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Effects of phenylbutazone and oxyphenbutazone on basic drug detection in high performance thin layer chromatographic systems*†

W. E. WOODS, S. CHAY, T. HOUSTON, J. W. BLAKE & T. TOBIN

Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, University of Kentucky, Lexington, Kentucky, U.S.A.

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Interference or 'masking' in thin layer chromatography occurs when the presence of one drug on a thin layer plate physically obscures or interferes with the detection of another drug. We investigated the ability of phenylbutazone and oxyphenbutazone to mask or interfere with the detection by high performance thin layer chromatography (HPTLC) of basic drugs used illegally in horse racing. Of fifty-five basic drugs called 'positive' since 1981 by laboratories affiliated with the Association of Official Racing Chemists (AORC), forty did not comigrate with phenylbutazone or oxyphenbutazone and could not, therefore, be masked. When 75 μg/ml of oxyphenbutazone was spiked into urine samples, subjected to an extraction procedure for basic drugs, and then run in our routine HPTLC systems, no 'spots' due to oxyphenbutazone appeared. 'Masking' by oxyphenbutazone, therefore, did not and could not occur in our test systems. When phenylbutazone at a concentration of 30 μg/ml was spiked into urine samples and run in the routine HPTLC system, phenylbutazone spots were visible under ultraviolet light and after certain specific oversprays were used to visualize basic drugs. These spots, however, did not interfere with routine thin layer testing for basic drugs. It was concluded that phenylbutazone and oxyphenbutazone had no significant ability to interfere with detection of the parent forms of these basic drugs under the conditions described in these experiments.

T. Tobin, Kentucky Equine Drug Research and Testing Programs, Department of Veterinary Science, University of Kentucky, Lexington, KY 40546-0076, U.S.A.

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INTRODUCTION

Phenylbutazone is a non-steroidal anti-inflammatory drug widely used in the treatment of musculoskeletal problems in racing horses (Tobin, 1981). As it is commonly used in the treatment of horses in training, blood and urinary residues (Tobin, 1979; Tobin *et al.*, 1981) may appear in both pre- and post-race testing samples taken for analysis in medication control programs (Tobin, 1981).

Some racing chemists hold that residues of phenylbutazone and its metabolites (oxyphenbutazone and γ-hydroxyphenylbutazone) in equine urine can 'mask' or interfere with the detection of other drugs. Popularly, the phenomenon is called 'masking' and phenylbutazone may be thought of as 'masking' or interfering with the detection of illegal drugs (Takade & Vassilaros, 1982).

Masking occurs when the presence of a substance on a thin layer chromatographic plate physically obscures or interferes with the detection of a drug (Takade & Vassilaros, 1982). However, the ability of phenylbutazone to 'mask' or interfere with the detection of other drugs has been controversial. For example, some workers hold that phenylbutazone masking is a serious problem, while others hold that the problem is trivial and can readily be overcome by the analyst (Takade & Vassilaros, 1982; Tobin, 1983). Further adding to the confusion on the 'masking' problem is the fact that there is no published scientific literature whatsoever on this subject.

Because of the regulatory and forensic importance of the concept of masking (Tobin, 1983) or interference, we have commenced a study of this problem. In this communication, we report on the potential of phenylbutazone (PB) and oxyphenbutazone (OPB) to interfere with high performance thin layer chromatographic (HPTLC) screening for basic drugs. The other major PB metabolite, γ-hydroxyphenylbutazone (γ-OHPB), occurs in two interchangeable forms (Girod *et al.*, 1957; G. Haas and K. Scheibli, personal communication). We are currently examining the role of these two forms of γ-OHPB in the masking problem and will present these results at a later time.

OPB was selected as the metabolite for study because it occurs in relatively high concentrations in equine urine (Houston *et al.*, 1983a, b). As such, OPB and PB present in urine samples may coextract with prohibited basic drugs and may interfere with their detection. As basic drugs include most of the stimulants, depressants, anesthetics and tranquilizers used as illegal medications, this represents an experimental approach to the question of whether PB or its metabolites can interfere with, or mask, the detection of other drugs, particularly stimulant and narcotic drugs.

MATERIALS AND METHODS

Drugs selected as candidates for possible masking by PB and OPB were those called 'positive' by AORC laboratories for the calendar year 1981 (Johnston, 1982). In addition, as further records became available, drugs called positive since 1981 were added to the candidate list. From this master list, basic drugs were selected for this study. One mg of each drug standard was made up in 10 ml of methanol and, depending on the detectability of the drug, from 20 µl to 100 µl of each standard was used for spotting.

Standard solutions of OPB in methanol were added to equine urine to give two concentrations. One, 24 µg/ml, was the mean level of OPB in about 200 Thoroughbred urines from horses racing in Kentucky. The second, 75 µg/ml, was equivalent to the highest concentrations of OPB in the urine of about 200 Thoroughbