, perhaps under the care of some incredibly ancient and learned university professor, is a large book, full of lists of intended uses for drugs. Furthermore, since nobody knows where these lists come from or whether the lists just spring into existence, there must therefore exist lists of "intended" and proper uses for those drugs that man has not yet discovered.

This whole concept of "intended" uses for drugs so dear to the hearts of certain people is, of course, just so much rubbish. There are no "intended" uses for drugs. All but one of the 4,000 drugs in common use today were discovered by chance, and *all* drug actions and effects have been discovered by trial and error. There are no "intended" uses for drugs, there never have been, and there never will be. The uses for drugs in man and horses have been discovered by man by careful observation, and the clinical use of phenylbutazone and furosemide in performance horses certainly comes under that heading.

The final argument, presented to cap all arguments about phenylbutazone and

furosemide, is that these medications do not cure any of the conditions that they treat. However, one must remember that in this imperfect world there are no drugs that "cure" anything. For example, if the reader is unfortunate enough to catch an infectious disease, he or she may be left with the feeling that the doses of penicillin cured it. Well, you are, of course, cured, as long as you stay away from the cause of the problem, but if you are reexposed to the infection, the chances are that you are going to have to keep taking penicillin. Similarly, the horse on phenylbutazone will heal and cure, spontaneously, with or without phenylbutazone, but if you run him to the point that brought on the condition in the first place, then it is going to recur. Which brings us to the last point. The alternative to phenylbutazone that is commonly suggested is to "lay up" the horse. Laying up, of course, does not cure anything either, because the condition will only recur when the horse is returned to the track, the environment in which the condition developed in the first place.

In summary, therefore, controlled medication programs represent one approach to the substantial technical and regulatory problems that are involved in medication control. These programs were carefully thought out and implemented and were well accepted in the racing community. When used in conjunction with good pre-race examinations and good laboratory support, they represent a very workable approach to the problem of medication control. Controlled medication programs are, however, peculiarly susceptible to attack from individuals and groups who feel that it is basically wrong to allow horses access to modern therapeutic medication during a competitive event.

THE LONG-TERM EFFECTS OF MEDICATION

The final problem, and by far the most difficult question to answer is what the use of medication is going to do to what is rather loosely called the "breed." The prophets of doom hold that permissive medication will give rise to a "breed" of horses that will only be able to run on "bute." The essence of this position was put forward by the stewards of the turf authorities of Ireland, Great Britain, and France, who issued a joint statement on this problem in 1971, which read as follows:

> It has been suggested that the Rules of Racing in our three countries should be relaxed to permit the use of phenylbutazone and similar anti-inflammatory drugs.
>
> In a number of American states the use of phenylbutazone is permitted, but only under strict supervision, and it is significant that, where this happens, a horse once given the substance must continue to receive it throughout the season, whether its physical state requires it or not, in order to prevent any possibility of discrepancies in form.
>
> This practice we consider completely undesirable and we are advised that the use of phenylbutazone can seriously jeopardise the soundness of our blood lines and would be liable to have a most damaging effect on the breeding industry.
>
> We wish to make it absolutely clear that under no circumstances should this situation be allowed to occur and we therefore reaffirm our intention to adhere strictly to the existing Rules of Racing and will severely penalise those responsible for any breach of the rules.

Responding promptly to this statement from the powers that be in European Thoroughbred racing, the Royal College of Veterinary Surgeons immediately

EQUINE MEDICINE AND SURGERY

THIRD EDITION
VOLUME ONE

Edited by

R.A. MANSMANN, VMD, PhD
Santa Barbara Equine Practice
Goleta, California

E.S. McALLISTER, VMD, MS
Diplomate, American College of
Veterinary Internal Medicine
Centre Equine Practice
Centre Hall, Pennsylvania

P.W. PRATT, VMD
Book Editor
American Veterinary Publications
Santa Barbara, California

AMERICAN VETERINARY PUBLICATIONS
DRAWER KK, SANTA BARBARA, CALIFORNIA
1982

CHAPTER 6

Phenylbutazone is generally used to relieve the pain of musculoskeletal disorders because of its low cost, unless there is concern regarding IV administration. Phlebitis and collapsed jugular veins are a complication of IV use of phenylbutazone.[12] In the event that benefits are incomplete, signs of toxicity occur or prolonged therapy is necessary, use of an alternative prostaglandin inhibitor may be preferable.[12,16] Phenylbutazone is a safe drug apart from the possibility of phlebitis; however, large doses may cause depression, and daily administration at 10 mg/kg body weight for 7-14 days has caused oral ulceration, depression, GI edema, and decreased concentrations of plasma protein and Ca in ponies.[18]

Benefit from meclofenamic acid administration may take 72 hours or longer to appear. In contrast, the effect of phenylbutazone, naproxen and flunixin meglumine can be detected as early as 1-2 hours after administration, with a maximum benefit in 8-14 hours. Some benefit may be detected as long as 24-30 hours after administration.[8,12,15] In view of this duration of effect, treatment with these products more than once daily may be necessary. In any case, benefits are not dose-related beyond the recommended dosage level.[15] The effect of phenylbutazone may, however, be prolonged when higher doses are used.[12] As much as 48-60 hours may be required for phenylbutazone and naproxen to disappear from the urine.[12,13] Naproxen may be especially appropriate for soft tissue and muscular disorders, while clinical impressions favor the use of flunixin meglumine or meclofenamic acid for skeletal disorders.[15]

These products may also be used prophylactically since they do not impair healing or immunity.[8] They are useful to control postsurgical pain and swelling, and may prevent soreness and injury associated with training.[12,13,15] Phenylbutazone administered 23 hours before time trials has improved performance, perhaps due to the relief of subclinical lameness.[12] In a controlled study, naproxen prevented training complications.[19] Intermittent (2-3 times weekly) administration of similar products resulted in apparent clinical benefit.

Prostaglandin inhibitors suppress the prostaglandin-mediated responses of inflammation, pain and fever. The extent to which they accomplish such benefits varies according to the product and tissues involved. Product variability may account for clinical impressions that concurrent use of more than one prostaglandin inhibitor may enhance their benefit.[8] These products are generally considered safe; however, they may cause an increased propensity for gastric ulcer formation, depression and GI irritation or edema. High dosages (6-8 mg/lb) of meclofenamic acid have caused oral ulcers and depression.[18] It is important that suppression of prostaglandin synthesis is relatively nonspecific and a variety of body functions may thereby be influenced.[8] Gastrointestinal lesions are possibly related to suppression of prostaglandins important to mucosal function; in some species, they influence blood pressure, luteal function, duration of pregnancy and pulmonary ventilation-perfusion relationships.[8] This broad spectrum of influence should be considered relative to potential adverse reactions.

Antihistamines

Just as prostaglandin inhibitors suppress the second (histamine-independent) phase of inflammation, so do antihistamines alleviate the initial histamine-dependent phase of inflammation.[4,5,16,20] Significant anti-inflammatory and antipyretic effects result from supplementation of a corticosteroid-antibiotic combination with chlorpheniramine maleate.[2,15] The antihistamine significantly alleviated signs of inflammation, fever and malaise within 2-4 hours, in contrast to the benefits of the corticosteroid therapy alone, which were not apparent until 8-12 hours after initial therapy. Such a use for the antihistamine is a slight variation from the more traditional recommendations for its use in allergies, hypersensitivities and laminitis. Unless a single mediator is responsible for a complex process such as inflammation, any single antagonist can have only limited efficiency. The concurrent administration of anti-inflammatory agents to attack various mediators, especially in different stages of inflammation, is a valid therapeutic procedure. Clinical observations have suggested that concurrent use of antihistamines and antiprostaglandins or corticosteroids is indeed beneficial for the early relief of acute inflammations, such as laminitis.[5,7,15]

Steroidal Anti-Inflammatory Drugs

While corticosteroids appear to influence primarily the histamine-independent or second phase of inflammation, their action is complex.[4,5,7,21] Corticosteroids impair prostaglandin production by preventing the release of fatty acid substrates necessary for prostaglandin synthesis. They are antimitotic, suppress the immune response (especially cell-mediated im-

Pharmacology Review: The Nonsteroidal Anti-Inflammatory Drugs. I. Phenylbutazone.

Thomas Tobin, D.V.M., Ph.D.

Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, Kentucky 40546.

The anti-inflammatory drugs in use in equine medicine fall into two principal categories, the corticosteroids, which we have dealt with previously[1] and all the others. These other drugs are known collectively and logically as nonsteroidal anti-inflammatory drugs" (NSAID).[2] The original member of this group is aspirin. The second oldest is phenylbutazone, which was introduced into human medicine around the time of World War II and shortly thereafter into veterinary medicine. Phenylbutazone was widely used in human medicine for a number of years,[16] but it soon became apparent that its use was associated with a small incidence of specific and serious toxicities.[6] Because phenylbutazone has caused deaths in humans from aplastic anemia and agranulocytosis, its use in human patients is now restricted. With the realization of the therapeutic limitations of the corticosteroid derivatives and phenylbutazone, and the huge market for a good anti-inflammatory drug, drug companies have researched the nonsteroidal anti-inflammatory field thoroughly in the last 10 years. The upshot of this has been that a large number of new nonsteroidal anti-inflammatory drugs have been developed and have recently become available in both human and equine medicine.

The nonsteroidal anti-inflammatory drugs all share a number of properties which seem to be required for their anti-inflammatory action (Figure 1). All are acidic drugs, with a pKa of 4.5 or less.[2,3] The acidic nature of these drugs means that they are all between 95% and 99% bound to plasma proteins. Because of this, drugs of this group do not pass into saliva, and saliva testing is essentially useless for detection of this important group of drugs. Their acidic nature also seems to be important for their action and has led to suggestions that their acidity enables them to accumulate in inflamed tissues, which also tend to be acidic.[2] This accumulation would then allow them to have more effect in inflamed areas than in normal tissue. However, these agents also tend to accumulate in the stomach, small intestine and kidney,[1] and we will see later that the toxicities produced by these agents are usually associated with these tissues (Figure 2). Also, because prostaglandins are involved in the generation of fever, all these drugs are antifever or antipyretic agents (Figure 2).

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ARE:

(1) HIGHLY ACIDIC DRUGS

(2) HIGHLY PROTEIN BOUND IN PLASMA

(3) DIFFICULT TO DETECT IN PLASMA

(4) ACT BY INHIBITING PROSTAGLANDIN SYNTHESIS

(5) ARE LIKELY TO BE DILUTED IN URINE BY FUROSEMIDE

ARQUEL (SHOWN BELOW) IS A SIMPLE NONSTEROIDAL ANTI-INFLAMMATORY DRUG.

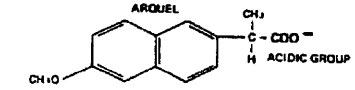


Figure 1. Properties of nonsteroidal anti-inflammatory drugs.

Among the nonsteroidal anti-inflammatory agents, phenylbutazone remains the most popular and widely used in equine medicine. After its introduction into equine medicine, phenylbutazone established itself as a very effective anti-inflammatory agent. It soon made its appearance at the racetrack, and pressure from horsemen for permission to use it during racing

NONSTEROIDAL ANTI-INFLAMMATORY DRUGS:

(1) INHIBIT ENZYMES WHICH FORM PROSTAGLANDINS AND THEREFORE SLOWLY REDUCE TISSUE PROSTAGLANDINS

(2) REDUCE PAIN IN INFLAMED TISSUES BY REDUCING TISSUE PROSTAGLANDIN LEVELS

(3) REDUCE FEVER (ARE ANTIPYRETIC)

(4) ACCUMULATE IN STOMACH, KIDNEY AND SMALL INTESTINES AND TEND TO PRODUCE LESIONS IN THESE TISSUES.

Figure 2. Effects of nonsteroidal anti-inflammatory drugs.

[a] Members of the nonsteroidal anti-inflammatory group of drugs include aspirin, ibuprofen, naproxen, flufenamic acid, fenamic acid, mefenamic acid, indomethacin (the most potent), niflumic acid and meclofenamic acid. All these drugs share the same basic mechanism of action and the same general pharmacology. Miscellaneous nonsteroidal anti-inflammatory drugs currently used in veterinary medicine include orgotein and hyaluronic acid. Because the mechanism of action of these agents is quite distinct from the antiprostaglandin agents they are usually not thought of as typical NSAID's.

increased. In 1958, Colorado approved phenylbutazone for use in racing, and the era of controlled medication was born.[a]

Phenylbutazone and the other nonsteroidal anti-inflammatory drugs produce their anti-inflammatory effects by inhibiting the production of chemicals called "prostaglandins."[14] Prostaglandins are produced in large amounts in inflamed tissues and are involved in the blood vessel changes which cause the reddening, heat, swelling, pain and loss of function that we associate with inflammation. They also have another peculiar action. In that they act to supersensitize pain receptors in the inflamed area to agents which cause pain. Anybody who has had anything as simple as a good sunburn is familiar with the great increase in sensitivity to even the lightest touch which occurs in sunburned tissue. This hypersensitivity is a typical effect of certain prostaglandins. In fact, if minute amounts of prostaglandins are injected into a normal joint, that joint soon becomes excruciatingly painful to move. This sensitizing action of the prostaglandins is represented schematically in Figure 3. It is clear from this mechanism of action that if the concentration of prostaglandins in inflamed tissues can be reduced, the signs of inflammation, swelling, and particularly the perception of pain in that area will also be reduced.

When phenylbutazone is injected intravenously it takes about 30 minutes to distribute throughout the horse and to begin blockage of formation of the prostaglandins. There are, however, unusually large amounts of prostaglandins already present in an inflamed tissue.[15] Therefore, even though the formation of new prostaglandins is blocked, phenylbutazone will not appear to take effect until these high prostaglandin levels have been reduced. This process usually takes about three to four hours, and once the excess prostaglandin has dissipated, the supersensitivity to pain reduces and the swelling and heat in the area begin to decline. Loss of these signs of inflammation will require an additional period of time, and it may take up to 12 hours or more for the full pharmacological action of phenylbutazone to become apparent.[12]

Since all the nonsteroidal anti-inflammatory agents produce their effects in more or less the same way, they all take a period of at least several hours to begin producing effects, even when they are given by intravenous (IV) injection. On the other hand, when blood levels of these drugs decline, the concentration of the prostaglandins in the inflamed tissues builds back up again, and the pain and other signs of inflam-

[a] Bierhaus, G.H.: Personal communication. Colorado Racing Commission Veterinarian (1979).

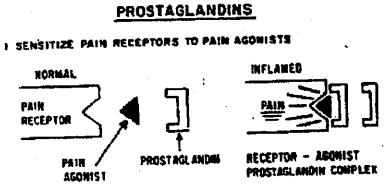


Figure 3. Sensitizing action of the prostaglandins.

mation begin to return. It is then time for another dose of phenylbutazone. It is clear from this mechanism of action that the therapeutic effects of phenylbutazone are directly related to its ability to reduce inflammation, and that any analgesic action it may have is secondary to and dependent on the anti-inflammatory effect. Phenylbutazone has no anesthetic actions and is not an anesthetic. It is simply a very effective anti-inflammatory drug, and as such, prevents the appearance of pain, which is one of the cardinal signs of inflammation.

In an average-sized horse (1000 lbs), a dose of 2 to 3 g/day of phenylbutazone IV, or about 4 g orally should produce the optimal anti-inflammatory effect. Two grams daily should then maintain the effect.[11] As the dose of the drug is increased, the blood levels increase and the plasma half-life of the drug is also increased (Figure 4).[9] If the drug is given orally instead of IV it takes about five hours for peak blood levels to be attained, and the time to attain peak pharmacological effect will be further delayed (Figure 5).[11] Urinary levels of phenylbutazone are usually higher than plasma levels of the drug, and urinary levels are detectable for at least 24 hours after a 2 g/1000 lb dose IV (Figure 6). Since the anti-inflammatory effect is not good for more than about 24 hours, daily dosing with phenylbutazone is required.[11]

For a long time the only way to assess the anti-inflammatory and lameness-alleviating efficacy of phenylbutazone was simply by "eyeballing" a treated horse. This led to some confusion about how effective the drug really was, how long it took to act, and for how long a period it was effective. Recently, however, Professor Pratt and his co-workers at the Massachusetts Institute of Technology have developed an instrument called a "force plate" which can provide an

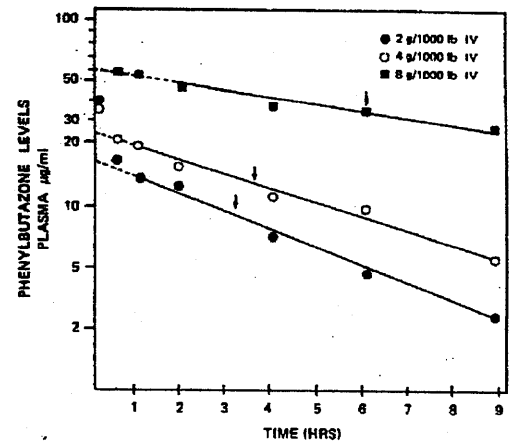


Figure 4. Plasma levels of phenylbutazone in a horse after administration of three different doses (after Piperno *et al.*).[11]

objective measurement of lameness and the effects of drugs on lameness.[12] This instrument measures small variations in the load on a horse's leg. It turns out that a horse is very unsteady on a lame leg, and that this unsteadiness, in the form of constant small readjustments of weight on this leg, can be measured by means of the "force plate." After administering phenylbutazone, the horse becomes much more comfortable and thus steadier on his sore leg, which begins to be comparable with his good leg. Using this instrument, 2 g phenylbutazone orally has been shown to take several hours to act after administration of an IV dose and to be largely over by 24 hours. If the dose is given intravenously, the onset of action is a little faster, but probably not much faster, because the reduction of tissue prostaglandins by phenylbutazone and other NSAIDs takes some time to develop.

Though the pharmacological action of phenylbutazone is over within 24 hours, the time for the animal to "eliminate" phenylbutazone is indefinite, as it is with any drug given to a horse. Since phenylbutazone is relatively easy to detect, it can be detected for a very long period indeed if the analyst so desires. Figure 7 shows some of the best data on plasma levels of phenylbutazone in the horse that has come to my attention.[10] In this experiment horses were given varying doses of phenylbutazone for up to three days, and plasma and urinary levels of the drug followed. Plasma levels started at about 20 to 30 μg/ml, declined with a half-life of about nine hours, and were still detectable in plasma eight days after dosing and, in some urine samples, on the ninth day after dosing. This experiment, when taken in conjunction with the previous data, emphasizes the point that while the pharmacological actions of phenylbutazone may be over within 24 hours, detection of phenylbutazone in plasma and urine after treatment with this drug is possible for very long periods indeed.

Although the "clearance times" for phenylbutazone in plasma and urine obviously depend on the

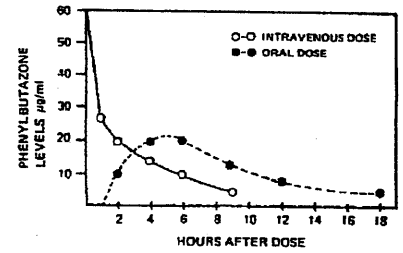


Figure 5. Plasma levels of phenylbutazone following 4 g/1000 lb (453.6 kg) administered by the oral and intravenous routes (after Piperno *et al.*).[11]

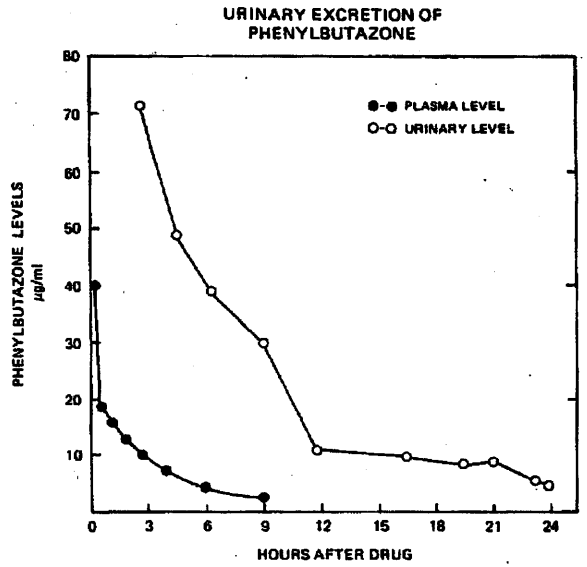


Figure 6. Plasma and urine levels of phenylbutazone in a horse following 2 g/1000 lb (453.6 kg) IV (after Piperno *et al.*).[11]

methods that the analyst elects to use, it is usually safe to assume that plasma and urine are clear by 48 to 60 hours after the last dose. Judging from the data of Figure 7 and what we know about the pharmacology of phenylbutazone, there appears to be little need for a phenylbutazone positive to be called when plasma or urinary levels of the drug are less than 1 μg/ml.

At the other end of the scale, some racing authorities have rulings designed to discourage race-day medication of horses with phenylbutazone. These rulings state that if certain levels, often 165 μg/ml, of phenylbutazone or metabolites are found in postrace urine, that this level indicates race day medication and that a "no medication on race day" rule has been infringed. In the absence of published data, the scientific status of these claims is somewhat certain, and rulings based on this information should be treated with caution.

As far as the horse is concerned, phenylbutazone is a very safe drug. The number of doses administered to horses over the years must be astronomic, while the incidence of reported side effects is small. The principal dangers with phenylbutazone are associated with its improper injection. If phenylbutazone is injected around the jugular vein by mistake, it may cause severe inflammation, abscessation, and eventual loss (sloughing) of the vein. This, however, can occur with many drugs and is a problem with the injection technique rather than with phenylbutazone. Phenylbutazone may also be injected directly into the carotid artery in the neck by accident. If this happens the horse immediately becomes excited, falls prostrate and may die. Some veterinarians and trainers report that phenylbutazone will occasionally depress a horse, especially with large doses in Standardbred horses. However, to judge from the number of horses running on phenylbutazone at some tracks, this effect must either be very rare or else horses get over it very rapidly.

There is only one single report in the literature in which horses treated with massive doses of phenylbutazone developed stomach ulcers and liver problems.[2] Thus, all in all, phenylbutazone, when administered with normal care and in reasonable doses, is a very safe and effective drug in the horse with minimal side effects and toxicities.

This pattern of minimal toxicity due to phenylbutazone in the horse contrasts sharply with the serious toxicities seen in man. In man, some type of side effect is seen in from 10 to 40% of patients, including peptic ulcer, liver and kidney problems and, most seriously, inability to form red cells or white cells in the blood. A number of phenylbutazone-dependent deaths have been recorded, and for this reason its use is restricted in man, and it is best used only in short-term therapy.*

Because a dose of phenylbutazone persists a long time in the blood of man (half-life about three days) compared with its relatively short plasma half-life in the horse (six to eight hours), it has been suggested that its lack of toxicity in the horse is due to its rapid metabolism by the horse. This theory ignores observations that drug toxicities are very often due to drug metabolites rather than to the drug itself, metabolites which are of course formed at a much greater rate in the horse than in man.

The question about the effect of phenylbutazone on the performance of horses has often been raised and has not been satisfactorily answered. Reviewing

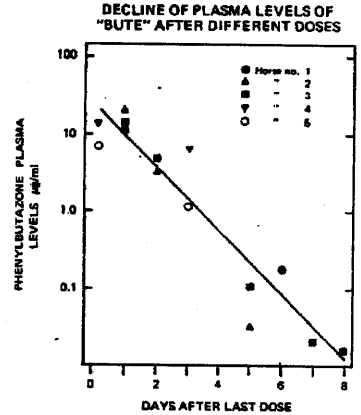


Figure 7. Symbols show plasma levels of phenylbutazone in horses which had been dosed with 2, 4, and 6 g phenylbutazone for up to three days before measurement of plasma levels. Phenylbutazone was detectable in plasma for eight days and urine for up to nine days (after Northeim *et al.*).[10]

the subject of phenylbutazone and furosemide* in racing horses, 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 him to race nearer to his maximum capacity.[6] Studying the effects of phenylbutazone in time trials in horses, Sanford and his colleagues found that phenylbutazone administered intramuscularly 23 hours before "time" trials improved performance in their horses.[12] These workers were apparently rather surprised by this result and concluded that phenylbutazone had acted to relieve subclinical lameness rather than to stimulate the horses.[13] This interpretation is well supported by some experiments from our laboratory, which show no effect of phenylbutazone on fentanyl-stimulated trotting in horses, minimizing suggestions that phenylbutazone either stimulates or depresses horses at the usually used clinical doses.[1]

Another important question with phenylbutazone is whether or not it interferes with the detection of other drugs, the popularly called "masking" effect. While there is no doubt that the presence of any drug must make the detection and unequivocal identification of another drug somewhat more difficult, the consensus of the Veterinary Chemists Advisory Committee was that the usual doses of phenylbutazone given on race day may or may not interfere with the detection of other medications, depending on the analytical methods used *and, perhaps just as importantly, on what other drugs are being tested for.**

---

* Lasix, National Laboratories Corporation, Somerville, NJ.
* Italicized portion represents this author's comments.

Part two of this review on nonsteroid anti-inflammatory drugs will appear in the July issue of The Journal.

References

1. Arnold, L., Collins, C., and Stamper, G.A. Renal and Gastric Lesions after Phenylbutazone and Indomethacin in the Rat. *Pathology*, 6, (1974) 303-313.
2. Brune, K. How Aspirin Might Work: A Pharmacokinetic Approach. *Agents & Actions*, 4, (1974) 231-232.
3. Brune, K., Glan, M., and Graf, P.: Mechanism of Action of Anti-Inflammatory Drugs. *Gen Pharmacol*, 7, (1976) 27-33.
4. Combie, J., Shults, T., Dougherty, J., and Tobin, T.: The Pharmacology of Narcotic Analgesics in the Horse. V. Selective Blockade of Narcotic-Induced Locomotor Activity in the Horse. *J Eq Med Surg*, submitted for publication (1979).
5. Ferreira, S.H. and Vane, J.R.: New Aspects of the Mode of Action of Nonsteroid Anti-Inflammatory Drugs. *Ann Rev Pharmacol*, 14, (1974) 57-73.

6. Gabel, A.A., Tobin, T., Ray, R.S., and Maylin, G.A.: Phenylbutazone in Horses. A Review. *J Eq Med Surg*, 1, (1977): 221-225.
7. Gabriel, K.L. and Maran, J.E.: Phenylbutazone. Short Term Versus Long Term Administration to Thoroughbred and Standardbred Horses. *JAVMA*, 140, (1962): 337-341.
8. Goodman, L.S. and Gilman, A. *The Pharmacological Basis of Therapeutics*. 5th ed., MacMillan Publishing Co., New York, (1975).
9. Maylin, G.A.: The Disposition of Phenylbutazone in the Horse. *Proc AAEP*, 20, (1974): 243-248.
10. Norheim, G., Hale, R., Frosbe, A., and Bergsjo, T.H.: Gas-Chromatographic Determination of Small Amounts of Phenylbutazone and Oxyphenbutazone in Horse Plasma and Urine. *Fresenius Z Anal Chem*, 289, (1978): 267-268.
11. Pipemo, E., Ellis, D.J., Getty, S.M., and Brody, T.M.: Plasma and Urinary Levels of Phenylbutazone in the Horse. *JAVMA*, 153, (1968): 196-198.
12. Pratt, G.: Breakthrough on Understanding Lameness. *The Blood-Horse*, 103, (1977): 3758-3760.
13. Sanford, J.: Effects of Drugs on Performance of the Horse. In *Symposium on Large Animal Therapeutics*. University of Surrey, Guildford, Surrey (1978).
14. Tobin, T.: Pharmacology Review: The Corticosteroids. *J Eq Med Surg*, 3, (1979): 10-15.
15. Vane, J.R.: Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-Like Drugs. *Nature New Biol*, 231, (1971): 232-235.
16. Van Rechenberg, H.K.: *Phenylbutazone*. 2nd ed., Edward Arnold Ltd., London, (1962).

Publication No. 53 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, College of Agriculture, University of Kentucky. Published as Kentucky Agricultural Experiment Station Article No. 79-4-78 with the permission of the Dean and Director, College of Agriculture, and Kentucky Agricultural Experiment Station.

Viewpoints

Studies in Equine Biomechanics

James R. Rooney, D.V.M.

For those who may have missed articles recently published on *in vitro* testing of horse forelegs, I should like to summarize some of the findings.

Based on earlier *in vivo* experiments[1] it appeared that the foreleg, from just above the carpus distad, functioned largely as a passive automatic system. For the *in vitro* studies the limb was cut off above the carpus, leaving the superior check ligament-superficial flexor tendon (SF) intact, the inferior check ligament-deep flexor tendon (DF) intact, and the suspensory ligament (SL) intact. The leg was placed in a high-speed testing machine which was capable of delivering force to the leg at almost the same acceleration and in the same time as in the galloping horse. Two principal findings emerged. First, the leg *in vitro* behaved in a manner almost identical to that of the *in vivo* leg, but there was one consistent difference between the live and dead leg.

The vertical force developed by a horse galloping over a force plate buried in the racetrack is shown schematically in Figure 1. The initial bump immediately after impact is characteristic of both human and horse and is called the "heel-strike." Its exact nature is not known. Obviously, the vertical force on the foreleg rises rapidly to a maximum at midsupport and then decreases, becoming zero when the foot leaves the ground at lift-off. It may be noted that the maximum vertical force is aproximately two times the horse's body weight (*i.e.* for a 1000 lb horse, the dynamic load is 2000 lb).

The dead leg in the testing machine was subject to the same loading as the live leg and the curve in Figure 2 resulted. The graphic record of the vertical force is essentially identical except for the high frequency vibrations which appeared in the first moments after impact.

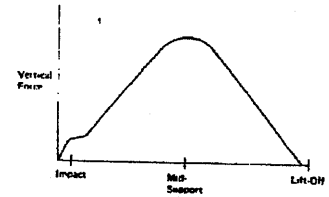


Figure 1

The conclusion is that the muscles of the foreleg serve primarily to prevent vibration of the leg immediately after impact, since vibration is not present in the live horse and is present, consistently, in the leg deprived of muscle.

The forearm muscles, then, as has been emphasized elsewhere[1,2] are not primarily concerned with movement of the leg; their primary function is to prevent movement—to prevent vibration.

(continued on page 292)

Pharmacology Review: The Nonsteroidal Anti-Inflammatory Drugs. II. Equiproxen, Meclofenamic Acid, Flunixin and Others

Thomas Tobin, D.V.M., Ph.D.

Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40546.

In Part I of this series, we reviewed the pharmacology of phenylbutazone, the standard nonsteroidal anti-inflammatory drug used in the horse.[11] In this portion of the review we compare phenylbutazone with a number of the more recently introduced nonsteroidal anti-inflammatory drugs (NSAIDs).

Equiproxen.[a] Equiproxen is another nonsteroidal anti-inflammatory agent with analgesic and antipyretic actions. It is recommended for the relief of pain, inflammation, and lameness associated with myositis and soft tissue disease of the horse. Its therapeutic effects in the horse have been studied in some detail by E.W. Jones and co-workers.[9,10] These workers induced "tying up" in horses by injecting lactic acid into the back muscles and then studying the effects of equiproxen on length of stride, pain, lameness and tissue swelling.[9]

Length of stride in the horse is a sensitive measure of lameness, and Kilian and Jones[10] were able to show that equiproxen greatly reduced lameness caused by lactic acid injections. In the untreated horses lameness peaked at two days after injury was induced and persisted for up to 14 days, while in equiproxen-treated horses the lameness was greatly reduced and virtually eliminated within three days. Comparison of the areas under the curves for the treated and nontreated horses suggests that equiproxen treatment was at least 80% effective in reducing lameness. Similarly, equiproxen treatment had marked effects in reducing swelling, and other data showed that it also equivalently reduced pain. These data constitute the best and most clear-cut experimental work demonstrating the therapeutic effects of a nonsteroidal anti-inflammatory drug that this author is aware of, and clearly delineate the therapeutic efficacy of equiproxen.

[a] Naproxen. Hamond Laboratories, Des Moines, IA.

Based on this experimental model, a study in which phenylbutazone and equiproxen were directly compared led Jones and Hamm[9] to conclude that in the equine myositis model, equiproxen was superior to phenylbutazone for more rapid relief of inflammatory swelling and associated lameness.

In other work, the response to equiproxen obtained in these studies was compared with the response in naturally occurring "tying up" disease. In the natural disease the average time for remission was found to be about five days, and a favorable response was observed in more than 90% of the animals.

Hamm[7] also evaluated the effects of continuous administrations of equiproxen during training to 50 yearling Quarter Horse colts. These colts were assigned to two equal groups at the beginning of a four-month training season, the yearlings assigned to the untreated (control) groups receiving standard medication for musculoskeletal disease as required. The test horses received about 5 g daily of equiproxen in feed during the training session. Hamm observed a marked and highly significant reduction in time lost during training and in the racing season in the horses on equiproxen, who lost only 3% of their training time compared with a 13% loss in training time in the control group. The appearance of musculoskeletal problems was delayed in the equiproxen-treated group, and when musculoskeletal problems did appear, their duration was reduced compared with the control group. The horses treated with equiproxen raced significantly more often than the control horses, and the overall frequency of musculoskeletal injuries was dramatically reduced four-fold during the training phase and 30-fold during the racing phase, for the horses on continuous equiproxen. This is very provocative data, and if independently confirmed, makes a clear case for the benefits of anti-inflammatory medication of young horses in training and in racing.

After oral administration of equiproxen the drug is about 50% absorbed. The recommended dose is 10 mg/kg or about 5 mg/lb. The plasma half-life following oral or intravenous administration has been estimated at about four hours. After oral administration, plasma levels of the drug peak at about 25 μg/ml between two and three hours after dosing and then decline to less than 1 μg/ml at 24 hours. If the drug is given twice a day, as recommended, one would then get a second peak of drug which should decline to less than 5 μg/ml by 24 hours. Equiproxen thus appears likely to have little tendency to accumulate in the horse.

Equiproxen is a very easy drug to detect in the horse because it absorbs well in the ultraviolet range, either as the parent drug or as its major metabolite, 2-(6-hydroxynaphtyl) propionic acid, both of which are excreted in high concentrations in urine. The approximate half-life of these compounds in equine urine is about six hours, and it appears that at least 60 hours should be allowed for equiproxen to clear the urine of the horse.

Equiproxen appears to be a relatively safe drug in the horse, in that three times the recommended dose can be given for up to 42 days with no attributable lesions. In mares, equiproxen was administered in late pregnancy without apparent effect on the mare or the foal, and these mares were subsequently rebred and conceived normally.

Flunixin Meglumine.[b] Flunixin is another member of the NSAID family[3] which has recently been approved for use in equine practice. It appears to be more potent than other members of this group in that a dose of about 1.0 mg/kg produces good clinical effects. The pharmacology and pharmacokinetics of flunixin in the horse have been reported by Houdeshell and Hennessey.[8] These workers reported that an intravenous dose of flunixin improved lameness by 55% and swelling by 34% at 30 hours post-injection, compared with a 52% and 23% improvement due to phenylbutazone. While these results might seem to suggest that flunixin is more effective than phenylbutazone, the apparent comparison of these drugs at 30 hours postdosing may be misleading, as phenylbutazone was only effective for 24 hours in these particular studies. A more appropriate period for evaluating the relative merits of phenylbutazone and flunixin would be the times of peak drug effect, or after the horse had been on both drugs for a period. Because of the uncertainty of the times at which the actions of phenylbutazone and flunixin were compared, it is difficult to evaluate the relative effectiveness of these drugs in this study. Flunixin produced approximately the same response whether it was given orally, intravenously or intramuscularly.

Field trials with flunixin for a typical clinical spectrum of musculoskeletal disorders showed that remission of clinical signs occurred after two to three days of therapy. Overall, 40% of the horses were rated as having an excellent response, 34% as having a good response, 14% as fair, and 12% as poor. Unfortunately, no control data or positive control information with a well-characterized drug such as phenylbutazone were presented for comparative purposes.

[b] Banamine® Schering Corporation, Kenilworth, NJ

It is characteristic of the NSAIDs that individual members of this group are more effective in some conditions rather than other conditions. Both literature and clinical experience suggest that flunixin is particularly useful in the treatment of colic and rapidly alleviates pain and distress associated with this condition. In studies on the use of this drug in animals with predominantly spastic and flatulent colics, Vermmb and Hennessey[13] found an excellent or good response in 93% of flatulent colics, in 72% of spastic colic cases, 52% of static colics and 60% of other types. Fifteen percent of horses showed a fair response and another 15% showed no response. The onset of the response to flunixin was also remarkably fast, about 40% of horses showing improvement in 15 minutes, and some evaluators reporting favorable changes in four to eight minutes. These are remarkably rapid changes to be induced by an antiprostaglandin agent, which, as pointed out previously, must reduce existing tissue prostaglandin levels to normal before they act. In fact, a response within four minutes, which is not more than two circulation times for the horse, must be accounted a very rapid response indeed. The duration of relief after flunixin was more in keeping with the known time course of action of this drug, and averaged six to eight hours after a single dose.

While flunixin is effective at 1.0 mg/kg, and 4.4 mg/kg of phenylbutazone is needed to produce an equivalent effect, this potency of flunixin should not in any way be taken as a particular advantage for flunixin. As far as the clinician and horsemen are concerned, potency is primarily an academic concept. What counts in the clinical setting is the quality of the clinical response and the ratio between the dose producing a good clinical response and that which produces a toxic effect. The actual number of milligrams of a drug required to produce the clinical effect is, per se, of little practical significance, as long as its use in effective doses is not associated with side effects or toxic effects.

The plasma half-life of flunixin in the horse is remarkably short, being only about 1.6 hours after its intravenous injection. After this dose plasma levels peaked at 1.6 μg/ml, while urinary levels of the drug peaked at 60 μg/ml two hours after injection and then declined. After dosing horses with 1.1 mg/kg daily for four days, plasma levels of 2.9 μg/ml were observed, and traces of flunixin were still observed in urine 48 hours after the last dose.

Although the plasma half-life of flunixin in the horse is very short (1.6 hours), the peak pharmacological response to flunixin occurs at 12 hours after dosing and the effect persists for 30 hours. This is an unusually long period of action for a drug with such a short plasma half-life, and may raise some questions about the mechanism of action or the active species of flunixin.

Flunixin appears to be a relatively safe drug in the horse, with doses up to five times the recommended dose for five days producing no drug related problems or toxicities.

Meclofenamic Acid.[c] Meclofenamic acid is another member of the nonsteroidal anti-inflammatory group of drugs. It is available as oral granules, reportedly quite palatable and can be mixed with the grain ration once a day. The usual dose rate is about 1.0 mg/lb of body weight for from five to seven days.[4]

Meclofenamic acid is an unusual drug among the NSAIDs in that its onset of action can be relatively slow, taking from 36 to 96 hours to develop.[4,12] After dosing with 1 g/1000 lbs in the horse, plasma levels of the drug peak within one to four hours at a little more than 1 μg/ml. They then decline with an apparent half-life of about six hours and clear the plasma by 24 hours. Because the plasma is essentially cleared after 24 hours, there is no tendency for meclofenamic acid to accumulate in plasma in the normal horse.

If meclofenamic acid is fed orally for five days, urinary concentrations of the drug run at about 25 μg/ml throughout the dosing period. After the last dose of meclofenamic acid, the drug is reportedly detectable in urine for 96 hours, although the levels are very low from 48 hours on. Again, since meclofenamic acid doses do not tend to accumulate in the body, the same rate of decline for meclofenamic acid in urine should be followed after single- or multiple-dose regimens. About 10 to 14% of the drug administered is eliminated in the urine, and it is thought that a good proportion of the drug is eliminated via the bile and feces. No data are available on the metabolism of meclofenamic acid in the horse.

In clinical trials on 304 horses suffering from osteoarthritis, navicular disease, laminitis and soft or bony tissue conditions, it was found that 78% of the cases with navicular disease improved, 76% with laminitis improved and 61% of the cases with osteoarthritis improved.[4] Since all these cases were carefully screened to exclude horses which might improve with stall rest, these improvements are difficult to compare with reported improvements on other drugs. These workers also reported that it was not possible to predict which horses would improve on meclofenamic acid and which would not.

[c] Arquel Parke-Davis and Company, Detroit, MI

Signs of toxicity to meclofenamic acid appear at high dose levels (6 to 8 mg/lb) and include mouth ulcers, loss of appetite, depression, edema and loss of weight. If the dosage rate is kept low (1 mg/lb), however, meclofenamic acid is a safe drug and useful in the treatment of musculoskeletal disease in the horse.

Aspirin. The oldest and best known of the nonsteroidal anti-inflammatory group of drugs is aspirin, or acetylsalicylic acid. The fact that aspirin is a household remedy and very readily available should not delude one into thinking that it is anything but a very effective drug. Aspirin has long been the drug of choice in the treatment of human arthritis, but it is unfortunately not nearly as effective a drug in the horse.

Where aspirin is concerned, nature has played a number of tricks on horsemen. The urine of horses and herbivores normally contains small quantities of salicylate. This salicylate either comes from the grass and hay that the horse ingests or is a product of the horse's metabolism.[5] Whatever its origin, the result is that it is difficult for a chemist to call a salicylate "positive," because salicylate is a natural constituent of horse urine.

While salicylate might thus seem to be an answer to the horseman's prayer, nature helped to set the record straight by making salicylate very rapidly excreted by the horse. It turns out that, as an acidic drug, salicylic acid is very rapidly eliminated in the basic urine of the horse. The half-life of salicylate in the blood of a horse with basic urine is less than one hour, so it is very difficult to maintain plasma levels of the drug.[6] Thus if you want to use salicylate effectively in the horse, you must give large doses of the drug and give them relatively close to the time at which you want the drug to take effect.

It would help considerably with the effects of salicylate if one could give aspirin intravenously or intramuscularly and in this way obtain a high blood level of the drug. However, since salicylate itself is not available as an injectable, this approach cannot be taken. On the other hand, a close relative of acetylsalicylic acid, thiosalicylate, is available and can be administered by injection.

Thiosalicylate is chemically a slightly different molecule from salicylic acid, in which an oxygen atom has been replaced with a sulfur atom. This chemical

change results in this particular salicylate being available in an injectable form, which means that higher blood levels and thus effective concentrations of the drug are readily attained. No information is available on the half-life of thiosalicylate in the horse; however, it is presumably much like that of salicylate itself. Thiosalicylate was once much used in some racing jurisdictions as a substitute for phenylbutazone, particularly in those states which have upper limits on postrace urinary concentrations of phenylbutazone. Unfortunately, because of the presence of the sulfur molecule, it is possible to distinguish this drug from normal salicylate, and a number of thiosalicylate "positives" have been called. Even though it makes little pharmacological sense to allow phenylbutazone and aspirin and to ban thiosalicylate, this apparently is the status of thiosalicylate in racing chemistry at the moment.

Orgotein.[4] Orgotein is the generic name adopted for drug versions of a copper- and zinc-containing metallo-protein called superoxide dismutase. In 1964 it was discovered to be a potent anti-inflammatory agent, and it was soon shown to be an enzyme, though beyond this little was understood about the mechanism of its anti-inflammatory action, other than that is was different from most of the other anti-inflammatory drugs in current use.[1]

Superoxide dismutase has recently been shown to be an intracellular enzyme which is part of the body's defense against the highly toxic $^{-}O_2$ or superoxide radical. Blood cells such as neutrophils and macrophages generate significant amounts of the superoxide radical as a killing agent in their attacks on bacteria, but these superoxides can also kill the phagocytes themselves.[12] When administered as a drug, superoxide dismutase apparently scavenges this excess superoxide radical and prolongs phagocyte life.

Clinical trials in humans have reportedly shown that orgotein is a safe, effective nonanalgesic anti-inflammatory drug. Its effects apparently take from two to six weeks to develop and persist for up to one month after termination of treatment. Orgotein may be administered systemically or, when the pathology is localized, injected into and around a site of a chronic inflammation.[11]

In Sweden, Ahlengard and co-workers[1] studied the efficacy of intra-articular orgotein in a series of cases of noninfective traumatic arthritis in horses. These workers concluded that intra-articular orgotein produced beneficial results, the effects being most marked (94% recovery) in horses which had shown lameness for less than six months, and less marked (49%) in horses which had been lame for more than two months before treatment.

Decker and co-workers[6] also reported on the treatment of 70 horses with local and/or intra-articular injection of orgotein. These workers considered the obtained response excellent, with 53 of the horses returning to racing within a few days, and some winning their races. Of 20 horses with fetlock problems, 16 responded to treatment and most of these after only a single dose of the drug.

As naturally occurring metallo-protein of high molecular weight, orgotein is not likely to enter equine urine in significant amounts. Even if it did it would be difficult to recover, detect and prove it to be of exogenous origin by currently used drug testing techniques.

Hyaluronic Acid. Hyaluronic acid is an essential component of synovial fluid and articular cartilage which has recently been introduced as an intra-articular therapy for joint problems. High molecular weight hyaluronic acid, which is the form injected into joints, inhibits lymphocyte migration and phagocytosis and also reduces the permeability of the synovial membrane. Usually between 20 and 40 mg of the sodium salt of hyaluronic acid (10 mg/ml) is injected into the joint, with an equivalent volume of synovia being removed. Reporting on a study of the effects of hyaluronic acid treatment in racing horses, Ashelm and Lindblad[2] had "frequently very good effects" with hyaluronic acid. These good effects were seen in foals which had previously been point-fired, blistered and sometimes treated with intra-articular corticosteroids. These authors considered it remarkable that in most cases injection of 1 to 2 ml of hyaluronic acid was sufficient to cure the lameness. The only factors which appeared to predictably interfere with therapy were pronounced bony changes in the joint or prior treatment with costicosteroids.

The mode of action of hyaluronic acid is not clear, but it appears to persist in the joint for days after an injection. One theory is that hyaluronic acid has good surface-protecting properties. Because of its high molecular weight and the large number of electrical charges carried by hyaluronic acid, diffusion of hyaluronic acid away from an intra-articular injection site and its detection in urine by current routine screening methods is highly unlikely.[10]

In summary, reviewing the properties of the nonsteroidal anti-inflammatory drugs, it is apparent

[4] Palosein® Diagnostic Data, Inc., CA.

that phenylbutazone is still the standard against which other drugs are compared. All these drugs share a number of broadly similar characteristics in that dosages are approximately equivalent from drug to drug, their time courses of action are broadly similar and their detection in blood or urine is not particularly difficult. There are, however, subtle differences in their clinical effectiveness against certain conditions, which makes certain agents the drugs of choice for specific conditions. In this way flunixin is apparently more effective against colic than other members of this group and is the drug of choice in this area. Similarly, equiproxen appears to be the drug of choice for muscle soreness, and is reported to be particularly effective in young horses with minor muscle problems. Meclofenamic acid has acquired a reputation for being useful in foot and hoof problems, and other specific strengths of individual members of this group will doubtlessly become apparent as clinical experience with these drugs increases. Therefore, despite the basically similar mechanism of action of the NSAIDs, they are all far from clinically equivalent, and it is a good idea to try different NSAIDs on clinical conditions which might be expected to respond to these drugs if the response to one member of this group is poor.

References

1. Ahlengard, S., Tufvesson, G., Petterson, H., and Andersson, T.: Treatment of Traumatic Arthritis in the Horse with Intra-Articular Orgotein (Palosein®). *Eq Vet J*, 10, (1978): 122-124.
2. Ashelm, A. and Lindblad, G.: Intra-articular Treatment of Arthritis in Race Horses with Sodium Hyaluronate. *Acta Vet Scand*, 17, (1976): 379-394.
3. Clofelo, V.B., Latrenyi, M.G., Patel, J.R., and Taber, R.I.: Flunixin Meglumine: A Non-narcotic Analgesic. *J Pharm Exp Ther*, 200, (1977): 501-507.
4. Conner, G.H., Riley, W.F., Buck, C.C., and Coppock, R.W.: Arquel (CI-583), A New Non-steroidal Anti-Inflammatory Drug for Horses. *Proc AAEP*, 19, (1973): 81-90
5. Davis, L.E. and Westfall, B.A.: Species Differences in Biotransformation and Excretion of Salicylate. *Am J Vet Res*, 33, (1972): 1253-1262.
6. Decker, W.E., Edmonson, A.H., Hill, H.E., Holmes, R.A., Padmore, C.L., Warren, H.H., and Wood, W.C.: Local Administration of Orgotein in Horses. *MVP*, 9, (1974): 773-774.
7. Hamm, D.: Continuous Administration of Naproxen to the Horse During Training. *J Eq Med Surg*, 2, (1978): 125-128.
8. Houdeshell, J.W. and Hennessey, P.W.: A New Nonsteroidal Anti-Inflammatory Analgesic for Horses. *J Eq Med Surg*, 1, (1977): 57-63.
9. Jones, E. W. and Hamm, D.: Comparative Efficacy of Phenylbutazone and Naproxen in Induced Equine Myositis. *J Eq Med Surg*, 2, (1978): 341-347
10. Kitas, J.G., Jones, E.W., Hamm, D., Riley, W.F., and Avalkin, E.: The Efficacy of Equiproxen (Naproxen) in a Unique Equine Myositis Model. *Proc AAEP*, 20, (1974): 201-215.
11. Linton, J.A.M.: The Use of Orgotein in the Treatment of Soft Tissue Injuries of the Horse. *Irish Vet J*, 30, (1976): 53-56.
12. Riley, W.F., Romane, W.M., Ellis, D.J., and Hacey, K.W.: Preliminary Report on a New Nonsteroidal Anti-inflammatory Agent in the Horse. *Proc AAEP*, 17, (1971): 293-300.
13. Saito, M.L. and McCord, J.M.: Free Radicals and Inflammation Protection of Phagocytosing Leukocytes by Superoxide Dismutase. *J Clin Invest*, 56, (1975): 1319-1323.
14. Tobin, T.: Pharmacology Review: The Nonsteroidal Anti-inflammatory Drugs. I. Phenylbutazone. *J Eq Med Surg*, 3, (1979): 253-258.
15. Vaneman, G.D. and Hennessey, P.W.: Clinical Studies on Flunixin Meglumine in the Treatment of Equine Colic. *J Eq Med Surg*, 1, (1977): 111-116.
16. Wigren, A., Falk, J., and Wik, O.: The Healing of Cartilage Injuries Under the Influence of Joint Immobilization and Repeated Hyaluronic Acid Injections. *Acta Orthop Scand*, 49, (1978): 121-133.

Publication No. 55 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, College of Agriculture, University of Kentucky. Published as Kentucky Agricultural Experiment Station Article No. 79-4-104 with the permission of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

(continued from page 296)

October 28–November 2, *United States Animal Health Association 83rd Annual Meeting.* For more information contact: Dr. W.L. Bendix, Suite 219–220, Byrd Building, 1910 Byrd Avenue, Richmond, VA 23230.

November 17–18, *AAHA Regional Short Course.* Sponsored by AAHA and The Ohio State University College of Veterinary Medicine, Division of Continuing Education, Fawcett Center for Tomorrow, Ohio State University, Columbus, OH. For more information contact: AAHA, 3612 East Jefferson Boulevard, P.O. Box 6429, South Bend, IN 46660.

Phenylbutazone in Horses: A Review

Albert A. Gabel, D.V.M., M.S.
Thomas Tobin, D.V.M., Ph.D.
Richard S. Ray, D.V.M., M.S., Ph.D.
George A. Maylin, D.V.M., M.S., Ph.D.

From Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by Gary P. Carlson, University of California; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; and David Snow, University of Glasgow; and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Finley, A.V. Villatico, Robert E. Vessiny, Dr. Francis Ozog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Bierhaus, and General Wayne Kester.

1. Phenylbutazone is a very effective nonsteroidal anti-inflammatory agent in horses. Through its anti-inflammatory action, it is an analgesic and an antipyretic. It is not an anesthetic.

2. Phenylbutazone does not change a horse's innate ability to race, but by relieving inflammation it may enable him to race nearer to his maximum capability.

3. Its mechanism of action is thought to be by inhibition of prostaglandins.

4. Onset of action occurs within a few hours, and optimum effect lasts for less than 48 hours after the last dose.

5. In horses phenylbutazone is safe; side effects and toxicity are rare at the usual doses.

6. In blood, phenylbutazone can be detected for 24 hours and occasionally up to 48 hours.

7. Although the quantity of phenylbutazone in the urine is not reliable for the determination of the time that the last dose of phenylbutazone was administered, reasonable estimates can be made by a comparison of the ratios of phenylbutazone to its major metabolites.

8. Trace amounts of phenylbutazone and its metabolites can be detected in urine for up to 96 hours by utilizing electron capture derivative formation and detection by gas chromatography.

9. The usual doses of phenylbutazone given on race day may interfere with the detection of other medications, depending upon analytical methods used.

Introduction

Phenylbutazone is a safe, effective nonsteroidal anti-inflammatory drug* (NSAID) with antipyretic and analgesic activity. In the recommended doses, there is no evidence that it changes a horse's innate ability to race, except to make him perform more nearly normal if he has pain due to inflammation of part of his musculoskeletal system.

Mechanism of Action

Phenylbutazone is the (NSAID) most widely used in horses. Its mechanism(s) of action is(are) not clearly understood. Studies involving the mechanism(s) of action of phenylbutazone in the horse have not been reported. Most evidence indicates that (NSAID)'s exert their action by inhibiting the biosynthesis of prostaglandins (at the last step of biosynthesis) which directly and/or indirectly mediate the inflammatory and pain response.[1,32] This inhibition probably reduces the concentration of prostaglandins in inflamed tissues.[31,32] Prostaglandins increase permeability of blood vessels and result in swelling and inflammation of tissue.[32] Prostaglandins also sensitize pain receptors in tissues to inflammatory and pain mediators: this blockade of prostaglandin formation results in reduced pain.[32] Because accumulation of phenylbutazone in the tissue is slow and the drug only prevents formation of new prostaglandins, the anti-inflammatory action of the drug develops slowly as preformed tissue prostaglandin concentrations must first decline.[4] Blockade of prostaglandin synthesis thus slowly returns the inflamed area toward normal. The analgesic action of phenylbutazone occurs directly in the inflamed tissue (not in the brain) and is directly related to its anti-inflammatory effects.

Phenylbutazone (and other NSAID) are all highly protein bound acidic drugs which accumulate in inflamed tissues (which is important for their action), but they also accumulate in the stomach, small intestine, and kidney.[4] Thus, all drugs in this group have the potential to produce lesions of the stomach, small intestine, and kidney tissue.[4] Since prostaglandins are involved in the cause of fevers, members of this group of drugs are also antipyretic. Even large doses do not reduce liver glycogen content in experimental animals, as do the salicylates.[32] Phenylbutazone interferes with several enzymes of amino acid and amino sugar metabolism.[32] It also inhibits mucopolysaccharide biosynthesis and collagen formation and uncouples oxidative phosphorylation. The other (NSAID)'s share these inhibitory properties.[25]

*Other members of the nonsteroidal anti-inflammatory group of drugs are flunixin meglumine, aspirin, ibuprofen, naproxen, flufenamic acid, fenamic acid, mefenamic acid, indomethacin, niflumic acid, and meclofenamic acid. All these drugs share the same basic mechanism of action and general pharmacology.

Clinical Efficacy

Currently, phenylbutazone is the most effective and dependable anti-inflammatory agent on the market for the equine. It is especially effective in cases of bone and joint inflammation and laminitis[4,9,14] as well as being useful in soft tissue inflammation.[13,23]

At low doses, the effect of the drug appears to be dose related.[1,4] In response to a single administration intravenously or orally, a dose of 4 g has an optimal effect for a 1,000-pound horse. Two grams are required daily to maintain optimal effect. First response to the drug can be seen a few hours after administration. Optimum effect is variable but occurs approximately 12 hours after administration. Intravenous injection appears to speed onset of action, on the average by about 2 to 4 hours compared to oral use.[1] Onset of action is variable after oral administration, depending on whether the horse eats before or after the drug is given.[26] Optimal clinical effect appears to continue for a variable time, usually for somewhat less than 48 hours after the last dose of the drug, depending on the dose and length of the course of treatment.[2,10] In other species, several drugs not commonly used in horses can be altered in their degradation when phenylbutazone is used with them.[32] Therefore, their duration and intensity of action change. This action has not been demonstrated in horses. Conversely, several drugs known to inhibit drug metabolism in other species have been of little or no effect on the half-life of phenylbutazone in the horse.[21]

Metabolism

Phenylbutazone is almost completely metabolized in the horse. In separate studies, only 3.7% of a 2 g/1000 lb dose was recovered in the first 24 hours,[21] and by dosing 2 g/1000 lbs at consecutive 24-hour periods, between 1 and 2% of the parent drug was recovered in the urine.[19] The principal metabolites reported in the equine are oxyphenbutazone and γ-hydroxyphenylbutazone, the "alcohol metabolite." Together, these account for about 25% of the drug administered.[19] A second minor alcoholic metabolite has been found, whose structure has tentatively been determined by nuclear magnetic resonance to be a primary alcohol.[18] Other metabolites and conjugated forms have been reported in the rat,[2] but to date 75% of the metabolites have not been accounted for in the horse.

Because the pharmacologically inactive γ-hydroxyphenylbutazone is less tightly protein bound than either phenylbutazone or oxyphenbutazone,[2] it is readily excreted in urine and is the principal metabolite found in urine (14%) after a single dose of the drug.[19] However, if dosing is continued, the proportion of the alcohol metabolite excreted drops to 6% of the dose.[19] This change in the proportion of different metabolites in the urine as dosing

proceeds is likely related to the dose-dependent kinetics of phenylbutazone due to inhibition of the drug's metabolism by one of its metabolites. Oxyphenbutazone has been shown to inhibit the metabolism of phenylbutazone in the rat and the horse.[17-21] Thus, a metabolite of phenylbutazone (probably oxyphenbutazone) becomes bound to and inhibits liver microsomal drug metabolism enzymes[5] and alters both the metabolite pattern of phenylbutazone and the plasma half-life of the drug.[20]

Blood (Plasma) Levels

Phenylbutazone is well absorbed in the horse after oral administration: a dose of 4 g/1000 lb gives peak plasma levels of about 20 µg/ml (parts per million) 5 hours after dosing. The same dose by rapid intravenous injection gives plasma levels which decline from about 30 µg/ml to 5 µg/ml at 9 hours.[20] The half-life of phenylbutazone in the horse is dose-dependent, increasing from 3.5 hours at 2 g/1000 lb to 6 hours at 8 g/1000 lb.[22] It has been reported that the plasma half-life of phenylbutazone increased after repeated doses at 2 g/1000 lb from 5.1 hours to 6.1 hours after the fourth daily dose.[19] This increasing half-life is evidence that accumulation of phenylbutazone in the body can occur the first 4 days of administration.[19] In another study, similar half-lives (5.46 hours at 3 g/1000 lb) were reported.[21] Phenylbutazone is at least 95% protein bound in plasma at therapeutic drug concentrations.[20] Thus, at plasma levels of 30 µg/ml only about 1.5 µg/ml of drug is free to interact with the drug receptors.[20] The ability of these acidic drugs to concentrate in inflamed tissues appears to be important for the mechanism of action.[4]

In the blood, phenylbutazone can easily be detected by the routine prerace blood test after more than 24 hours and can occasionally be detected up to 48 hours post-administration, especially if the horse has been medicated for several days.[20,23]

Phenylbutazone and its metabolites may interfere with analysis for unauthorized drugs in blood and urine, depending upon the concentration of phenylbutazone, the sensitivity and specificity of the analytical techniques, and the expected scope of drug coverage.[20,22,23]

Urinary Levels of Phenylbutazone and Metabolites

a. Phenylbutazone

After intravenous administration of 2 g/1000 lb of phenylbutazone, it was detected spectrophotometrically in equine urine at 31 hours but not at 48 hours.[12] In other studies, 2 g/1000 lb[19] and 3 g/1000 lb[21] of phenylbutazone resulted in detection of the drug in the urine at 24 hours by gas chromatographic methods.

b. γ-Hydroxyphenylbutazone

This metabolite appears to be excreted less rapidly than phenylbutazone but more rapidly than oxyphenbutazone.[19-20,22] After a single dose of phenylbutazone, the bulk of the material found in the urine for the first 10 hours is γ-hydroxyphenylbutazone.

c. Oxyphenbutazone

Oxyphenbutazone is the most persistent of the urinary metabolites studied to date. After 4 daily doses of 2 g/1000 lb, about 5 µg/ml of oxyphenbutazone was found 48 hours after terminating dosing.[19] In another study, oxyphenbutazone was found up to 53 hours after dosing with 2 g/1000 lb intravenously, but later urine levels were not determined.[12] Others were able to find this metabolite for at least 96 hours, using electron capture derivatives and gas chromatography following a single intravenous dose of 4 g/1000 lb.[19,20]

d. Combined Metabolite Studies

Tracer isotope ^{14}C was observed for about 65 hours in acidic equine urine and for up to 150 hours in basic urine after administration of ^{14}C phenylbutazone.[21] As no chemical identification of the ^{14}C was reported, it is difficult to relate these results to studies on specific metabolites. In horses dosed orally with increasing amounts of phenylbutazone, the drug and its metabolites were measured spectrophotometrically.[14] After 4 g orally, no traces of the drug or its metabolites were observed at 48 hours, but the drug or its metabolites were observed at 72 hours after 8 or 16 g. After 3 consecutive doses of 8 g, it took 120 hours for urine levels to fall to 0.5 µg/ml of drug or metabolites.[14] A number of studies show that the metabolite pattern in the urine is nearly constant regardless of the route or dose.[18,19,22,24,25] High levels of γ-hydroxyphenylbutazone occur between 0–15 hours postmedication. Oxyphenbutazone levels appear more slowly, exceeding the γ-hydroxyphenylbutazone levels after 12–18 hours. Following the final administration of phenylbutazone, the interrelationships of phenylbutazone and its metabolites and their decline in the urine make it possible to reasonably estimate the time of that administration up to about 48 hours.

Side Effects and Toxicity

Side effects due to phenylbutazone are unusual in horses, there being few in clinical experience or in horses in racing.

a. Local

Accidental injection of phenylbutazone into the carotid artery will cause immediate excitement, prostration, and sometimes death. Rapid intravenous injection of the undiluted solution can cause serious phlebitis since it is irritating. Extravascular injection causes severe inflammation at the site, with phlebitis, permanent occlusion of the jugular vein in some cases, and very rarely death due to sloughing of the vein and exsanguination.

b. Behavior

Although there is no objective evidence, the observation has been made by veterinarians that a small percentage of horses, more commonly Standardbreds, will appear to be slightly sleepy or depressed by phenylbutazone, especially after recent large doses.[4,26] If this is a valid observation, it is not known whether this affects their speed in the race.

c. Systemic

Although there is often retention of water and electrolytes in man, this apparently does not occur in horses.[12] Toxicity to phenylbutazone is almost never seen in equine patients in spite of close observation and periodic blood counts done during thousands of courses of therapy.[4,12,15,20] The drug has been given at the level of 2 g orally daily for 6 months to a number of patients[15] and to others for a period of 2 to 3 years[4] with no deleterious effects clinically nor changes in the blood counts. Postmortem studies done on equine patients which have died of various diseases and injuries while receiving phenylbutazone have not revealed lesions.[12] There has been 1 undocumented report of low hematocrit and hemoglobin following "prolonged periods" of phenylbutazone administration.[27] With the usual doses of the drug in equine patients, no substantiated evidence of blood dyscrasias (untoward changes),[7] allergic skin reactions, or gastrointestinal effects[18] have been reported. Only 2 reports in the literature present evidence for toxicity. One of these is a report, with evidence of several technical inconsistencies, on a single equine patient which showed a reversible hypoplastic anemia.[7] In the other study,[14] 4 times (8 g) and 8 times (16 g) the usual daily maintenance dose was administered to 2 horses of undescribed age and health for 9 days. In these horses "some ulcers of the oral mucosa and erosions of the fundus of the stomach" were found. In 1 experimental horse after 32 days of phenylbutazone administration, the horse was euthanatized and "necrotizing phlebitis of the portal veins" was found. It is difficult to reconcile this study with the clinical experience of minimal toxicity problems with phenylbutazone in the horse. In man, these and many other toxic and side-effects are fairly commonly seen with the drug.[20] However, it continues to be widely used in man because of its efficacy.[28] It has been suggested that the apparent low toxicity of phenylbutazone in the horse is due to the short half-life when compared to that of man.[28,29]

Effect on Incidence of Horses Breaking Down

In Thoroughbred horses in California and Colorado where there is a close inspection for soundness before racing and good control of medication, the percentage of horses sustaining serious injuries requiring destruction has been the same or slightly less in horses which have been given phenylbutazone compared to those which have not been given the drug.[3,10] The incidence of serious injuries to the horses racing in these states has not increased since the controlled use of phenylbutazone has been permitted.[3,10]

References

1. Arnold, L., Collins, C., and Starmer, G.A.: Renal and Gastric Lesions after Phenylbutazone and Indomethacin in the Rat. *Pathology*, 6, (1974): 303-313.
2. Bakke, O.M., Draffan, G.H., and Davies, D.S.: The Metabolism of Phenylbutazone in the Rat. *Xenobiotica*, 4, (1974): 237-254.
3. Bierhaus, G.H., Colorado Race Commission Veterinarian: Unpublished data, 1977.
4. Brune, K., Glatt, M., and Graf, P.: Mechanism of Action of Anti-Inflammatory Drugs. *Gen Pharmacol*, 7, (1976): 27-33.
5. Cho, A.K., Hodshon, B.J., and Brodie, B.B.: Effects of Phenylbutazone on Liver Microsomal Demethylase. *Biochem Pharmacol*, 19, (1970): 1817-1823.
6. Davidson, A.H., and Franks, W.C.: Anti-Inflammatory Agents in Equine Surgery. *Mod Vet Pract*, 27, (Nov. 1966): 46-49.
7. Dunavant, M.L., and Murray, E.S.: Clinical Evidence of Phenylbutazone-Induced Hypoplastic Anemia. In *Proc 1st International Symposium on Equine Hematology*, (1975): 353-355.
8. Dunn, P.S.: Symposium (1) A Clinician's Views of the Use and Misuse of Phenylbutazone. *Equine Vet J*, 4, (1972): 63-75.
9. Ebert, E.F.: Clinical Use of Phenylbutazone in Large Animals. *Vet Med*, 57, (1962): 33-35.
10. Edmondson, A.H., California Racing Commission: Unpublished data, 1977.
11. Ferreira, S.H., and Vane, J.R.: New Aspects of the Mode of Action of Nonsteroid Anti-Inflammatory Drugs. *Ann Rev Pharmacol*, 14, (1974): 57-73.
12. Finocchio, E.J., Ozog, F.J., Oehme, F.W., Johnson, J.H., and Oshaldiston, G.W.: Detection of Phenylbutazone and Oxyphenbutazone in the Urine of Thoroughbreds. *JAVMA*, 156, (1970): 454-456.
13. Gabel, Albert A., The Ohio State University, Columbus, Ohio: Unpublished data on postsurgical equine patients, 1977.
14. Gabriel, K.L., and Martin, J.E.: Phenylbutazone: Short Term Versus Long Term Administration to Thoroughbred and Standardbred Horses. *JAVMA*, 140, (1962): 337-341.
15. Hackett, Richard P.: Analgesics for Horses. *Vet Anesthesia*, 3, (1976): 116-120.
16. Hopes, R.: Use and Misuses of Anti-Inflammatory Drugs in Race Horses–I. *Equine Vet J*, 4, (1972): 66-70.
17. Janchen, E., and Levy, G.: Inhibition of Phenylbutazone Elimination by its Metabolite, Oxyphenbutazone. *Proc Soc Exp Biol Med*, 141, (1972): 963-965.
18. Maylin, G.A.: Disposition of Phenylbutazone in the Horse. Annual Meeting of the AORC, 1976.
19. Maylin, G.A.: The Disposition of Phenylbutazone in the Horse. In *Proceedings*, 20th Ann Meeting, Amer Assoc Equine Practitioners, 1974: 243-248.
20. Maylin, George: Unpublished data, 1977.
21. Moss, M.S., and Haywood, P.E.: Persistence of Phenylbutazone in Horses Producing Acid Urines. *Vet Rec*, 93, (1973): 124-126.
22. Neusbaum, S.: Report on Maylin's Findings. Annual Meeting of NASRC, 1973.
23. Oehme, F.W.: Phenylbutazone in the Treatment of Soft Tissue Reactions of Large Animals. *Vet Med*, 57, (1962): 229-231.
24. Panel on Pharmacology and Physiology of Drugs Used in Racing–Poll of Practitioners. AAEP, 1976.

25. Pipenia, E., Ellis, D.J., Getty, S.M., and Brody, T.M.: Plasma and Urine Levels of Phenylbutazone in the Horse. *JAVMA*, 153, (1968): 195-198.
26. Ray, R., The Ohio State University, Columbus, Ohio: Unpublished data, 1977.
27. Roberts, D.V.: Panel Report. *Mod Vet Pract*, 53, (1972): 67-69.
28. Tharp, V.L., Murdick, P.W., and Ray, R.S.: Annual Report to Harness Tracks of America, 1971.
29. Tharp, V.L., Murdick, P.W., Ray, R.S., and Blake, J.W.: Supplement to the Annual Research Report to The Ohio State Racing Commission, Nov. 1971.
30. Tobin, T., University of Kentucky: Unpublished data, 1977.
31. Tobin, T., Blake, J.W., and Valentine, R.: Drug Interactions in the Horse: Effects of Chloramphenicol Quinidine and Oxyphenbutazone on Phenylbutazone Metabolism. *Amer J Vet Res*, 38, (1977): 123-127.
32. Vane, J.R.: Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-Like Drugs. *Nature New Biol*, 231, (1971): 232-235.
33. Von Rechenberg, H.K.: *Phenylbutazone*. 2nd ed. Edward Arnold Ltd, London, 1962.

Pharmacology Review: Testing for Drugs in Horses

Thomas Tobin, D.V.M., Ph.D.

From the Kentucky Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, KY 40506.

The testing of body fluids from horses for the presence of drugs was first introduced into racing in Europe about 1910. In the year 1912, a horse called Bourbon Rose won the gold cup at Maisons Lafitte in France, but was disqualified because it yielded the first "positive" dope test. Following a pattern, which has since become all too familiar, the owners sued, but lost their case. Routine testing for drugs in horses had arrived and had been supported in the courts.

Although equine drug testing has been with us now for more than 70 years, most veterinarians know relatively little about the testing process and how an analyst arrives at the conclusion that he has a "positive." Consequently, most veterinarians are not likely to be in a position to even begin to evaluate an analyst's evidence or advise a client in the event of a "positive" being called. This review is to outline the process of drug testing as it is currently conducted and to help the veterinarian in assessing the significance of the kind of evidence on which an analyst may "call" a "positive."

Before we outline the various methods used by analysts, it should be clearly understood that analytical methods which are quite acceptable in experimental work may be of very limited value in forensic work. In an experimental situation, where one administers a known drug to a horse, relatively simple analytical methods will often suffice.[1] This is because these horses can be tested before and after the experiment to insure that they are not yielding "false positives." In the forensic situation, however, no control (i.e. predrug or postdrug) samples are available from the horse, and samples are being drawn from a large number of horses under widely differing conditions. These horses will have been treated with many different drugs, feed additives, and domestic remedies, and exposed to many different varieties of plant life, and may have been exposed to any one of the approximately 4.2 million known chemical entities.[2a] On scientific grounds, therefore, scrupulous care must be taken in any forensic test merely to insure accuracy, quite apart from the consideration that personal and professional reputations and livelihoods are usually involved. Because of these considerations, drug "positives" should be "called" only on the strongest scientific grounds. The purpose of this article is to help the veterinarian recognize these grounds and to differentiate between excellent and perhaps marginal analytical work. In writing this article I have drawn heavily on a review of the subject by Dr. Bruce Stein[2] and his co-workers in *The Wisconsin Law Review*.

On the assumption that the biological samples in question were properly taken, numbered, sealed with evidence tape, and delivered to the laboratory, their receipt in the laboratory should be recorded by identifying numbers in a bound laboratory log dated for that day. The log should record the approximate volume received, any comments on their appearance, and the pH of a urine sample. The laboratory should also have an up-to-date loose-leaf manual on the testing procedures in use on that particular day, as methods in all laboratories change with time, and sometimes rapidly. In addition, the laboratory should have clear-cut written criteria set up in advance to be adhered to for differentiating between data points. For example, in thin layer chromatography work by Sunshine, *et al.*,[3] all Rf's differing by more than +0.05 were considered distinguishable. Similar criteria should also be established for GC retention times and other quantative data.

If the procedure of the International Olympic Committee is followed, as it is by many European laboratories, the samples arrive in two bottles labelled "A" and "B." The analysis is started on the A sample and the B sample is stored frozen. In the event of a "positive," the B sample is then available for confirmatory analysis either in the presence of a referee or by an independent laboratory. This procedure safeguards the interests of the owners, and in many European laboratories analysis is not started in the absence of a referee's sample.[4] This system has proven its worth in Europe, because at least one "positive" called this spring (1978) was not confirmed by an unquestionably competent refereeing laboratory in another country. Unfortunately, in the United States many jurisdictions do not require a referee sample, and it is all too easy to dispose of the remains of a test sample.

[1] There are about 4,000 active drug ingredients, 63,000 chemicals in everyday use, and, as of December 1977, about 4,000,000 chemical entities, a number which was increasing at the rate of about 6,000/week.[2]

The Testing Procedure

The first step in drug analysis is usually the taking from the sample two aliquots of about 10 to 20 ml. One of these aliquots is made acidic with about 5 ml of saturated acid phosphate buffer, and one is made basic with a saturated solution of sodium tetraborate. Then an equal volume of an organic solvent, such as benzene or dichloromethane, is added to each system and the whole shaken up together. When the organic solvent settles out, it is found that the acidic drugs will have migrated into the organic solvent under acidic conditions. In this way, acidic drugs such as phenylbutazone, furosemide, naproxen, and so forth will predominate in the acidic extract, while basic drugs such as amphetamine, ritalin, cocaine, and the local anaesthetics will predominate in the basic extract.[4] This process is technically called solvent extraction. Other drugs, such as caffeine and reserpine, which do not carry any dominant electrical charge, are not easy to separate from other drugs at this stage (Figure 1).

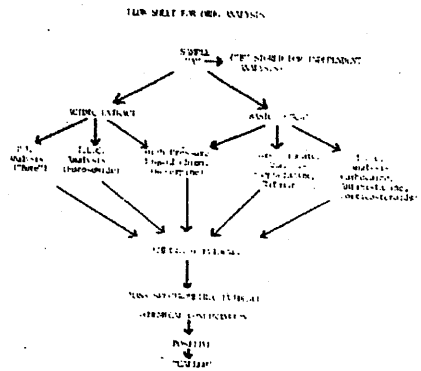


Figure 1. General flow sheet for drug analysis.

Once the solvent extraction has been made, the solvent is evaporated down to a very small volume to concentrate the extracts. A small portion of the acidic extract is then spotted on a "thin layer plate" and chromatographed by a process called Thin Layer Chromatography (TLC). Other common manuevers with acidic extracts include Ultraviolet Spectrometry (UV analysis) and, increasingly, High Performance Liquid Chromatography (HPLC). If positive indications for the presence of a drug appear in any or all of these test systems, the urine extract may be examined by Gas Chromatography-Mass Spectrometry (GCMS).[4]

The same general procedure is followed with the basic extract. While a portion of the extract may be subjected to thin layer chromatography, an increasingly common procedure is to react the extract with a highly reactive "marker" chemical such as pentafluropropionic anhydride (PFA) and examine the result by gas chromatography. This method is useful for picking up very small quantities of central nervous system stimulants.[2] Again, if suspicious peaks are found, the material may be subjected to gas chromatography-mass spectrometry. A general flow sheet for drug analysis is presented schematically in Figure 1.

Horses excrete some drugs such as apomorphine and pentazocine in their urine by linking these drugs to highly water-soluble sugar molecules. Before analysis for these drugs can commence, the drug portion of this complex must be split out by enzymatic hydrolysis.[10] Once this process, which takes two to three hours, is complete, testing for these drugs proceeds by acidic or basic extraction as previously.

The Analytical Methods

The essence of all these procedures is that they give varying kinds of evidence about the presence of a drug. At the first sign of something suggesting the presence of a drug, the analyst has what he calls a "suspicious sample." As he continues to gather evidence, he must ask himself how good or useful the evidence is and at what point he should call it a "positive."[5] Before we can second-guess him on this judgement, we will have to look more closely at the analytical techniques he uses and the quality of information that has been provided him.

Ultra Violet Spectrometry (UV Spectrum)

In ultraviolet spectrometry, light of shorter and shorter wavelengths is directed through the drug solution. If a drug is present in sufficient concentration and absorbs UV light, the instrument will plot out a graph of the drug's light absorbance at each wavelength. A typical UV absorbance spectrum for flunixin, extracted from horse urine, is shown in Figure 2. From the shape of the UV curve and the wavelengths at which the peaks occur, the analyst may suspect the presence of a certain drug. He will then change the pH of the system, and if the shape of the absorbance curve changes

[5] For the purpose of this report, a "positive" is a drug finding which violates a medication rule. An analyst reports a positive when there is sufficient data to substantiate the presence of a specific drug or foreign chemical substance. Partial data which suggests but does not satisfy the analyst that a specific drug is present constitutes a "suspicious." A "suspicious" may be upgraded to a positive by the acquisition of more data depending on the medication rules of the particular jurisdiction.[11]

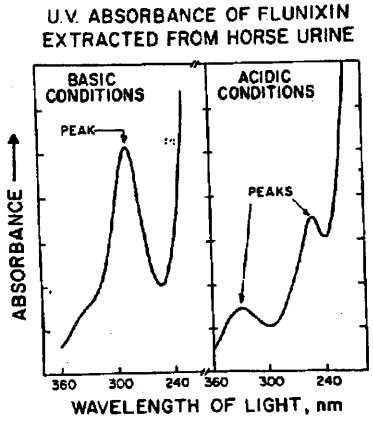


Figure 2. UV absorbance pattern of flunixin. The right-hand panel shows two absorbance peaks for flunixin at 250 nm and 330 nm under acidic conditions, while the left-hand panel shows a single peak at 290 nm under basic conditions.

in a way which supports his opinion as to which drug is present, he will have made a tentative drug identification.

There are two principal problems with UV data as a basis for drug identification. The first is that many compounds share broadly similar UV absorption patterns. For example, all barbiturates generate similar UV absorption spectra, so the test cannot distinguish between barbiturates. Since not all barbiturates are depressants, the method cannot be relied on to identify barbiturate drugs.

The second problem is that the portion of the UV region used by analysts is only about 200 nm wide, which means that there are at the most about 200 different values for UV absorption peaks. If only about 20% of the compounds absorb in the UV, then there are about 4,000 different candidate compounds for each UV peak. The problem is further complicated by the fact that extracts of horse urine contain unknown compounds which also absorb in the UV and will tend to confuse the picture. Because no control (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. UV is therefore considered a screening technique by most analysts.[5]

Thin Layer Chromatography (TLC)

Thin layer chromatography is another useful screening technique. It derives its name from the fact that the experiment is performed in a thin layer of silica gel or other adsorbent coated on a glass or metal plate. The drug extract is spotted about 1 cm from the edge of the plate, and the plate is stood on edge in a solvent system (Figure 3). As the solvent system migrates up the plate, the compounds in the spot move at varying speeds depending on their affinity for either the solvent (mobile phase) or the gel (stationary phase). Compounds which spend about half their time in each phase will migrate about one-half the way up the plate and are spoken of as having an Rf of about 0.5. Other compounds may spend most of their time absorbed to the silica gel and barely move at all from the point of origin. Others may spend most of their time in the solvent and migrate close to the "solvent front"* and thus have an Rf in the region of 0.9 to 1.0.

When an analyst suspects the presence of a drug, he makes an educated guess as to which drug it might be. He then reruns the experiment with his best guess running right beside his unknown. If the spots do not migrate the same distance relative to the solvent front, he can be quite sure that the drug and the unknown are not the same substance. If the spots do migrate together, they may be the same substance, but they are not necessarily the same substance.

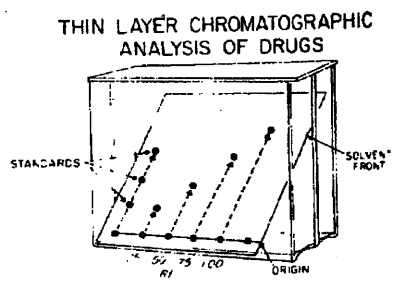


Figure 3. Thin layer chromatography. Drug standards and unknowns are spotted at the origin and the plate stood on end in the solvent. The fraction of the distance migrated by the drug in comparison with the distance migrated by the solvent front is the Rf of the drug.

The reason one can never be sure about identification with TLC is statistical. The maximum number of spots that one can hope to 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 2,000 possible candidates for each spot. Specific extraction conditions would reduce this number even further, but a considerable probability of overlap in TLC systems still exists. This is the basic problem with trying to use TLC for definitive identification.

* The solvent front is the leading edge (front) of the solvent as it moves up the TLC plate.

Since most forensic chemists are aware of the lack of specificity of TLC, they usually elect to compare the drug and the unknown in a number of different TLC systems. If both spots again migrate the same distance, the chances that the analyst is right are improved. In an experimental test of this procedure, Sunshine and his co-workers[7] chromatographed 138 drugs in four different solvent systems. They were unable to separate 25 of these drugs in four solvent systems, and in experiments with even seven TLC systems, overlaps were still found. A reasonable conclusion from these experiments is that the number of solvent systems required to separate just the four thousand drugs in common use without risk of overlap is both astronomic and unlikely to be run by the average drug-testing laboratory. TLC is therefore a test which can readily prove the absence of a drug but can never prove its presence. It is consequently not considered a specific test by most forensic authorities.[6]

Gas Chromatography (GC)

Gas chromatography functions on the same principle as TLC with the difference that the mobile phase is a gas, and the stationary phase may be any one of a huge variety of materials. Because the mobile phase is gaseous, the substance to be chromatographed must be volatile, which restricts GC analysis to the approximately 400,000 volatile chemicals and perhaps another 400,000 which can be volatilized after appropriate treatment.

Gas chromatography is like TLC in that some drugs will flow right along with the gas and some will stick to the column (stationary phase) near the origin and never come out. However, if one has chosen the right column pack (stationary phase), mobile phase (gas) temperature, gas flow, and so forth, one can arrange for a nice separation of drugs by GC. To start the experiment, the unknown is injected onto the column and allowed to percolate through the column. Given a suitable detector at the end of the column, one can record the exit from the column of everything the detector will detect. Some typical gas chromatographic records for procaine in horse urine are presented in Figure 4.

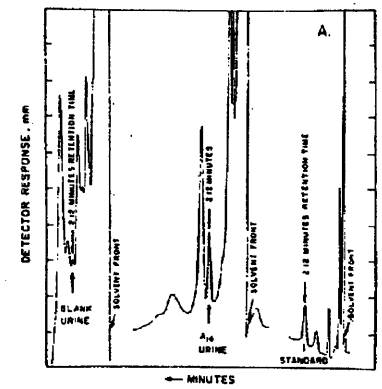


Figure 4. Gas chromatographic analysis of procaine derivatives in horse urine. The right-hand chromatogram is that of a standard solution of 10 ng/ml of procaine derivatized with heptafluorobutyric anhydride (HFBA); the center chromatogram is that of a urine sample from a horse pretreated with procaine HCl and similarly treated; the left-hand chromatogram is that of urine from a control horse similarly derivatized.[9]

Now the question of how does one know what each peak the pen writes represents, and for that matter, how can one know that a peak does not contain two or more drugs, obviously arises. The answer to this question is the same as before: you guess at what a peak may be, then test your guess by running a pure (authentic) sample of the drug you suspect. If the pure sample and the unknown come off the column at exactly the same time, and the peaks look exactly the same shape, you may have guessed right. On the other hand, if the pure standard and the suspect peak came off at different times, you know for sure that you are wrong and will have to try another guess.

With respect to the problem of specificity, exactly the same principles apply to GC as apply to TLC. On a 200 m capillary GC column, one can separate about 400 compounds in a four-hour experiment. However, most drug detection laboratories, and especially prerace laboratories, do not have this kind of time to play with, so they use short columns (1 to 2 m) and short retention times. With these systems, one cannot hope to separate more than about 100 compounds. Since there are about 800,000 compounds which can be volatilized, there are 8,000 possible candidates for each peak. The usual procedure at this point is to further test one's guess by changing the column tem-

perature once or twice to see whether or not the material continues to co-chromatograph with the analyst's best guess. Then the pair (suspect and authentic) are usually run on a different GC column at three different temperatures. If the unknown and suspect continue to chromatograph together, many analysts will conclude that they have identified a drug.

A helpful test rarely used is to mix an equivalent amount of the suspect and authentic material and chromatograph them together. If they are indeed the same substance, the suspect peak should double in size, remain symmetrical, and show no tendency to "split," no matter what the chromatographic conditions. This is a particularly useful test, because in its absence one is relying on stopwatch timings of peaks by a person who is paid to match unknown peaks in urine samples with drug peaks. Personally, therefore, I would very much like to see combined samples in every GC run where an unknown is being compared with an authentic for forensic identification.

Again, 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 one can say is that the unknown and the authentic compound are indistinguishable in the systems in which they were compared. While GC is unquestionably more accurate than TLC, just how accurate it is is not clear. To my knowledge, there is no study on the resolving power of GC to compare with the Sunshine[7] study on TLC. Some analysts suggest a 10% error rate in drug identification based on TLC and GC, while other authorities hold that co-chromatography on three distinct column systems is very good evidence for identity,[d] and that the most important factor is the skill and ability of the analyst.

The Gas Chromatograph—Mass Spectrometer (GC—MS)

In the gas chromatography-mass spectrometer, the unknown materials forming the peaks which come off the gas chromatograph are fed directly into a mass spectrometer, which functions as a detector for the gas chromatograph. However, as well as simply detecting the peak, the mass spectrometer can measure its molecular weight and determine its fragmentation pattern. This gives what is sometimes called a molecular "fingerprint" for each drug, and a good mass spectrum is considered among the best evidence available as to the identity of a drug.[e]

[d] Personal communication from Dr. H. W. Dorough, Department of Entomology, University of Kentucky, Lexington, KY

The principle of mass spectrometry is quite straightforward. The unknown drug peak from the gas chromatograph is introduced into a vacuum chamber where it is bombarded with an electron beam. The electron beam positively charges the drug molecules and, depending on its energy, may fragment the molecules. These charged particles are then accelerated through a magnetic or electrical field which separates them on the basis of their mass/charge (m/e) ratio. At the end of the analysis tube, the impact of the ions is recorded on an ion detector, and the number of each mass is tallied. Changing the strength of the magnetic field changes the mass/charge ratio of the ions hitting the detector and in less than a second yields a mass spectrum for a drug.

A pair of such mass spectra are shown in Figure 5, one of which (B) is authentic dipyrone and one of which is a racetrack sample identified as containing dipyrone (A). In these experiments, dipyrone (molecular weight 351.4) broke down to yield a fragment of molecular weight 217.2, present in quite small amounts and a series of smaller fragments, all of which are represented essentially equally in both spectra. It is this kind of detailed evidence of similar chemical structures which makes mass spectrometry such a useful tool for identifying drugs. Since each known chemical, and thus its fragmentation pattern, is unique, this type of information yields a virtual "fingerprint" of the chemical in question. In addition, the mass spectrome-

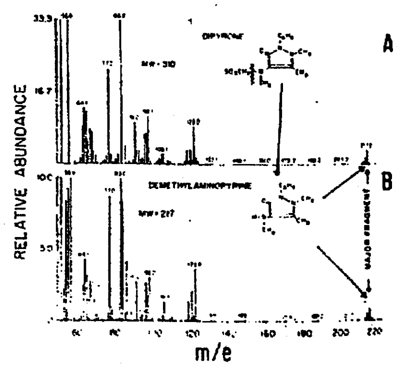


Figure 5. Mass spectrometric analysis of dipyrone in horse urine. Panel A shows the mass spectrum of a dipyrone standard, while panel B shows the spectrum of an unknown recovered from horse urine. The largest fragment present in both spectra is of mass 217.2, consistent with breakdown of dipyrone to demethylaminopyrine in both samples. Many others ions of similar m e are present in similar abundances, suggesting that sample B does indeed contain dipyrone.

ter is able to detect nanogram quantities of drugs and as such is sufficiently sensitive to be useful for drug detection in body fluids of horses.[4]

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography is broadly similar to GC except that the mobile phase is a liquid under very high pressure. Because solvents for most chemical compounds are available, the potential field of application of HPLC is much wider than that for GC which requires volatile compounds. However, at this time HPLC is only beginning to be used in routine drug testing.

Radioimmunoassay (RIA)

In RIA, a specific antibody to a drug molecule is used to bind both a radiolabeled drug and whatever unlabeled drug may be added with the unknown plasma or urine sample. If a significant amount of unlabeled drug is added to the system, sufficient radiolabeled drug is displaced for the reduction of radioactivity in the system to be measurable. 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 can never be a chemically specific test.

"Calling" a "Positive"

Having briefly reviewed the analytical methods available to the analyst, we can now look over his shoulder as he examines his data. The average analyst will have on hand about 200 standards with which he will try to match any suspect peaks or spots in his test systems. Because the 200 standards cover many of the drugs used routinely on the track, he will be able to match them up successfully much more often than odds of 200 knowns to 4,000, 63,000 or 4.2 million unknowns might suggest. If the analyst has GC-MS with a computerized library, he will have access to about 40,000 chemical spectra, which improves the scope of his search 200-fold.

At some point in this matching procedure, the analyst may decide to call a "positive." He will do this when he is satisfied that he has attained certain analytical criteria 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 three analytical systems, one of which must be GC-MS. Most United States racing jurisdictions, however, do not have any explicit statements on what the analytical requirements for a "positive" are. Therefore, in these jurisdictions the criteria on which positives are called are the analyst's own. While this system allows the analyst considerable flexibility, it can also leave him open to pressure to call "positives" against his better judgement.

A very real problem arises in "calling positives" when GC-MS data is not available for either economic or technical reasons. If MS data is not available, positives called on TLC, GC, or other empirical methods are "positives" called on evidence with a distinct probability of error. Data from the Cornell University Drug Testing Program, which has the longest experience with GC-MS confirmation of analytical data, suggests that up to 10% of drug identifications obtained with TLC and GC may turn out to be incorrect when tested by MS.[f] Analysts who call positives in the absence of MS data should keep such estimated error levels in mind and only "call positives" on the very best of empirical evidence.

Calling positives on empirical or any other kind of evidence means that the analyst should be prepared to present all his evidence for examination if required. This should include all chart records such as presented in Figures 2, 4, and 5, and all actual physical evidence such as thin layer plates and microcrystals, or good quality color photographs of these if the plates cannot be preserved. The physical evidence should be presented with accompanying evidence from suitable controls matched as closely as possible with the test samples and run *simultaneously* with the tests in question.[8] Just producing typed statments that "UV spectra typical of drug X" were observed is good evidence of an ability to type but totally useless as chemical evidence for the presence of drug "X." Though affidavits and sworn statements carry considerable weight in law, they carry no weight in science, where physical evidence and ability to reproduce the evidence or events are the only criteria. Therefore, the remains of the original duplicate sample should be preserved at all costs for independent analysis. Only in this way can the potential for error inherent in any human process be reduced and all concerned be assured that the matching process on which drug detection depends has been as good as possible.

[f] Personal communication from Dr. Jack Henion, New York Racing and Wagering Board, Drug Testing and Research Program, Cornell University, Ithaca, NY

References

1. Atti Della Conferenza Internazionale sul Doping. Stampa Degli Atti a Cura Dello Studio Ego Congress, Viale Tiziano, Roma, Italia 146-147

2 Blake, J.W. and Tobin, T.: The Gas Liquid Chromatograph and the Electron Capture Detector in Equine Drug Testing. *Brit J Sports Med.* 10, (1976): 129-132.

3 Maugh, T.H., III: Speaking of Science. Chemicals: How Many Are There? *Science*. 199, (13): (1978): 162

4. Maylin, G.A.: Pre-Race Testing. *Com Vet.* 64, (1974): 325-334.

5. Shapiro, R.H.: Chemical Defenses in Drug Cases. *J Crim Def.* 2, (1976): 131-144.

6. Stern, B., Laessig, R.H., and Indrikson, A.: An Evaluation of Drug Testing Procedures Used by Forensic Laboratories and the Qualifications of their Analysts. *Wis Law Rev.* 3, (1973): 726-789.

7. Sunshine, I., Fike, W. F., and Landesman, H.: Identification of Therapeutically Significant Organic Bases by Thin Layer Chromatography. *J Forensic Sci.* 11, (1966): 428-439.

8. Tobin, T.: A Review of the Pharmacology of Reserpine in the Horse. *J Eq Med Surg.* 18, (1978): 433-438.

9. Tobin, T., Blake, J.W., Sturma, L., Ament, S., and Truelove, J.: Pharmacology of Procaine in the Horse: Pharmacokinetics and Behavioral Effects. *Am J Vet Res.* 38, (1977): 637-647.

10 Tobin, T., Roberts, B. L., and Miller, J.R.: The Pharmacology of Furosemide in the Horse. I. Effects on the Disposition of Procaine, Methylphenidate, Phenylbutazone and Pentazocine. *J Eq Med Surg.* 1, (1977): 402-409.

11 Tobin, T., Maylin, G.A., Harrion, J., Woodward, C., Ray, R., Johnston, J., and Blake, J.W.: An Evaluation of Pre- and Post-Race Testing and Blood and Urine Testing in Horses. *J Eq Med Surg.* (1979), in press.

Publication No. 44 from the Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY. Published as Kentucky Agricultural Experiment Station Article No. 78-4-186 with the permission of the Dean and Director, College of Agriculture, Agricultural Experiment Station. The assistance of Mr. Brian L. Roberts in preparing this article is gratefully acknowledged.

Effect of Urine pH on Urine Levels of Oxyphenbutazone in Racing Horses

Teresa Houston, Thomas Tobin and J.W. Blake

Kentucky Equine Drug Testing and Research Programs
Department of Veterinary Science
University of Kentucky
Lexington, KY 40546-0076

Published as Kentucky Agricultural Experiment Station Article 83-4- , with the approval of the Dean and Director, College of Agriculture and Kentucky Agricultural Experiment Station.

Publication #87 from the Kentucky Equine Drug Testing and Research Programs, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky.

Supported by a grant entitled "Masking by Phenylbutazone in Equine Drug Testing: An Analysis" from the Kentucky Equine Drug Research Council and the Kentucky State Racing Commission.

Correspondence should be addressed to Dr. Houston.

INTRODUCTION

The use of phenylbutazone in racing horses is often regulated by quantitating urine levels of phenylbutazone and its metabolites. Oxyphenbutazone is the principal metabolite of phenylbutazone found in equine urine (1). Because of the forensic importance of the levels of oxyphenbutazone found in equine urine, we elected to investigate the effects of urinary pH on blood and urine levels of oxyphenbutazone in racing horses.

Urine pH in racing horses is unusual in that it may range from pH 4.5 to pH 10.0 . Beyond having such a wide range, the distribution is bimodal, with population peaks at about pH 5.0 and pH 8.0 (1). This wide range of urine pH values in racing horses suggests that urinary levels of either phenylbutazone or its metabolites may be substantially influenced by urinary pH (2) independently of other factors such as the time which has elapsed since the last dose of phenylbutazone. Despite the well recognized role of pH in affecting the distribution and disposition of drugs (1,2), and its potential forensic importance (1) only two studies in this area have been reported (3,4).

To determine the effect of pH on urineconcentrations of oxyphenbutazone, we measured blood and urinary levels of oxyphenbutazone in post-race urine samples from Thoroughbred horses racing in Kentucky. The data show that while oxyphenbutazone levels in equine blood are not significantly influenced by urine pH, urine concentrations of oxyphenbutazone are highly dependent on urine pH , and may vary up to 1,000-fold, depending on whether the urine is acidic or basic.

MATERIALS AND METHODS

Phenylbutazone (PB), oxyphenbutazone (OPB) were obtained from Ciba Pharmaceuticals (Summit, NJ) and γ-hydroxyphenylbutazone (γ-OHPB) from Ciba-Geigy

(Switzerland). Liquid chromatographic grade methanol and water was obtained from Burdick-Jackson (Alltech, Deerfield, IL). All other solvents and reagents used were of analytical grade from Fisher Scientific (Louisville, KY).

All blood and urine samples tested were post-race blood and urine samples submitted to the Kentucky Equine Drug Testing laboratory by the Kentucky State Racing Commission. Blood samples were drawn and urine samples collected when the horse voided urine post-race. All samples were stored at 4° C and shipped to the University of Kentucky for analysis within 48 hours. Phenylbutazone is a permitted medication for Thoroughbred horses racing in Kentucky.

A Beckman 341 liquid chromatograph equipped with a 421 controller, an Altex model 100 pump, and an Altex model 153 UV detector (254 nm) was used. A 20 µl loop was used on the 210 sample injector valve. An Ultrasphere-ODS 5 µ column (4.6 mm x 25 cm) with a guard column (Pellicular C-18, 4.6 mm x 5 cm, Alltech) between the injector and analytical column was used for the separation of the compounds.

The chromatographic procedure used was based on that described by Marunaka et al (5). The mobile phase was a linear gradient of 50% methanol-50% 0.01 M sodium acetate (pH 4) as the initial concentration and 100% methanol (at 5%/min) as the final concentration. The column was maintained at room temperature with a flow rate of 1.0 ml/min and the eluted compounds were recorded by the detector at 254 nm.

Blood samples were collected in 20 ml Vacutainer tubes containing potassium oxalate and sodium fluoride. Urine samples were collected in glass jars when the horses voided. To 1 ml of plasma was added 4 ml saturated KH_2PO_4 and 6 ml dichloromethane at room temperature. The samples were rotoracked 6 min, centrifuged for 2 min at 2000 rpm and the organic layer was transferred to a clean

tube and evaporated to near dryness in a water bath (65° C). The samples were completely dried under a stream of N_2. To the residue, 100 µg methanol was added and 20 µl of the sample injected onto the column. The urine samples were prepared in a similar manner except 4 ml of pH 3.3 saturated KH_2PO_4 was added.

Standard curves were prepared for the determination of phenylbutazone, oxyphenbutatone and γ-hydroxyphenylbutazone by adding known amounts of authentic standards to blank plasma or urine and assaying by the same extraction procedure. Concentration ranges of 0.5-5.0 µ gm/ml and 10-50 µ gm/ml were used. Peak heights were plotted against the concentration.

RESULTS

The insert in Fig 1 shows the distribution of urine pH values observed in 103 post-race urine samples. The pH values observed ranged from about 4.8 to 8.5. The distribution observed is consistent with the bimodal distribution for urine pH values in several thousand horse urines reported by Moss et al and Nakajima et al (1). The pH range represented in Fig 1 covers the great bulk of the range reported by these authors.

Figure 1 shows the plasma and urinary levels of oxyphenbutazone found in these horses, plotted against urinary pH values. The data show that the plasma levels of oxyphenbutazone do not vary with pH since similar plasma levels were found in the urine of horses forming acidic or basic urines. The data suggest that the plasma levels and plasma pharmacokinetics of oxyphenbutazone in the horse are not dependent on or substantially influenced by urine pH.

In contrast, urine concentrations of oxyphenbutazone were very variable and highly dependent on urinary pH. At urinary pH 6.0, plasma and urinary

levels of oxyphenbutazone were broadly similar. At about pH 5.0, however, urinary concentrations of oxyphenbutazone were too low to measure by our analytical method. These values are reported as 0.1 µmg/ml, but some of these values are probably less. On the other hand, at pH 8.0 and above, urinary concentrations of oxyphenbutazone averaged 50 µg/ml or more, about 50 times the plasma levels of oxyphenbutazone observed in these horses. The data suggest that urinary concentrations of oxyphenbutazone in horses are influenced by urinary pH.

DISCUSSION

These experiments suggest that the concentrations of oxyphenbutazone in post-race urine samples of Thoroughbred horses racing in Kentucky were highly influenced by urinary pH. If the pH of the urine sample was 5.0 or less, the concentration of oxyphenbutazone in the urine was too low to measure. If the pH of the sample was 8.0 or above, the concentration was 50 µg/ml or more. In each case, the mean plasma level of oxyphenbutazone was about 1 µg/ml. The data suggest that oxyphenbutazone levels in equine urine may vary up to 1,000-fold, depending principally on the pH of the urine sample.

Based on the Henderson-Hasselbach equation (2) and the known distribution of urinary pH values in horses, one can readily calculate the theoretical pH-dependent distribution of oxyphenbutazone in equine urines of different pH values (1). Based on these calculations, one may expect about a 10,000-fold concentration of the drug and a urinary pH range of 4.5 to 10.0. Prior to this report, however, no data suggesting that such pH-dependent effects might occur in the urine of racing horses. The data demonstrate close to a 1,000-fold variation in urinary levels of oxyphenbutazone depending on urinary pH. It

appears likely that given more sensitive analytical instrumentation and the wider range of pH values which obtain in larger numbers of samples, the full 10,000-fold theoretical range of oxyphenbutazone levels in equine urine would be observed.

Since all these samples were racing samples, the doses of phenylbutazone or other drugs which may have been administered to these horses are not known. However, the plasma levels of oxyphenbutazone observed in these experiments are relatively low and did not vary significantly with pH. On the other hand, urinary levels of oxyphenbutazone varied directly with pH and in a manner quantitatively consistent with that predicted by pH theory. The data, therefore, strongly suggest that urinary pH is a major determinant of urinary oxyphenbutazone levels in horses.

The data reported here also shed light on the problem of "masking" (1). "Masking" is thought to occur when high levels of oxyphenbutazone in a urine sample interfere with the detection of other drugs. One of the principal objections to the use of phenylbutazone in racing horses is that horsemen can use it to "mask" the presence of other drugs. However, these data show no evidence for high blood levels of oxyphenbutazone, as might be expected if horsemen were attempting to cause "masking" problems. Rather, these results suggest that high urinary concentrations of oxyphenbutazone are found in horses forming alkaline urine, in which ionized oxyphenbutazone is "trapped". High urinary concentrations of oxyphenbutazone are therefore more likely caused randomly by the physiology of the horse than by any ability or intent of horsemen to "load" urines with high or "masking" levels of oxyphenbutazone.

As a practical matter, these results bear on the relative merits of blood

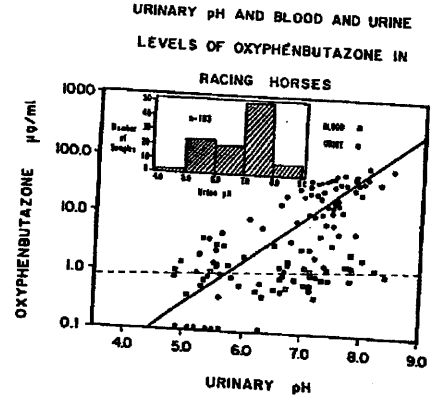
as the regulation of phenylbutazone use is concerned, urine sampling is therefore likely to be a about 100-fold less satisfactory as a regulatory medium than blood.

These observations are forensically important, since many racing jurisdictions regulate the use of phenylbutazone by measuring urinary levels of phenylbutazone and its metabolites. Most commonly, racing authorities have rules which state that medication with phenylbutazone within 24 or 48 hours of race time is not permitted. Some authorities enforce these rules based on the assumption that urinary levels of phenylbutazone and its metabolites are directly related to the time of administration of the last dose of phenylbutazone. In support of such rules, pharmacokinetic experiments on small numbers of horses (usually 6 or less) have been performed and reported in the literature (6) and elsewhere (7,8). However, no investigators have studied the urinary kinetics of phenylbutazone and its metabolites over the full range of physiological pH values of equine urine. Because of this, the validity of these studies as a basis for regulatory and forensic decisions on racing horses is at best questionable.

REFERENCES

1. Tobin, T. Drugs and the Performance Horse, Charles C. Thomas, Springfield, Illinois, 1981.
2. Baggot, J.B. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. Philadelphia, 1977.
3. Evans, J.A. and Lambert, M.B.T. Veterinary Record 95:316-318 (1974).
4. Moss, M.S. and Haywood, P.E. Veterinary Record 93:124-125 (1973).
5. Marunaka, T., Shibata, T., Minami, Y., and Umeno, Y. J. Chromatography 183:331-338 (1980).
6. Maylin, G.A. Proc. AAEP 20:243-248, 1974.
7. Soma, L.R., Rudy, J.A., Gallis, D.E., Railing, F.A., Woodward, C.B. and Cochran, T.A. Proc 35th Meeting of the Association of Official Racing Chemists, p 118-136, 1981.
8. Soma, L.R., Gallis, D.E., Rudy, J.A., Davis, W.L., Cochran, T.A., and Woodward, C.B. A.J.V.R., in press, 1983.

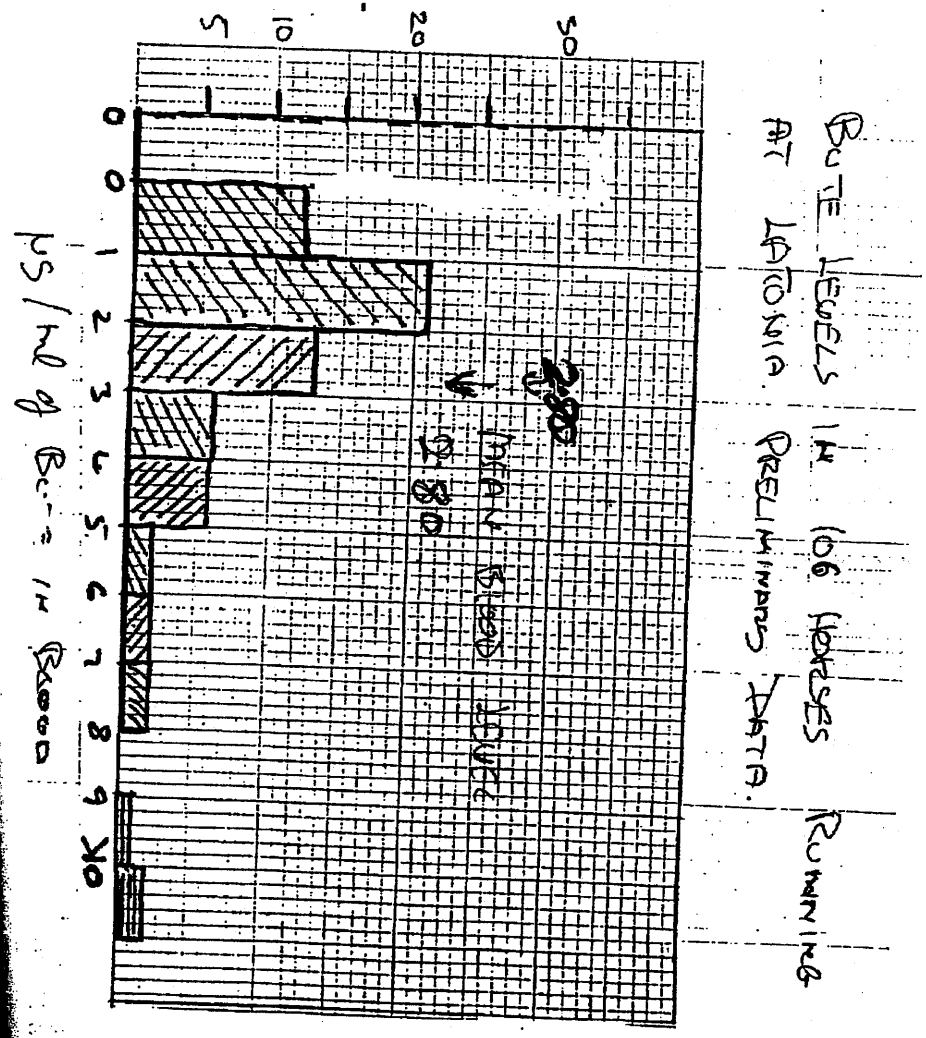
Fig 1 *Urinary pH and Blood and Urine Levels of Oxyphenbutazone in Racing Horses.*- The solid circles and line (●—●) show urine levels of oxyphenbutazone plotted against urinary pH (r=0.92), while the open squares and dotted line (□---□) show concentrations of oxyphenbutazone in the corresponding plasma samples (r=0.19). The inset shows a frequency distribution of pH values of the urine samples used in these experiments. All lines were fitted by the method of least squares.



SUMMARY OF LATONIA RESULTS, February 1983

Date	Total Tested	# of Bute	Mean Bute Level
2/5	13	4	2.0 µg/ml
2/8	14	9	2.25 µg/ml
2/9	13	4	3.35 µg/ml
2/10	16	11	3.61 µg/ml
2/12	13	8	2.82 µg/ml
2/16	12	10	2.43 µg/ml
2/17	8	6	5.00 µg/ml
2/19	17	10	1.66 µg/ml
	106	62	2.83 µg/ml

	Mean	High Bloods
1980 NASRC Study	4.3	13.0
Latonia, 1983	2.8	>10.0
Florida Study	1.0	3.0 (estimates)



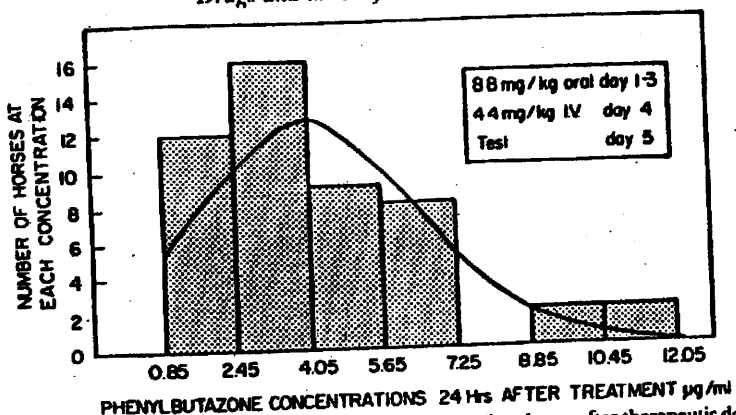


Figure 23-2. Phenylbutazone levels in forty-nine horses twenty-four hours after therapeutic doses of phenylbutazone. Forty-nine horses were dosed with 8.8 mg/kg (about 4.0 gm/1000 lb.) of phenylbutazone orally for three days and then given 4.4 mg/kg (2 gm/1000 lb.) on the fourth day. Twenty-four hours after the IV dose, blood samples were drawn from each and assayed for phenylbutazone. The vertical bars show the number of horses found with the indicated blood levels of phenylbutazone, while the solid line represents a population curve fitted to these data. These data are reproduced with the permission of the National Association of State Racing Commissioners. The experimental protocol was prepared by the NASRC Blue Ribbon Medication Committee. The horses were dosed and analytical facilities were provided by Doctor George Maylin, Cornell University; Doctor Cliff Woodward, Pennsylvania; Doctor Richard Sams, The Ohio State University; and Doctor Thomas Tobin, University of Kentucky. Data analysis was performed at Cornell and Kentucky. These experiments were completed in the spring of 1980.

four-hour" rule, for about 50 per cent of the horses will have blood levels of phenylbutazone below 4.13 µg/ml, 50 per cent will also have blood levels higher, and a small number may be expected to have much higher blood levels. The actual spread of the data from this experiment is shown in Figure 23-2. In this illustration, each hatched bar represents the number of horses found to have blood levels within a particular range. While the "average" horse had a blood level of about 4.13 µg/ml, the blood levels in some horses were much higher, and two had blood levels of between 10.5 and 12.05 µg/ml. If two horses in forty-nine can show these high blood levels, it is obvious that the number of horses in a population of 10,000 that show high blood levels of the drug is going to be substantial. The problem, then, becomes where to put the cutoff for the higher blood levels, or conversely, how one determines whether one has an unusual blood level or a medication violation.

To do this, one measures the variability in the population by determining what is called the standard deviation for the data. Within plus or minus of what is called one standard deviation of the mean of the population, one finds 67.4 percent of the population, and within 1.96 standard deviations of the mean one has about 95 percent of the population. Therefore, if one sets the limit at the mean plus 1.96 standard deviations, one is going to catch a certain percentage of horsemen who overmedicate, along with the about 2.5 percent of all horsemen who comply with the rules. While a 2.5 percent level of error does not sound like much, it means

POSITIVE CALL RATES FOR "HARD DRUG POSITIVES"*
IN CONTROLLED MEDICATION AND NO MEDICATION JURISDICTIONS

State	⌀ # Samples/yr.	⌀ # Positives/yr.	⌀ Call Rate/yr.	N
(with bute)				
Kentucky	6,693**	19.4	2.9/thousand	5
California	19,903**	8.7	0.4/thousand	7
(without bute)				
New York	175,393***	137.5****	0.8/thousand	4
Canada	77,792**	46.4	0.6/thousand	5

*"Hard drug positives" are positives that would be called in Kentucky (stimulants, depressants, narcotics, tranquilisers, local anesthetics)

**Post-race urines

***Pre- and post-race bloods and urines.

****NOTE: THESE NEW YORK "POSITIVES" LIKELY CONTAIN AT LEAST 50% OF MEDICATIONS LEGAL CALIFORNIA AND KENTUCKY.

Sources:

Ky. : University of Ky. Equine Drug Testing Program, JW Blake.

Cal.: Robert Vessiny, Chief Racing Chemist, Truesdail Laboratories, Inc.

NY. : Norman Lewis, Chief Racing Chemist, NY State Racing and Wagering Board.

Can.: J. Easton, Associate Director, Race Track Division of Agriculture Canada.

Equine Communications

A digest of clinical notes and letters.

Letters may include preliminary communications discussing recent developments, first observations of a new disease, a new pathological finding, or any other brief article or case history of outstanding importance or general interest.

Practical review articles and case histories should be written to provide instructive information on a particular technique, disease, or condition that will be relevant for the equine veterinarian in practice.

"Equine Communications" is intended to complement the Journal's editorial and to open the lines of communication among the equine practitioners. Your frequent participation is encouraged. All contributions will be promptly acknowledged.

Pharmacology Review: A Review of Recent Research on Furosemide in the Horse

—Thomas Tobin, Kentucky Equine Drug Research Program, Department of Veterinary Science, University of Kentucky, Lexington, KY

Furosemide* is a member of the "high ceiling" group of diuretics and is a safe and very effective diuretic in the horse.[4] Outside of the usual medical indications for diuretics, furosemide is also useful in racehorses because it is believed to be effective in the control of epistaxis (nosebleed). Epistaxis, however, only occurs in about 2% of horses, while up to 80% of the horses running in some meets may be on furosemide.[5] This article will review recently acquired knowledge concerning furosemide in the horse and may suggest some reasons for the widespread use of furosemide in racing horses.

Furosemide was introduced into the equine medical literature by Dr. Marvin Beeman and his colleagues in Colorado.[3] It is useful in edema from all causes. It is particularly useful in cases of pulmonary edema because it acts to rapidly remove this fluid, even before it begins to move fluid out through the kidneys.[1] Its ability to increase the volume of urine may also be used to dilute out substances in urine and prevent renal damage. Thus, when a horse "ties up," the released myoglobin may deposit in and damage the kidneys. In situations such as this, the increased urine volume due to furosemide greatly reduces the possibility of renal damage.[4]

The diuretic action of furosemide is due to its inhibition of active reabsorption of chloride in the renal tubules. Sodium and water reabsorption normally follow chloride reabsorption, so administration of furosemide leads to greatly increased losses of sodium, chloride, and water.[2]

In a 1,000-pound horse, 4 ml of furosemide (50 mg/ml) produces about 4 liters of urine in about 40 minutes. This is the dose recommended for use in epistaxis (nosebleed) in the horse. A 10-ml dose, which is a common clinical dose, produces about 9 liters of urine, mostly within 1 hour. Forty milliliters of furosemide will produce about 21 liters of urine within about 2 hours, and this is close to the maximum response in a normal horse.[2]

The diuretic action of furosemide is therefore relatively short lived, especially after small intravenous (IV) doses. This short lived action of furosemide is not due to any inability of the horse to respond, as a second dose of furosemide will usually produce a very good response. Rather, the action of furosemide in the horse is brief because furosemide is rapidly pumped out of the blood into the renal tubules.[6] This pumping accounts for the short plasma half-life ($T_{\frac{1}{2}}$ = about 35 minutes) of the drug, its very rapid onset of action in the kidneys, and the fact that up to 60% of the drug is found unchanged in the urine. This is a very high proportion of a drug to be excreted unchanged in the horse.[6]

Because furosemide is secreted directly into the urine, it is found there in very high concentrations. A good analyst can easily detect furosemide in equine urine for 12 hours after a 10-ml dose, and the drug can be detected for up to 52 hours. Because the pharmacological actions of furosemide are largely over within 2 hours after an intravenous dose, there appears to be no need for detection of the drug for more than 12 hours after its administration. Racing chemists should bear this in mind when setting the level of sensitivity of their test systems.[6]

If furosemide is given intramuscularly (IM), its plasma half-life is greatly increased ($T_{\frac{1}{2}}$ = 86 minutes), and the diuretic response also lasts longer. After IM injection of 1 mg/kg of furosemide, about a 50% greater urinary response is observed than after its in-

* All the work reported here refers to injectable Lasix®, 50 mg/ml from Hoechst Laboratories Corp. subsidiary of American Hoechst Corp., Somerville, NJ.

Equine Commun., continued from p. 314.

travenous injection. Onset of the diuretic effect is, however, almost as fast as after IV injection, so IM injection would appear to be the route of choice if a prolonged or greater diuretic effect is required.[6]

Like the diuretic effects of furosemide, the cardiovascular effects of furosemide are also transient. After intravenous injection, furosemide reduces pressure in the pulmonary circulation within minutes. This effect occurs independently of the diuretic effect and may be the basis of the action of furosemide against epistaxis. In the systemic circulation, furosemide produces a decrease in cardiac output with a small increase in heart rate. Furosemide does not alter blood pressure in the systemic circulation. After 1 mg/kg (about 10 ml) intravenously, these effects are usually over within 2 hours.[4]

After intravenous injection, 10 ml furosemide increases total plasma solids about 10%, hematocrit by about 5%, and rapidly reduces plasma K^+ levels. These effects peak between 10 and 30 minutes after administration of the drug and decline back to control levels by 2 hours postdosing.[3,6]

In studies in a number of laboratories, furosemide was found not to reduce the plasma levels of any drugs tested to date.[4] Furosemide does not, therefore, act to "flush" drugs out of the blood or plasma of horses and should not interfere with blood testing for drugs.[7] Similarly, it did not significantly affect the urinary concentrations of a number of drugs tested, such as procaine or methylphenidate, and a closely related drug did not affect amphetamine.[4] These drugs are all basic, relatively lipid soluble drugs, and it appears likely that furosemide has little or no effect on urinary concentrations of this group of drugs.[7]

On the other hand, furosemide treatment caused up to 50-fold reductions in the urinary concentrations of both phenylbutazone and the major metabolite of pentazocine, which is a water soluble pentazocine glucuronide conjugate. In the case of phenylbutazone, this reduction in concentration was sufficient to render the drug difficult to detect in our routine screening tests on racetrack samples at the University of Kentucky. Since both furosemide and phenylbutazone are permitted medications in Kentucky, we now routinely take both blood and urine samples for testing. In our opinion, blood samples are necessary to accurately test for medication with phenylbutazone if the use of furosemide is also permitted.[7]

Some racing jurisdictions, such as Illinois or California, have rules which set upper limits on the concentration of phenylbutazone permitted in horse urine on race day. These rules make little sense if furosemide is also a permitted medication. This is because simple administration of furosemide either IV or IM as close to race time as possible will easily reduce the concentration of phenylbutazone in the postrace urine to below the critical level.[7]

By and large, however, the actions of furosemide on phenylbutazone concentrations in urine are not a serious problem for the racing chemist, in contrast with the likely actions of furosemide on some other groups of drugs. The 50-fold dilution of the major water soluble metabolite of pentazocine also probably occurs with all other drugs detected as water soluble metabolites, such as apomorphine, morphine, and related narcotics and the phenothiazine tranquilizers. Since these drugs are effective at very low plasma concentrations and are difficult or impossible to find in equine plasma, their dilution by furosemide in equine urine is a serious problem for the analyst. The potential for furosemide to interfere with detection of these drugs in urine is probably the most serious problem associated with the approval of furosmide for use in racing horses.[7]

The performance effects of furosemide have been studied in some detail at both the University of Kentucky and the Ohio State University.[8] As pointed out earlier, furosemide has a very rapid action against pulmonary edema and is thought by track veterinarians to greatly assist the breathing of horses with respiratory problems, such as follicular pharyngitis. However, double blind trials on healthy Standardbred horses at both the University of Kentucky and the Ohio State University showed no statistically significant effects of furosemide on the performance of Standardbred horses.[4,8]

Because the numbers of horses involved in these trials were small and the horses were all clinically normal, we elected to perform a further study on the effects of furosemide on racing performance under track conditions. To this end, the Kentucky Harness Commission made available to us the "Lasix list" for the Louisville Downs Summer 1977 Harness Racing Meet. 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 furosemide. From the "Lasix list," 58 horses were selected, and for these horses 160 times prefurosemide and 232 times postfurosemide were obtained. It turned out that the horses were actually 0.14 seconds slower on furosemide than before they went on furosemide, though this difference was not statistically significant. The only conclusion from this experiment seems to be that the horsemen at Louisville Downs or their veterinary advisors were, on the whole, not able to improve the performance of their horses with furosemide.[8]

Furosemide appears to be a very safe drug in the horse. The usual dose administered in the treatment of epistaxis is a relatively small dose, and when administered IV, its action is brief. Given access to water and salt, any drug-induced losses of fluid or ions should be rapidly replenished. In another series of experiments 10-ml (1 mg/kg) doses of furosemide were given daily to horses for 4 consecutive days, and a series of hematological parameters followed. No cumulative changes in any of these parameters over the 4-day test period were observed. However, if these same 4 doses were given at hourly intervals, a 40% drop in serum K^+ was observed. Thus, the only likely acute toxicity with furosemide would appear to be the possibility of inducing hypokalemia with rapidly (hourly) repeated doses.[8]

In conclusion, furosemide is a very effective, safe, and rapidly acting diuretic in the horse. It clearly acts to dilute out and interfere with testing for certain drugs in equine urine. It does not appear to affect the performance of Standardbred horses. Clinical experience suggests that furosemide is effective in the treatment of epistaxis, and it may be useful in horses with respiratory problems, but no hard evidence to support these clinical impressions is currently available.

References

1. Bouland, W.A., Day, D.K., and Williamson, H.E.: The Role of the Kidney in the Early Non-Diuretic Action of Furosemide to Reduce Left Atrial Pressure. *J Pharm Exp Therap*, in press, (1976).
2. Berg, M., and Stonar, L.: Renal Tubular Chloride Transport and the Mode of Action of Some Diuretics. *Ann Rev Physiol*, 38, (1976): 37—45.
3. Cook, W.R.: Epistaxis in the Racehorse. *Equine Vet J*, 6, (1974): 45—58.
4. Gabel, A.A., Tobin, Thomas, Rey, Richard S., and Haylin, G.A.: Furosemide in Horses: A Review. *J Eq Med Surg*, 1, (1977): 215—218.
5. Mair, W.W., Milne, D.W., and Skarda, R.T.: Acute Hemodynamic Effects of Furosemide Administered Intravenously in the Horse. *Amer J Vet Res*, 37, (1976): 1177.
6. Roberts, B.L., Blake, J.W., and Tobin, T.: The Pharmacology of Furosemide in the Horse. II. Its Detection, Pharmacokinetics, and Clearance from Urine. *J Eq Med Surg*, 2, (1978): 185—194.
7. Tobin, T., Roberts, B.L., and Miller, R.W.: The Pharmacology of Furosemide in the Horse. I. Effects on the Disposition of Procaine, Phenylbutazone, Methylphenidate, and Pentazocine. *J Eq Med Surg*, 1, (1977): 402—409.
8. Tobin, T., Roberts, B.L., Swerczek, T.W., and Crisman, M.: The Pharmacology of Furosemide in the Horse. III. Dose and Time Response Relationships, Effects of Repeated Dosing and Performance Effects. *J Eq Med Surg*, 2, (1978): 216—226.
9. Vail, C.D., Bessman, G. GM., and Johnson, H.W.: Furosemide in Equine Practice. *Vet Med*, 62, (1967): 881—884.

Piperazine Toxicity in Horses

—P. H. McNeil, B.V.Sc. and G. B. Smyth, B.V.Sc., M.A.C.V.Sc., Dept. of Veterinary Clinical Sciences, University of Melbourne, Veterinary Clinical Centre, Werribee, 3030, Victoria, Australia

Clinical signs of horses involved in an epidemic of iatrogenic piperazine citrate poisoning are described. The disease was reproduced by similarly overdosing an experimental horse. No treatment was necessary for a complete recovery.

Introduction

Toxic reactions subsequent to oral administration of piperazine compounds for the treatment of helminth infestations of man and domestic animals are uncommon. Vomiting, urticaria, blurred vision, weakness, nystagmus, and convulsions may occur with normal or excessive dosing in humans.[1,3,10,12] Occasional emesis, diarrhea, and transient neurotoxic symptoms are seen with excessive dosing in small animals.[4,5,16,18,20] Mild gastrointestinal signs are reported after administering high doses to calves.[15] Therapeutic activity is attributed to the neuromuscular blocking activity of these compounds in the parasite, which allows normal intestinal peristalsis of the host to expel the intact parasite from the gut. The effects of the drug on neuromuscular activity within the host are minimal.[10,18]

Reports of toxicity in horses are rare. Toxic signs were not produced in foals treated with 6 times the normal dosage levels of piperazine adipate.[17] However, in a more recent study, involving trials with both levamisole and piperazine, neurotoxic signs were produced in a horse dosed with piperazine monohydrochloride at 6 times the normal dosage levels.[7] This paper reports the clinical signs associated with an outbreak of iatrogenic piperazine citrate poisoning on a Standardbred stud and the experimental reproduction of the disease.

Case History

A group of 10 weanling Standardbred colts were running in a 50-acre paddock grassed with improved pasture. All had suffered a mild upper respiratory tract infection during the 3 weeks prior to anthelmintic treatment. There was no other history of illness in any of the horses. During a 2-day period, every horse on the property had been treated via nasogastric intubation with piperazine citrate and thiabendazole pow-

Furosemide in Horses: A Review

Albert A. Gabel, D.V.M., M.S.
Thomas Tobin, D.V.M., Ph.D.
Richard S. Ray, D.V.M., M.S., Ph.D.
George A. Maylin, D.V.M., M.S., Ph.D.

From the Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by: Gary P. Carlson, University of California; Harold E. Garner, University of Missouri; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; William W. Muir, Ohio State University, and David Snow, University of Glasgow, and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Fraley, A.V. Villatico, Robert E. Vestry, Dr. Francis Ozog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Bierhaus, and General Wayne Kester.

1. Injectable furosemide[1] is a potent, effective, safe diuretic which at the usual dose has its peak diuretic effect in about 20 minutes in horses, at which time it increases urine output up to 40-fold for a short time. Most of its diuretic effect is within the first 2 hours after injection.

2. During the first 2 hours, it decreases the pressure in the right side of the heart, decreases left atrial pressure, decreases cardiac output, and concurrently it increases peripheral (systemic) blood vessel resistance and heart rate.

3. It reduces edema in the lungs and airway of horses with certain respiratory diseases by 1 or more mechanisms, perhaps by lowering the pressure of the left atrium and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.

4. The mechanism by which furosemide helps prevent epistaxis may result from the beneficial effect outlined in (3) above since most horses bleed from the lungs. Lasix[1] also appears to affect the platelets of horses during exercise.

5. The blood (plasma) level of furosemide declines rapidly. It can be detected in the urine by screening methods for the first 24 hours and by more sophisticated methods for up to 48 hours after injection.

6. Furosemide does not affect the blood levels of other drugs.

7. Furosemide has little effect on the concentration of certain drugs, such as procaine, in the urine. However, during its peak diuretic effect, within 2 hours after injection, furosemide causes up to 40-fold dilution of certain other drugs such as phenylbutazone. However, furosemide administered more than 4 hours before race time does not significantly reduce the ability to detect those drugs studied to date.

[1] Lasix® National Laboratories, Inc., Somerville, NJ 08876

8. There is no evidence that furosemide directly or indirectly affects the behavior of the horse.

9. Furosemide cannot make horses race faster or perform better than their innate ability, but in many cases it restores normal performance of horses which bleed.

Introduction

There is no scientific evidence that furosemide will increase the speed or improve performance of a horse beyond its innate capability. In a recently completed double-blind study there was no significant difference in the times of time-trial miles of clinically normal Standardbreds after intravenous furosemide injection compared to saline injection, 2 hours and 10 minutes before the trials.[18] A mechanical prompter was used for uniform psychological stimulation. However, clinical observations indicate that the drug may help a horse to more nearly reach his racing capacity if he is a bleeder, or has certain other conditions, including perhaps early obstructive lung disease "heaves" or diseases in which pulmonary edema occurs, even transiently, during exercise.[12,14]

Mechanism of Action (Diuretic Effect)

The primary effect and use of injectable furosemide has been as a potent diuretic. It has a high degree of efficacy, low-inherent toxicity, and a high therapeutic index in horses.[11] Furosemide produces the increase in urine by inhibiting active electrolyte (inorganic ions such as chloride) transport in that part of the kidney known as the diluting segment of the ascending limb of the loop of Henle.[4,6,21,29] Furosemide produces increased losses of chloride, sodium, other electrolytes, and water.[11] There is little potassium loss in horses.[11] In normal horses, no dehydration or electrolyte imbalance occurs if they have access to water and salt before and after the injection.[22] Its onset of action is rapid.[13,22,29,31,39] The diuretic effect of furosemide peaks within 15 to 30 minutes, when there is up to a 40-fold increase in urine production for a short time.[24,39] Dosed at 1 mg/kg body weight, most of its diuretic effect occurs within 2 hours.[24,40] Doses between 0.01 and 1.0 mg/kg (the dose recommended by the manufacturer) produce a graded increase in urine volume and decrease in its specific gravity.[11,39] The average urine production during a 2-hour period after injection of the usual dose of furosemide is 3 to 12 liters and is dependent on the horse's state of hydration when the drug is given.[29,31,39] Only 1 liter of urine is produced during an average 2-hour control period.[39] Due to the loss of fluid during the period of increased urine production, horses which are dehydrated before the injection make less urine during several hours which follow; therefore, it is sometimes difficult to get a urine sample during this period.[31] Race Commission veterinarians do not find this to be a significant problem.[31]

Furosemide is used by many veterinarians in the treatment of acute tieing-up and azoturia to increase urinary output, since high concentrations of the pigment from damaged muscles can cause kidney shutdown.

Cardiovascular Effects

In resting horses, in addition to increased urine production, the following changes were found to be statistically significant ($P<0.05$)[39] from 15 minutes to 110 minutes after intravenous injection of the usual dose (1 mg/kg) of furosemide:

1. Decreased blood pressure in the right side of the heart, pulmonary artery, and left atrium. This may be due to hypovolemia and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.
2. Decreased venous return and therefore decreased cardiac output.
3. No significant change in systemic arterial blood pressure, although there was a significant increase in peripheral (systemic) vascular resistance and reflex increase in heart rate.
4. Hemoconcentration. Packed cell volume and total protein percentage in the blood increased.

In a recent double-blind study intravenous furosemide injection was compared to saline injection under simulated race conditions using Standardbreds.[18] Hemodynamic and biochemical data were recorded before and during the first "warm-up miles" 15 minutes after injection and before, during, and after the third (time-trial) mile 2 hours and 10 minutes after injection. After furosemide, compared to saline, pulmonary artery pressure was lower during the first warm-up mile, 15 minutes after injection, but not during the time-trial mile 2 hours and 10 minutes after injection. There was no difference in cardiac output immediately after and 10 minutes after each of the miles, nor in the pulse rate during or after each of the miles.

These experiments suggest that, if given during the 2-hour period prior to a race, furosemide might have a negative effect on performance because of a decreased cardiac output.

Respiratory Effects

A clinically important action of furosemide in both man and small domestic animals is its rapid action against pulmonary edema. Since this effect also occurs in patients in which this drug does not produce diuresis, and in patients which are kept hydrated, the volume change is not totally due to diuresis.[7,33]

Recent observations suggest that the pulmonary effects of furosemide may be mediated by prostaglandins released from the kidneys.[7,33] Another theory is that there may be a tendency for lung edema to develop when mean pulmonary artery pressure exceeds 40 mm Hg by very much.[7,32,33] Aver-

age mean pulmonary artery pressures of approximately 70 mm Hg have been measured in Standardbreds during time trials.[17] Edema (which may occur transiently) has a negative effect on breathing reflexly through the J-fibers of the vagus nerve.[27] The more severe the exercise, the greater the tendency for pulmonary edema to occur. This may explain why furosemide is used more in Thoroughbreds, which work harder, than in Standardbreds.[31]

The experience of race track veterinarians indicates that the drug appears to have an effect of producing a more nearly optimal performance in Thoroughbreds which have heavy or labored breathing while training or racing, in those which have nasal exudate after racing, and in some which become excited in the paddock.[24]

It is thought that horses with edema and narrowing of the upper airway, especially the pharynx by such conditions as follicular pharyngitis, may race more normally following the use of furosemide, because tissue fluid is removed, enlarging the airway toward normal size.[14,27]

Epistaxis

It is thought that in most cases the blood comes from rupture of small vessels in the lungs, and sometimes the upper airway, when horses have epistaxis following work.[6,14] The experience of many veterinarians shows that furosemide injection approximately 4 hours before exercise is effective in preventing epistaxis in a high percentage of Thoroughbred and Standardbred horses which bleed during or after racing or hard workouts.[22]

In a double-blind study under simulated racing conditions, Standardbred horses were injected with 1 mg/kg of furosemide or saline 15 minutes before the first warm-up mile.[15] Hemostatic function including the blood clotting mechanism and platelets were evaluated prior to injection, immediately following the first warm-up mile, and after the time-trial mile 2 hours and 10 minutes after the injection. There was a slight increase in the retention of platelets in glass bead columns, but this platelet function change could not be substantiated in a follow-up study using furosemide injections in nonexercised horses. No significant changes were found in screening tests of the blood clotting mechanism.

Furosemide may be effective in horses which bleed by reducing the blood pressure of the left atrium and increasing capacitance of the lung vessels.[9]

Blood (Plasma) and Urine Levels—Detection

Furosemide is usually administered to horses by intravenous (IV) or intramuscular (IM) injection at doses of 1 mg/kg or somewhat less. After IV administration of 1 mg/kg, its plasma levels decline rapidly, from about 10 μg/ml (parts per million) initially to 500 ng/ml (parts per billion) at 20 minutes. Thereafter, plasma levels fall more slowly with a half-life of about 30 minutes, to less than 20 ng/ml at 4 hours. Urinary levels of the drug start at about 30 μg/ml and fall rapidly over the first 12 hours to about 500 ng/ml. This is about the detection limit of the thin layer techniques used in routine drug testing screens. However, using gas chromatographic (G.C.) methods, urinary levels of the drug can still be detected up to 48 hours postdosing.

Effects of Furosemide on the Concentration of Other Drugs in Equine Blood and Urine

In no instance is furosemide known to significantly reduce the blood or plasma level of any drug.

Treatment with furosemide does not appear to significantly affect the concentration of basic lipid soluble drugs in equine urine. Thus, furosemide has no effect on either plasma or urinary concentrations of procaine in horses treated with 10 mg/kg of procaine HCl intramuscularly. Similar results were observed with methylphenidate[a]. In both cases, urinary output of the drug was increased by the greatly increased urine volume, and the concentration did not decrease enough to cause trouble in detection of the drugs. The same effect probably occurs with amphetamine. Glucuronide and sulfate conjugates of narcotics can be diluted up to several fold.[12]

In contrast, however, urinary concentrations of phenylbutazone are reduced up to 40-fold at peak diuresis. This concentration change can greatly reduce the reliability of routine UV screening for phenylbutazone.[25] Concentrations of phenobarbital also are probably affected, though not quite so severely.[8]

In doses usually used, furosemide can greatly reduce the urinary concentrations of some drugs, but only during the first 2 hours and for a much lesser extent up to 4 hours after it is administered; in other drugs it has little effect. In particular, use of furosemide can interfere with routine screening for phenylbutazone during this period of increased urine production (2 to 4 hours). For this reason, blood testing for phenylbutazone should be recommended where medication rules permit the use of furosemide within 4 hours of racing.

---

[a] Ritalin®, Ciba Pharmaceutical Co., 556 Morris Ave., Summit, NJ 07901.

References

1. Berman, L.B., and Ebrahimi, A.: Experiences with Furosemide in Renal Disease. *Proc Soc Exp Biol Med*, 118, (Feb. 1965): 333-336.
2. Biagi, R.W., and Badat, B.N.: Furosemide in Acute Pulmonary Edema. *Lancet*, 1, (1967): 849
3. Bierhaus, G.M., Race Commission Veterinarian, Colorado: Personal communication, 1977.

4. Burg, M., Stoner, L., Cardinal, J., and Green, N.: Furosemide Effect on Isolated Perfused Tubules. *Amer J Physiol*, 225, (1973): 119-124.
5. Bourland, W.A., Day, D.K., and Williamson, H.E.: The Role of the Kidney in the Early Non-diuretic Action of Furosemide to Reduce Elevated Left Atrial Pressure. *J Pharm and Exp Therapy*, (1976), in press.
6. Cook, W.R.: Epistaxis in the Race Horse. *Equine Vet J*, 6, (April, 1974): 45-58.
7. Cross and Zelis: *Peripheral Circulation: Patho-physiology of Pulmonary Edema*. Grune and Stratton, New York, NY (1975): 315-362.
8. Deetjen, P.: Mikropunktionsuntersuchungen zur Wirkung von Furosemid. *Pflugers Archiv fuer die Gesamte Physiologie*, 284, (1965): 184-190.
9. Edmondson, A.H., Racing Commission Veterinarian, California: Personal communication, 1977.
10. Frey, H.H., Fitzek, H., Wintzer, H.J., and Baumgartner, E.: Use of Bumetanide, A Potent Diuretic, to Obtain Urine Samples for Dope Testing in Horses. *Amer J Vet Res*, 37, (1976): 1257-1260.
11. Garner, H.E., Hutcheson, D.P., Coffman, J.R., Johnson, J.H., and Salem, C.: Urine Electrolyte and Diuretic Responses of Horses to Seven Dosage Levels of Lasix. *Proc AAEP*, (1975): 87-90.
12. Gillespie, J.R.: University of California Panel on Pharmacology and Physiology of Drugs Used in Racing, AAEP Meeting, 1976.
13. Hirsch, G.H., Pakuts, A.P., and Bayne, A.J.: Furosemide Accumulation by Renal Tissue. *Biochem Pharmacol*, 24, (1975): 1943-1946.
14. Johnson, J., University of Missouri, Columbia, Missouri: Personal communication, 1977.
15. Kociba, G., et al.: Hemostatic Chemistry Including Platelet Function of Standardbred Horses: Effect of Furosemide Under Simulated Racing Conditions—A Double Blind Study. *Amer J Vet Res*, (1977), in preparation.
16. Lindstrom, R., and Molander, M.: Gas Chromatographic Determination of Furosemide in Plasma Using an Extractive Alkylation Technique and an Electron Capture Detector. *J Chromatog*, 101, (1974): 219-221.
17. Maylin, George: Unpublished data, 1977.
18. Milne, D.W., Gabel, A.A., et al.: Effects of Furosemide on Performance and Cardiovascular Function of Standardbred Horses Under Simulated Race Conditions—a Double Blind Study. *Amer J Vet Res*, (1977), in preparation.
19. Muir, W.W., The Ohio State University, Columbus, Ohio: Personal communication, 1977.
20. Muir, W.W., Milne, D.W., and Skarda, R.T.: Acute Hemodynamic Effects of Furosemide Administered Intravenously in the Horse. *Amer J Vet Res*, 37, (1976): 1177-1180.
21. Muschaweck, R., and Hajdu, P.: Die salidiuretische Wirksamkeit der Chlor-N-(2-furylmethyl)-5-sulfamyl-anthranilsaeure. *Arzneimittel-Forschung*, 14, (1964): 44-47.
22. Muser, R.K., Animal Health Research and Development Department, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.: Personal communication, 1977.
23. Panel on Pharmacology and Physiology of Drugs used in Racing. Poll of Veterinary Practitioners, AAEP, 1976.
24. Ray, R., The Ohio State University, Columbus, Ohio: Unpublished data, 1977.
25. Roberts, B.L., Blake, J.W., and Tobin, T.: Drug Interactions in the Horse: Effect of Furosemide on Plasma and Urinary Levels of Phenylbutazone. *Res Commun Chem Pathol & Pharmacol*, 15, (1976): 257-265.
26. Schmidt, H.A.E.: "Animal Experiments with S35 Tagged Lasix in Canine and Ovine." Radio-chemical Pharmacological Laboratory, Farbwerke Hoechst, Frankfurt, West Germany.
27. Staub, C.N.: Pulmonary Edema. *Phys Rev*, 54, (1974): 678-811.
28. Suki, W., Rector, Jr., F.C., and Seldin, D.W.: The Site of Action of Furosemide and other Sulfonamide Diuretics in the Dog. *J Clin Invest*, 44(9), (1965): 1458-1469.
29. Timmerman, R.J., Springman, F.R., and Thoms, R.K.: Evaluation of Furosemide, a New Diuretic Agent. *Current Therapeutic Research*, 6(2), (Feb. 1964): 88-94.
30. Tobin, T., University of Kentucky, Lexington, Kentucky: Unpublished data, 1977.
31. Williamson, H.E., Bourland, W.A., Marchand, G.R., Farley, D.B., and VanOrden, D.E.: Furosemide Induced Release of Prostaglandin E to Increase Renal Blood Flow. *Proc Soc Exp Biol Med*, 150, (1975): 104-106.

The Pharmacology of Furosemide in the Horse. III. Dose and Time Response Relationships, Effects of Repeated Dosing, and Performance Effects

Thomas Tobin, D.V.M., Ph.D.
Brian L. Roberts, B.Sc.
T.W. Swerczek, D.V.M., Ph.D.
Mark Crisman

From the Kentucky Equine Drug Research Program, Department of Veterinary Science and the Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40506.

Publication #24 from the Kentucky Equine Drug Research Program, Department of Veterinary Science, College of Agriculture, University of Kentucky, Lexington, KY 40506. Published as Kentucky Agricultural Experiment Article #77-4-182 with the permission of the Dean and Director, College of Agriculture. The advice and assistance of Dr. J.B. Hook, Michigan State University, and Dr. George Maylin, Cornell University, is gratefully acknowledged. Particular thanks are due to Mr. Biff Lowry, General Manager, The Red Mile Racecourse, Lexington, KY, for allowing use of the Red Mile Track for the performance studies. The guidance and cooperation of Mr. Carl Larsen of the Kentucky Harness Racing Commission who made available to us Medication Data from the Louisville Downs Summer Meet is gratefully acknowledged. Thanks are also due to Mrs. Virginia White, Carl Czaky, Joy Dunn, and Marguerite Mayes, who assisted with the various analyses.

This work was supported by grants from the Kentucky Equine Research Fund.

Furosemide was administered intravenously (IV) and intramuscularly (IM) to Thoroughbred horses at various doses. After rapid IV injection, furosemide produced up to a 40-fold increase in the rate of urine formation with a concomitant decrease in its specific gravity. This effect peaked between 15 and 30 minutes after dosing and then declined rapidly.

At doses of up to 1 mg/kg IV, the bulk of the diuretic effect occurred within 1 hour. A second dose at 1 or 2 hours again produced substantial diuresis (70% of the initial response). Larger doses or intramuscular (IM) administration prolonged and enhanced the diuretic effect. The diuretic effect was closely related to plasma levels of the drug, and the transient response after IV administration was apparently due to the short plasma half-life of the drug. Increasing doses of furosemide produced a graded increase in urine volume and a fivefold increase in its Na^+ concentration at all doses above 0.01 mg/kg. In contrast, increasing doses produced a graded decrease in urinary K^+ concentrations. The net effect on K^+ excretion was that all doses tested produced a small and transient increase in urinary K^+ output.

At 1 mg/kg IV, furosemide increased packed cell volume (PCV) about 5%, total plasma solids by about 10%, and reduced plasma K^+ concentrations. These effects peaked between 10 and 30 minutes postdosing, then returned rapidly to control values.

Repeated doses of furosemide resulted in loss of the diuretic effect. Dosing every hour at 1 mg/kg, a third dose produced 37% of the diuresis of the first dose, while a fourth dose was about one-quarter as effective as the first dose.

This loss of diuretic effect was associated with no further changes in the blood picture. These repeated dosing experiments suggested that about 25 liters of fluid can be withdrawn from a 435 kg (1,000 lb) horse by furosemide. Marked reductions in plasma K^+ concentrations were seen after repeated doses of furosemide, but no clinically significant changes in blood enzymes were observed. Similarly, daily dosing with 1 mg/kg furosemide IV for 4 days produced no clinically significant cumulative changes in any of the blood values tested.

Time trials over a 1-mile course showed no statistically significant changes in the performance of Standardbred horses treated with 0.55 mg/kg furosemide IV 30 minutes prior to the test. Similarly, a study of the track times of 58 Standardbred horses running with and without furosemide at Louisville Downs showed no significant difference between times on and off furosemide.

Introduction

Furosemide (Lasix®[a]) is a member of the "high ceiling" group of diuretics and is the member of this group most widely used in equine medicine.[1] It is used in the treatment of various forms of edema, in azoturia, to reduce space-filling lesions, and more recently, in the prophylaxis of epistaxis in racing horses.[2] As well as its actions against epistaxis, it is suspected in racing circles of "diluting out" prohibited drugs in the urine of racing horses and also improving their racing performance.[17] In recent years its use for the prophylaxis of epistaxis in horses during races has been approved by some racing authorities; as a result, its use in horses racing in these jurisdictions has increased up to 80%, a frequency of use far beyond that which might be expected on the basis of the relatively small (<5%) incidence of epistaxis in Thoroughbred horses.[18]

Because of this widespread use of furosemide in racing horses, many questions concerning its actions and use in the horse have arisen. Surprisingly, even the diuretic effect of this drug in the horse is not well characterized,[3] in particular, with respect to dose and time response relationships. Beyond recent work from this laboratory,[14-16] no data whatsoever were available on its pharmacokinetics and clearance times from urine in the horse. Preliminary reports on the effect of furosemide on the disposition of other drugs in the equine have been published from this laboratory.[15,17,19] No studies on the efficacy of furosemide in the prophylaxis of nosebleed nor on the effect of furosemide on racing performance in the horse are available. Further, concern exists among horsemen regarding the effects of repeated doses of furosemide in the horse because of the possibility of producing hypokalemia, dehydration, or other forms of toxicity.[3]

In this study, we report on dose and time response relationships for the natruretic, kaluretic, and diuretic responses of horses to furosemide after its IV and IM injection. We further report on the performance effects and the effects of repeated doses of furosemide in the horse. Companion papers on the detection and pharmacokinetics of furosemide and its effects on the disposition of other drugs in the horse have been published,[15-17] and preliminary reports of this work have been presented.[20]

Materials and Methods

Mature Thoroughbred and half-Thoroughbred mares (375–550 kg), kept on pasture, were housed in individual box stalls with hay and water *ad libitum* unless otherwise noted on the days of an experiment. The horses were administered agents either intravenously into the left jugular vein or by intramuscular injection into the deep muscles of the neck. Blood samples were collected in 15-ml heparinized Vacutainer®[b] tubes from the opposite jugular vein. Urine samples were collected by bladder catheterization. Urine samples for analysis were collected "midstream" after catheterization. Bladders were drained completely at each time period, and volumes were recorded to calculate the quantity of drug or electrolytes excreted over a time period. Because the diuretic effect of furosemide administered IV was both very marked and very short lived (Fig 1), complete draining of the bladder at up to 15-minute intervals was important to accurately monitor the time course of the diuretic effect. Mean urinary flow rates were calculated by dividing urine volume for each period by the collection time in minutes. All samples were held on ice until they could be taken to the laboratory for analysis or for further storage at −40°C until the samples were analyzed.

Sodium and potassium concentrations in plasma and urine were analyzed by flame photometry.[c] Urine samples were centrifuged at 10,000 X g for 10 minutes to prevent clogging of the aspirator line. Sodium and potassium concentrations were recorded as milliequivalents/liter (meq/liter). Urine specific gravity was determined by means of a urinometer float. Total plasma solids were

[a] Lasix®, 50 mg/ml Hoechst-Roussel Pharmaceuticals, Somerville, NJ

[b] Becton-Dickinson Co., Rutherford, NJ

[c] Model 143 Instrumentation Laboratories, Inc., Lexington, MA

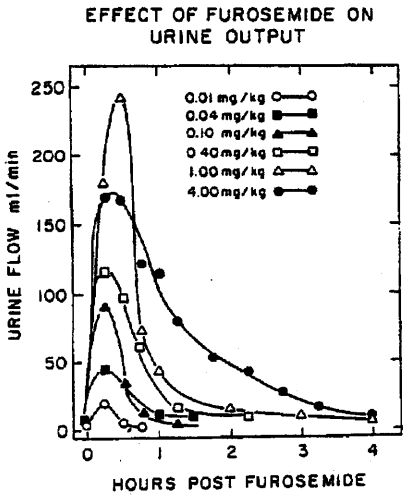


Fig 1—Time course of urinary flow rate response to intravenous furosemide. The symbols show flow rate in ml/minute after the indicated doses of furosemide intravenously. Open circles (○-○), 0.01 mg/kg; solid squares (■-■), 0.04 mg/kg; solid triangles (▲-▲), 0.1 mg/kg; open squares (□-□), 0.4 mg/kg; open triangles (△-△), 1.0 mg/kg; solid circles (●-●), 4.0 mg/kg. All experiment points except those for 0.01 mg/kg (2 horses) represent means of experiments on 4 different horses.

determined by refractometry.[4] Hematocrits were determined by centrifugation,[5] red blood cell counts on a Coulter counter,[6] and hemoglobin on a hemophotometer.[7] Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and creatine phosphokinase (CP) were determined by the Spin Chem method.[8] Other methods, drugs, and chemicals were as previously outlined.[10-17] Unless otherwise noted, all values are the means ± standard errors of the means of determinations on at least 4 different horses.

The effects of furosemide on performance were determined in Standardbred horses. Standardbred horses in training were purchased locally (commercial value $500–1,000) and entered into the training program. The horses were between 2 and 15 years of age. Training followed the traditional Kentucky pattern for Standardbred horses and consisted of daily jogging for 5 miles. Sundays and weather permitting, with 1 day of "full speed" training each week. When the horses attained what appeared to be their optimal performance, they were entered into the drug trial program. All drug trials were performed double-blind, with an equivalent number of control (saline injection) treatments randomly inserted into the drug sequence. Inasmuch as the diuretic effects of the dose of furosemide were occasionally observable, the driver/trainer was aware of the nature of the drug during the trials. Dosage was 0.55 mg/kg of furosemide (about 5 ml of 50 mg/kg furosemide to a 1,000 lb, 453 kg horse) 30 minutes before the timed run. Times were recorded by the driver by stopwatch. Data were analyzed for statistical significance by the t test and $P < 0.05$ was selected as the criterion for statistical significance.

For analysis of the effects of furosemide on racing performance at Louisville Downs, a list of the horses which elected to go on furosemide during this meet was made available to us. Furosemide was the only permitted medication allowed during this meet, and urine samples taken from these horses were checked for furosemide to monitor compliance with their declared furosemide status. Performance times for these horses obtained prior to going on furosemide were obtained from the track program and compared with those on furosemide. For statistical analysis a randomized block design was used with each horse representing a single block. Statistical significance was determined by an F test.

Results

Furosemide was administered IV to groups of 4 horses at doses of between 0.01 and 4.0 mg/kg. Figure 1 demonstrates changes in urinary flow rates produced by these doses. A base line urine flow of 6.6 ml/minute in the absence of the drug was determined by averaging flow rates for the 30 minutes just prior to injection of drug for all horses tested in this experiment.

The lowest dose tested, 0.01 mg/kg, briefly increased urine flow about threefold over base line, which then returned to the control rate by 30 minutes postdosing. At higher dose rates, urine flow peaked between 15 and 30 minutes after IV injection. Urinary output was largely complete within the first hour and had returned to close to control values by 2 hours postadministration for all but the largest dose, 4.0 mg/kg.

Figure 2 shows the effect of these doses on urinary specific gravity. Mean specific gravity in control urines was 1.031 ± 0.004, with a range of from 1.025 to 1.050. Urinary specific gravity was rapidly reduced after furosemide treatment, with peak reduction occurring between 20 and 40 minutes. At higher doses of furosemide, urinary specific gravity dropped to less than 1.005 and then slowly returned toward control values.

Figure 3 shows dose response curves for the indicated doses of furosemide on peak urinary output and the sodium and potassium content of urine at the times of peak flow. The data demonstrate that while urine volume responded with a graded increase to increasing dose, urinary potassium concentration declined in a similar graded manner. In contrast to these graded responses, urinary sodium content was minimally affected by the 0.01 mg/kg dose but was maximally affected by the 0.04 mg/kg dose. Thus, doses of furosemide above 0.04 mg/kg all produced the maximal increase in the concentration of Na^+ in equine urine in contrast with their graded and opposite effects on volume and K^+ concentration.

Figures 4 and 5 show the results of these effects on urinary excretion of Na^+ and K^+. While urinary Na^+ excretion increased with dose in a graded fashion, urinary excretion of K^+ was maximally increased by the smallest dose tested (0.01 mg/kg) and was not further increased by increasing the dose.

[4] Optical Refractometer, Bausch & Lomb, Rochester, NY.

[5] "Autocrit" Centrifuge, Clay-Adams, Inc., NY.

[6] ZBI Coulter Counter, Coulter Electronics, Inc., Columbus, OH.

[7] Hemophotometer, Fisher, Inc., Cincinnati, OH.

[8] Spin Chem, Smith-Kline Instruments, Ltd., Palo Alto, CA.

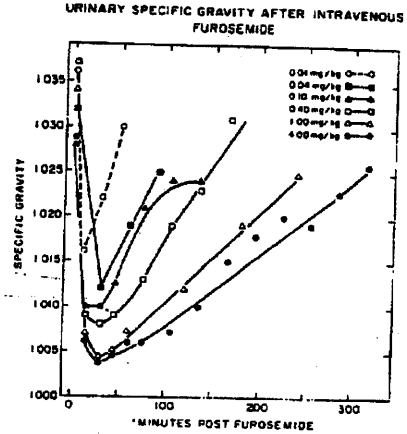


Fig 2—Effect of intravenous furosemide on urinary specific gravity. Furosemide at the indicated dose levels was administered to Thoroughbred horses by rapid IV injection. The symbols show urinary specific gravity after 0.01 mg/kg (open circles, ○-○); 0.04 mg/kg (solid squares, ■-■); 0.1 mg/kg (solid triangles, ▲-▲); 0.4 mg/kg (open squares, □-□); 1.0 mg/kg (open triangles, △-△); 4.0 mg/kg (solid circles, ●-●). All points except those at 0.01 mg/kg (2 horses) are the results of experiments on 4 different horses.

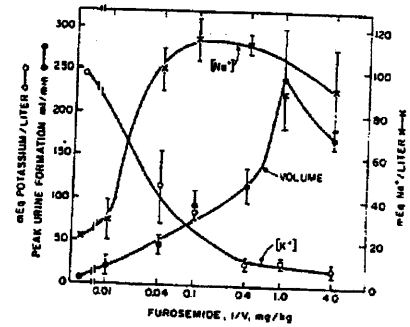


Fig 3—Dose response relationships of urinary output and cation concentrations at peak diuresis due to intravenous furosemide. Furosemide at the indicated concentrations was given by rapid intravenous injection to horses. The solid circles (●-●) show peak urinary output at each of the indicated doses. The crosses (X-X) and open circles (○-○), show urinary concentrations of Na^+ and K^+, respectively, at the indicated dosage levels. All experiments except those at 0.01 mg/kg (2 horses) are the means of experiments on 4 different horses ± standard deviation.

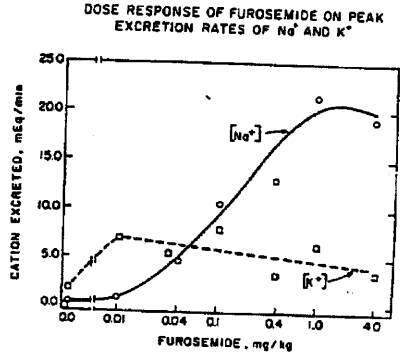


Fig 4—Dose response relationship for intravenous furosemide on cation excretion rates. All data points represent cation excretion rates at peak drug effect. The open circles (○-○) show peak urinary output of Na^+ after the indicated concentrations of furosemide, while the open squares (□-□) show peak urinary output of K^+ after the indicated doses of furosemide. All experiments except those at 0.01 mg/kg (2 horses) are the means of experiments on 4 different horses ± standard deviation.

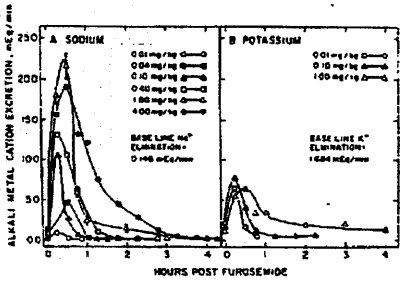


Fig 5—Effect of intravenous furosemide on rates of urinary loss of Na^+ and K^+. The left-hand panel shows the effect of the indicated doses of furosemide on Na^+ output/minute; the right-hand panel the effect of the indicated doses on K^+ loss/minute. All points are means ± standard deviation of determination on 4 different horses.

Figure 6 shows the effect of 1 mg/kg furosemide IV on a number of blood values. Packed cell volume increased by about 6%, which effect was accompanied by parallel increases in hemoglobin and red blood cell count (data not presented). Total plasma solids increased by about 10%, while serum K^+ concentrations showed up to a 20% drop. No consistent changes in plasma Na^+ concentrations were observed. All the changes observed were rapidly reversed, and all values had returned to control levels by 120 minutes postdosing.

Figure 7 shows the diuretic response obtained after IM injection of 1 mg/kg of furosemide. Peak flow rate achieved after injection by this route was less than that observed after IV injection, but the diuretic effect was more prolonged. Similarly, the effects on cation concentration in urine were of longer duration than after IV injection and paralleled the prolonged diuretic effect. As shown in Table 1, about 50% more water and 25% more Na^+ were eliminated after 1 mg/kg furosemide was given by the IM rather than by the IV route.

Figure 8 shows the relationship between plasma levels of furosemide and the diuretic response. The diuretic responses to 1 mg/kg furosemide IV or IM were replotted from Figs 1 and 6, while plasma levels of the drug were replotted from Figs 6 and 8 of Roberts *et al.*[14] Baseline urine flow rates were subtracted from all values presented in Figs 1 and 6, so that only the diuretic response to the drug was represented. The data show that in both cases the diuretic response to furosemide declined a little more rapidly than plasma levels of the drug.

Traditionally, the diuretic effect of furosemide has been thought to be limited by the amount of extracellular

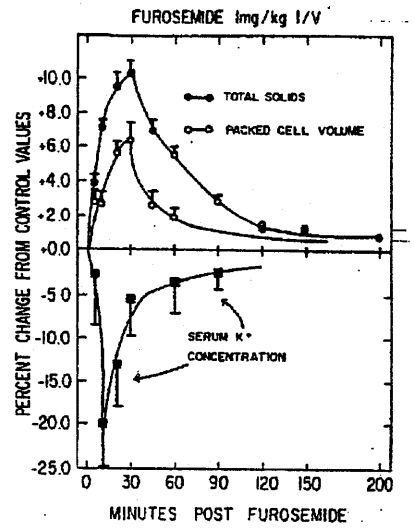


Fig 6—Effect of 1 mg/kg furosemide intravenously on blood values. Horses were administered 1 mg/kg furosemide intravenously and blood samples drawn at the indicated times. The solid circles (●-●) show the percent increase in total plasma solids, while the open circles (○-○) show the percent increase in packed cell volume following furosemide. The solid squares (■-■) show the percent decrease in serum K^+. Control serum K^+ levels were 3.78 ± 0.12 Eq/liter. All data are expressed as percent change from predrug controls and are the means ± standard error of the means of experiments on 6 horses.

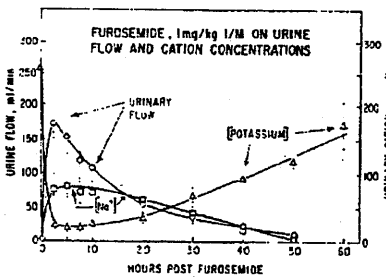


Fig 7—Effect of 1 mg/kg furosemide intramuscularly on urine volume and cation concentration. The open circles (○-○) show urinary output after the indicated dose of furosemide, while the open squares (□-□) and triangles (△-△) show urinary concentrations of Na^+ and K^+, respectively. All experimental points are means ± standard deviation of experiments on 4 different horses.

TABLE 1

Cumulative Water and Na^+ Loss After 1 mg/kg Furosemide Intravenously and Intramuscularly

Time after administration	1.0 mg/kg IV		1.0 mg/kg IM	
	Urine Vol. (liter)	Na^+ Excretion (mEq/liter)	Urine Vol. (liter)	Na^+ Excretion (mEq/liter)
1 hour	8.2*	732	8.4*	621
2 hours	9.0	744	12.6	887
4 hours	10.5	748	16.2	994

*n = 4 horses

fluid available to be excreted. However, because of the close correlation between the plasma half-life of furosemide and its pharmacological action (Fig 8), we decided to test this hypothesis by administering repeated IV doses of furosemide to horses. Figure 9 (Experiment 1) shows that a second dose of furosemide within 120 minutes elicited a prompt diuretic response of about 73% of the magnitude of the first response. Similarly, Fig 9 (Experiment 2) shows that about the same response was obtained if a second dose was administered within 60 minutes of the first dose. These experiments show that at the 1 mg/kg dose level considerable quantities of fluid remain available to furosemide at 1 hour after dosing. It thus appears reasonable to assume that the diuretic response to a single IV dose of furosemide at 1 mg/kg is limited primarily by rapid elimination of the drug.[11]

If, however, the 1 mg/kg IV dose of furosemide was repeated, the diuretic response decreased rapidly. Thus, a third dose produced a diuretic response of only 37% of the original response, and a fourth dose produced a response of about 25% of that observed initially. Since these horses were deprived of water during these experiments, it appears likely that the 21.3 liters of fluid withdrawn over the

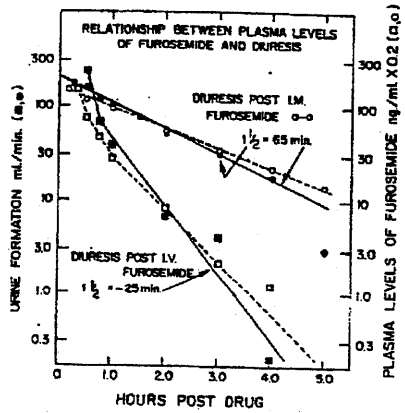


Fig 8—Relationship between plasma levels of furosemide and diuresis. The solid symbols and lines show rates of urine formation in ml/minute after IV injection (solid squares, ■-■, replotted from Fig 1) and after IM injection (solid circles, ●-●, replotted from Fig 6) of 1 mg/kg furosemide. Control rates of urine formation were subtracted from all values so the points represent diuresis due to furosemide only. The open squares (□-□) and circles (○-○) show plasma levels of drug after similar doses of furosemide, replotted from Roberts *et al.*[14] Plasma levels of furosemide were superimposed on urinary flow rates by multiplying all plasma levels by 0.2. The approximate half-lives for the diuretic effect after each route of administration compare with kinetically determined plasma half-lives for furosemide of about 30 and 86 minutes, respectively (Roberts *et al.*[14]).

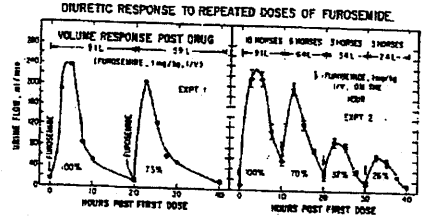


Fig 9—Diuretic response to repeated doses of furosemide. The left panel (Experiment 1) shows the diuretic response to 1 mg/kg of furosemide IV at indicated zero time and 2 hours, respectively. All data points are the means of experimental determinations on 4 different horses. The right-hand panel (Experiment 2) shows the effect of 4 doses of 1 mg/kg furosemide IV. The liter figures refer to the total volumes of urine voided between doses, while the percentage figures express the diuretic response as a percentage of the response to the first dose. No access to water was allowed during these experiments.

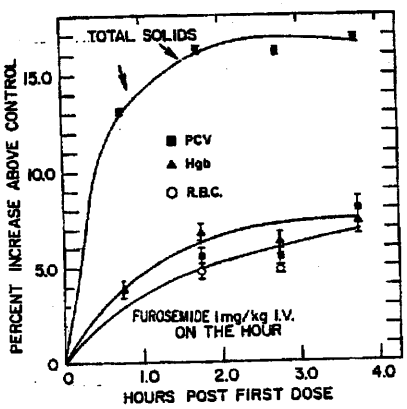


Fig 10—Effect of repeated doses of furosemide on total solids, hematocrit, hemoglobin, and RBC content. Blood samples were drawn from the horses of Experiment 2 (Fig 9) at 45 minutes after each dose of furosemide. The solid circles (●-●) show total plasma solids, the solid squares (■-■) hematocrit, the solid triangles (▲-▲) total hemoglobin, and the open circles (O-O) RBC. All values are plotted as percent increase over control values, ± the standard error of the means.

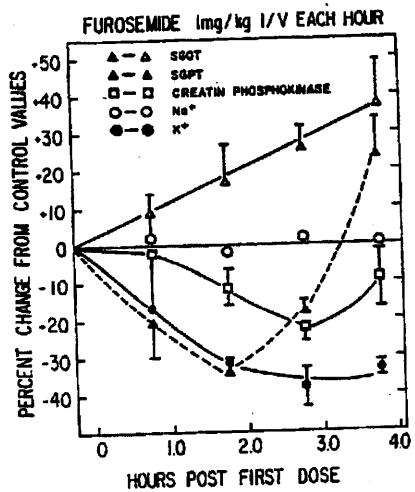


Fig 11—Effect of repeated doses of furosemide on plasma enzymes and monovalent cations. Blood samples drawn from the horses of Fig 9 (Experiment 2) at 15 minutes prior to furosemide and 45 minutes after each dose were analyzed for Na^+ (open circles, O-O), K^+ (solid circles, ●-●), SGOT (open triangles, Δ-Δ), SGPT (solid triangles, ▲-▲), and CP (open squares, □-□). All values represent means ± standard errors of the means on 3 or more horses, and all values are expressed as a percentage of control values at 15 minutes prior to furosemide.

course of experiments of Fig 9 approached the maximal amount of fluid available to this dose level of furosemide in these horses.

Throughout Experiment 2 (Fig 9) blood samples were drawn at 45 minutes after each IV dose of furosemide and analyzed for total protein, Na^+, K^+, SGOT, SGPT, CP, hematocrit, hemoglobin, and total RBC. Figures 10 and 11 show the changes observed. Hematocrit, hemoglobin, and total RBC all increased up to about 8%, whereas about twice this change in total plasma solids were observed. In each case the bulk of the effect was observed with the first dose, and the increases thereafter were considerably smaller.

Figure 11 shows the effect of repeated doses of furosemide on plasma Na^+, K^+, SGOT, SGPT, and CP. Again, no significant change was seen in plasma Na^+ concentration, while plasma K^+ was markedly reduced. No clinically significant changes in any of the plasma enzyme levels were observed.

In another sequence of experiments, 4 horses were dosed daily with 1 mg/kg furosemide IV for 4 days with blood samples being drawn 15 minutes before and 45 minutes after each dose. This experiment was designed to test for any cumulative change in these parameters, either predrug or at peak drug effect (45 minutes postdosing). Again, total protein, plasma Na^+, K^+, SGOT, SGPT, hematocrit, hemoglobin, and total RBC were followed. No clinically significant cumulative effects on any of these values were observed.

The effect of furosemide on the performance of Standardbred horses is presented in Table 2. The dose of 0.55 mg/kg IV or about 5 ml/1,000 lb (453 kg) horse was selected on the advice of Dr. Gene Bierhaus of the Colorado Racing Commission. The test time of 30 minutes after dosing was selected on the basis of the very brief pharmacological action of furosemide and its rapid elimination, demonstrated in this and earlier reports from this laboratory.[14] The data show no statistically significant effects of this dose of furosemide to improve the performance of these horses at 30 minutes after dosing. Table 2 shows the times observed for Standardbred horses racing on furosemide at Louisville Downs this summer. Pre and postfurosemide times were compared for horses which elected to go on furosemide medication during the meet.

TABLE 2

Effect of Furosemide on Time Trials in Standardbred Horses

Trial	Horse	Saline	Furosemide	Δ
1	Noelle	131	141	+10
2		135	134	− 1
3		135	129	− 6
4	Doc Stultz	134	135	+ 1
5		134	132	− 2
6	Beau	142	136	− 6
7		139	141	+ 2
8	Ballards Love Bug	143	142	− 1
9	Wilson	134	134	0
All Horses		136.33	136.00	−0.33

Time trials were performed as described in Methods. All values represent times in seconds required for these horses to pace 1 mile. The Δ symbol refers to the change from the matched control associated with furosemide administration. Applying a t test to all data points, $t = 0.2$, less than one-tenth the value required for significance at the 0.05 level.

The data show no significant difference betwen the times of these horses before and after they went on furosemide.

Discussion

The most surprising result from the present studies has been the apparently fast onset and decline in the diuretic effect of furosemide after IV injection (Fig 1). At all doses and by all routes tested (Figs 1 and 7), the diuretic effect of furosemide peaked between 15 and 30 minutes postinjection. At all IV doses up to 1 mg/kg, the bulk of the diuretic effect was over within 1 hour. Similar results have been reported by Muir *et al.*[12] who observed apparent spontaneous voiding of urine for the first 55 minutes after 1 mg/kg of furosemide IV and no voiding during the following hour. Other work by Garner *et al.*[8] did not report the time course of diuresis due to furosemide.

The rapid peaking and decline in the diuretic effect of furosemide appears to be due to rapid elimination of the drug. Inspection of the data of Roberts *et al.* (Fig 6)[16] shows that, between 10 and 60 minutes postinjection, plasma levels of furosemide fell from 3,000 ng/ml to about 150 ng/ml. This 20-fold drop in plasma concentrations of the drug is sufficient to account for the rapid decline in the pharmacological response to furosemide. The decline in response was not due to any limited availability of fluid to excrete, because a second dose 1 or 2 hours after the first immediately produced a good diuresis (Fig 9). The interpretation that the decay in the diuretic effect of furosemide is primarily due to clearance of the drug is well supported by the data of Fig 8, which shows a close relationship between diuresis due to furosemide and its plasma levels after IV or IM administration.

Intramuscular administration of furosemide resulted in a more prolonged diuretic effect that after IV injection (Fig 7) and in the excretion of about 50% more urine (Table 1). This prolonged diuretic effect after IM injection correlated well with the more prolonged plasma levels of furosemide observed after IM injection. Therefore, in a situation where a more prolonged or more effective diuretic response to a given dose of furosemide is required, IM injection would appear to be the route of choice.

In these horses, 0.04 mg/kg appeared to be the threshold dose for significant renal effect after IV injection. Below this dose, little effect on urinary Na^+, K^+, or volume was observed. At or above this dose level, urinary Na^+ concentration was maximally increased in what appeared to be an all or none effect (Fig 3). In contrast to this effect on Na^+ concentration, increasing doses of furosemide produced a graded increase in volume and a graded decrease in urinary K^+ concentration (Fig 3). The upshot of these relationships is that while increasing concentrations of furosemide produced an increasing output of urine and also of Na^+, increasing the dose had very little effect on K^+ output. As shown in Figs 4 and 5, loss of K^+ was approximately maximally increased by the lowest dose of furosemide tested and was not further increased by higher doses.

Studying the effects of doses of up to 18 mg/kg furosemide IV in horses, Garner *et al.*[8] reported no significant effects on K^+ excretion. The discrepancy between the results reported here and those of Garner and coworkers[8] is probably due to the fact that these workers pooled all urine samples obtained in the first 6 hours postdrug. As shown in Fig 5B, the effect of furosemide on K^+ output is small and occurs within the first hour. Therefore pooling samples for the first 6 hours would tend to dilute out the effect of furosemide on K^+ excretion and render it more difficult to detect. In addition, the dose response data of Fig 4 show, if anything, a tendency for the effect on K^+ output to remain the same or possibly decrease as the dose was increased. For this reason, even the larger doses used by Garner *et al.*[8] would not tend to overcome this problem.

After intravenous administration of furosemide, the changes in blood values (Fig 6) closely followed the time course of the diuretic effect. Hematocrit values increased about 6%, peaking between 20 and 30 minutes postdosing and then declining. Total plasma solids also increased, but to about 10%, and then declined back to baseline.

Plasma Na^+ levels showed no change, while plasma K^+ levels declined and then turned toward normal.

Muir and co-workers[12] observed similar discrepancies between the effects of furosemide on hematocrit and total solids, and Fregin and co-workers[8] suggested that the effect was due to albumin moving into the circulating compartment. The same effect has also been observed in human plasma, where total colloid osmotic pressure was increased about 15% after a dose of furosemide, compared with a 5% increase in hematocrit.[9] Fregin *et al.*[8] chose to interpret this phenomenon in terms of a furosemide induced net entry of albumin into the vascular compartment but failed to suggest a site or mechanism for such an effect. The reduction in plasma K^+ reported here is similar to that reported by Fregin *et al.*[8] and in good agreement with the observed increase in urinary loss of K^+ after furosemide (Fig 5).

The shape of the curves for the increase in total solids and hematocrit post-IV furosemide reported here (Fig 6) differs considerably from those reported by Muir *et al.*[12] These workers did not observe the sharp peaking of these values at 30 minutes and subsequent rapid return to control values reported here (Fig 6). The reason for these differences is not clear but may relate to the fact that the horses in these experiments had free access to water, while those of Muir *et al.*[12] were likely restrained in stocks and had no access to water.

Another unexpected finding from these studies was the close relationship between plasma levels of furosemide and its diuretic effect. Based on studies on the effects of probenecid on the saluretic effect of low doses of furosemide, Hook and Williamson[10] suggested that either cellular or luminal (urinary) furosemide levels would be most closely related to the natriuretic activity of this compound. However, inspection of Fig 9 in the paper of Roberts *et al.*[11] shows that, after IM injection of furosemide, its urinary concentrations rose steeply throughout the diuretic effect, while plasma levels of the drug were falling in parallel with diuretic response. The urinary data are in apparent contradiction with the observations of Burg and Stoner[4] who report that *in vitro* furosemide only acts from the luminal surface of the renal tubule. However, it is difficult to relate bladder concentrations of furosemide (such as were reported by Roberts *et al.*[11]) to concentrations at receptor sites in tubules, especially since urinary output was falling (and therefore drug concentration in urine rising) throughout the experimental period (Fig 9).[11]

In contrast to the apparent lack of relationship between urinary levels of furosemide and the diuretic effect, plasma levels of furosemide were closely related to the diuretic effect (Fig 8). This slightly faster decay of the diuretic effect when compared with plasma levels of the drug probably reflects the fact that we were unable to rehydrate these horses. As shown in Fig 9, the diuretic response to furosemide declined as fluid was eliminated by these animals. It appears probable that a much closer plasma level: diuretic effect relationship could be demonstrated in an experimental situation in which hydration of the test animals could be maintained.

These observations on the correlation between plasma levels of furosemide and its diuretic effect are also in good agreement with some recent work in man. Studying chlorothiazide[12] and more recently furosemide,* Brater has observed a probenecid-induced prolongation and enhancement of the diuretic response to both drugs in human subjects. Probenecid apparently inhibited tubular secretion of these drugs, prolonged their plasma half-life, and thus gave rise to a prolonged and enhanced clinical response. In the study reported here, IM administration of furosemide also acted to prolong its plasma half-life and similarly prolonged and enhanced its diuretic response (Fig 7). Similarly, Branch and co-workers[3] have recently reported a linear relationship between plasma levels of furosemide and the rate of Na^+ excretion in man, in good agreement with the results reported here.

Studies on the performance effects of furosemide posed the problem of selection of a dose and a suitable time period postdosing to conduct the test. The dose of 0.55 mg/kg (about 5 ml of 50 mg/ml furosemide IV to a 1,000-lb horse) was selected on the advice of Dr. Gene Bierhaus of the Colorado Racing Commission. A time of 30 minutes between dosing and exercise was selected because this was the time at which peak changes in total plasma solids and hematocrit were observed (Fig 6). Other workers[7] have selected doses of furosemide of 1 mg/kg IV and waited 130 minutes before their performance tests. Judging from data reported in this paper and elsewhere,[14] a 130-minute delay would allow 90% of the drug to be eliminated and most or all of the acute cardiovascular and hematological effects to dissipate.[12] Performing trials under these circumstances hampers interpretation of negative data, since the most likely interpretation becomes dissipation of the drug effect. We therefore elected to run the trials reported here as close to peak drug effect as possible. As shown in Table 2, no statistically significant effect of pretreatment with furosemide on the times to pace 1 mile were observed. These data are apparently in good agreement with those

* Brater, D.L., Department of Pharmacology, Univ. of Texas Southwestern Medical School, Dallas, TX: Personal communication.

of Gabel and co-workers[7] who reported a small but statistically nonsignificant improvement in the performance of horses pretreated with furosemide in drug trials similar to those reported here.

The principal problem with the drug trial study reported in Table 2 and that of Gabel *et al.*[7] is the small number of animals involved. Thus, if a single trial (trial #1) in which an unusually slow time for furosemide was observed is eliminated, furosemide then improved the performance of 4 out of 5 horses and had no effect in one. Under these circumstances the probability that furosemide improved performance becomes 1 in 16, close to but still less than the 1:20 required for statistical significance. We therefore decided to perform a retrospective study of the effect of furosemide on racing performance of Standardbred horses racing under the furosemide medication rule in Kentucky in 1977.

Through the cooperation of the Kentucky Harness Commission, the list of horses electing to go on furosemide medication during the 1977 Louisville Downs Summer Meet was made available to us. From the names of these horses and the date on which they went on furosemide, their performance times pre- and postfurosemide were obtained from the published race programs. A comparison of these times showed that the mean times to pace 1 mile of the 58 horses selected for study was actually increased by 0.1441 seconds in the horses treated with furosemide. This small slowing in their performance times by furosemide was not statistically significant. The data show that the average horsemen and presumably their veterinary advisors at Louisville Downs were not able to improve the performance of horses during this meet by medication with furosemide. It should be borne in mind that nothing is known about the doses of furosemide used, the times before racing that they were administered, or the conditions which prompted administration of the drug. These observations support the results obtained in the performance trials reported here and those of Gabel and co-workers[7] and suggest that furosemide has no effect on the time to pace 1 mile of Standardbred horses.

Because of the widespread use of furosemide in racing horses, questions arise concerning the safety of repeated doses of furosemide. Studies by the manufacturer indicate that the acute toxic IV dose of furosemide is not less than 300 mg/kg. Irreversible deafness has been reported in human patients receiving massive doses of furosemide.[5] Other workers have reported enhanced tubular necrosis in patients simultaneously receiving furosemide and cephaloridine.[3] Further, very high doses of furosemide in mice have been shown to be metabolized to a chemically reactive metabolite which produces massive hepatic necrosis.[11]

Because of these considerations we studied the effect of repeated dosing with furosemide on a number of blood values. Figure 9 shows that at 1 mg/kg IV the diuretic response to furosemide was self-limiting, and the data suggest that at this dose level about 25 liters of fluid can be obtained from a 1,000-lb (453 kg) horse. Once this amount of fluid was withdrawn, the animal became resistant to further 1 mg/kg doses, and little further diuresis or hemoconcentration was seen. No clinically significant changes in plasma enzymes were seen in this experiment, but plasma K^+ was markedly reduced. Hypokalemia would thus seem to be the most likely acute toxicity problem to be encountered during repeated dosing with furosemide in this horse. However, the horses in these experiments appeared clinically normal at the end of these repeated dosing experiments. Similarly, in the experiments in which 1 mg/kg furosemide was administered IV daily to horses for 4 days, no cumulative deviations

TABLE 3

Effect of Medication with Furosemide on the Performance of Horses Racing at Louisville Downs, Summer, 1976

	# of Horses	# of Trials	Mean Times	S.E.M.	
Prefurosemide	58	160	128·5925	0.2031	F = <0.00
With Furosemide	58	232	128·7366	0.1594	(F for significance should be >3.0)

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 postfurosemide treatment were obtained from the meet programs and compared. Only times on good or fast tracks were taken. For the 58 horses selected, 160 prefurosemide times were available and 232 postfurosemide 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).

from control values were seen in any of the parameters tested.

These results support the conclusions of Gabel and co-workers[7] that, at the commonly used doses, furosemide is a safe drug in the horse. From these studies it is clear that these are relatively small doses (0.5–1 mg/kg) of the drug, and that when administered IV their action is brief and the drug is rapidly eliminated. The 8–9 liters of water eliminated after a 1 mg/kg dose IV is only about 2.8% of the total body water in a 1,000-lb (453 kg) horse. If access to water is restricted, this deficit can presumably be distributed throughout total body water. Given access to water, this deficit is probably readily replaced and accounts for the rapid reversal of the hemoconcentration observed in Fig 6. The sharp decline in plasma K^+ after furosemide (Figs 6 and 11) must acutely reduce the turnover of membrane $Na^+ K^+$-ATPase[16] and increase the net flux of K^+ along the leak pathway to extracellular sites, accounting for the rapid reversal of the decline in plasma K^+ levels observed in Fig 6. Because of the large excess of K^+ in the normal equine diet and the ready availability of intracellular K^+ for the plasma pool, acute hypokalemia would seem an unlikely event in the normal horse after single doses of furosemide. However, the possibility of clinically significant hypokalemia after large or repeated doses of furosemide is very real (Fig 11) and deserves further investigation.

References

1. Branch, R.A., Roberts, C.J.C., Homeida, M., and Levine D.: Determinants of Response to Furosemide in Normal Subjects. *Brit J Clin Pharmac.* 4, (1977): 121-127.
2. Brater, D.C.: Accentuation by Probenicid of the Diuretic Effects of Chlorothiazide in Man. *Pharmacologist.* 19, (1977): 137.
3. Brown, C.G., Ogg, C.S., Cameron, J.S., and Bewick, M.: High Dose Furosemide in Acute Irreversible Intrinsic Renal Failure. *Scottish Med J.* 19, (1974): 35-39.
4. Burg, M., and Stoner, L.: Renal Tubular Chloride Transport and the Mode of Action of Some Diuretics. *Ann Rev Physiol.* 38, (1976): 37-45.
5. Dodd, M.G., and Foord, R.D.: Enhancement by Potent Diuretics of Renal Tubular Necrosis Induced by Cephaloridine. *Brit J Pharmacy.* 40, (1970): 227-236.
6. Fregin, G.F., Nicholl, T.K., Milne, D.W., Muir W.W., and Skarda, R.J.: Furosemide: Its Influence on Hematology and Blood Chemistry in The Horse Exercised by Swimming. *J Eq Med Surg.* 1, (1977): 195-202.
7. Gabel, A.A., Tobin, Thomas, Ray, Richard S., and Maylin, George A.: Furosemide in Horses: A Review. *J Eq Med Surg.* 1, (1977): 215-218.
8. Garner, H.E., Hutcheson, D.P., Coffman, J.R., Johnson, J.H., and Salem, C.: Urine Electrolyte and Diuretic Responses of Horses to Seven Dosage Levels of Lasix. *Proc AAEP.* (1975): 87-90.
9. Hesse, B., Nielsen, I., and Lund-Jacobson, H.: The Early Effects of Intravenous Furosemide on Central Hemodynamics, Venous Tone and Plasma Renin Activity. *Clin Sci Mol Med.* 49, (1975): 551-553.
10. Houk, J.G., and Williamson, H.E.: Influence of Probenicid and Alteration in Acid Base Balance on the Saluretic Effect of Furosemide. *J Pharm Exp Therap.* 149, (1965): 404-408.
11. Mitchell, J.R., Nelson, W.L., Potter, W.Z., Sasame, H.A., and Jollow, D.L.: Metabolic Activation of Furosemide to a Chemically Reactive Hepatotoxic Metabolite. *J Pharm Exp Therap.* 199, (1976): 41-52.
12. Muir, W.W., Milne, D.W., and Skarda, R.T.: Acute Hemodynamic Effects of Furosemide Administered IV in the Horse. *Amer J Vet Res.* 37, (1976): 1179-1180.
13. Roberts, B.L.: Pharmacokinetics, Pharmacodynamics and Forensic Implications of Furosemide in the Horse. MSc Thesis. Department Veterinary Science, University of Kentucky, Lexington, KY 40506.
14. Roberts, B.L., Blake, J.W., and Tobin, Thomas: The Pharmacology of Furosemide in the Horse. II. Its Detection, Pharmacokinetics, and Clearance from Urine. *J Eq Med Surg.* 2, (1978): 185-194.
15. Tobin, T.: A Review of the Pharmacology of Furosemide in the Horse. *Proc 3rd Intnl Doping Conf.*, (March, 1977), Rome, Italy.
16. Tobin, T., Akera, T., Han, C.S., and Brody, T.M.: Lithium and Rubidium Interactions with Sodium and Potassium-Dependent Adenosine Triphosphatase: A Molecular Basis for the Pharmacological Action of these Ions. *Mol Pharmacol.* 10, (1974): 501-508.
17. Tobin, T., Roberts, B.L., and Miller J.R.: The Pharmacology of Furosemide in the Horse. I. Its Effects on the Disposition of Procaine, Methylphenidate, Phenylbutazone, and Pentazocine. *J Eq Med Surg.* 1, (1977): 402-409.

Furosemide in Horses: A Review

Albert A. Gabel, D.V.M., M.S.
Thomas Tobin, D.V.M., Ph.D.
Richard S. Ray, D.V.M., M.S., Ph.D.
George A. Maylin, D.V.M., M.S., Ph.D.

From the Department of Veterinary Clinical Sciences, The Ohio State University, 1935 Coffey Road, Columbus, Ohio 43210 (Gabel, Ray); Department of Veterinary Science, University of Kentucky, Lexington, Kentucky 40508 (Tobin); and Equine Drug Testing and Research, Cornell University, Ithaca, New York 14853 (Maylin).

With additional input by: Gary P. Carlson, University of California; Harold E. Garner, University of Missouri; Jerry R. Gillespie, University of California; Jerry H. Johnson, University of Missouri; Dennis W. Milne, Ohio State University; William W. Muir, Ohio State University, and David Snow, University of Glasgow, and other members of the National Association of State Racing Commissioners Veterinary-Chemist Advisory Committee: Tom Finley, A.V. Villatico, Robert E. Vestey, Dr. Francis Ozog, Dr. Robert Schoenfeld, Dr. Joe Solomon, Dr. Marvin Beeman, Dr. Joe O'Dea, Dr. Gene Bierhaus, and General Wayne Kester.

1. Injectable furosemide[*] is a potent, effective, safe diuretic which at the usual dose has its peak diuretic effect in about 20 minutes in horses, at which time it increases urine output up to 40-fold for a short time. Most of its diuretic effect is within the first 2 hours after injection.

2. During the first 2 hours, it decreases the pressure in the right side of the heart, decreases left atrial pressure, decreases cardiac output, and concurrently it increases peripheral (systemic) blood vessel resistance and heart rate.

3. It reduces edema in the lungs and airway of horses with certain respiratory diseases by 1 or more mechanisms, perhaps by lowering the pressure of the left atrium and/or increasing capacitance (ability to accept blood) of the vessels of the lungs.

4. The mechanism by which furosemide helps prevent epistaxis may result from the beneficial effect outlined in (3) above since most horses bleed from the lungs. Lasix[*] also appears to affect the platelets of horses during exercise.

5. The blood (plasma) level of furosemide declines rapidly. It can be detected in the urine by screening methods for the first 24 hours and by more sophisticated methods for up to 48 hours after injection.

6. Furosemide does not affect the blood levels of other drugs.

7. Furosemide has little effect on the concentration of certain drugs, such as procaine, in the urine. However, during its peak diuretic effect, within 2 hours after injection, furosemide causes up to 40-fold dilution of certain other drugs such as phenylbutazone. However, furosemide administered more than 4 hours before race time does not significantly reduce the ability to detect those drugs studied to date.

[*] Lasix®, National Laboratories Inc., Somerville, NJ 08876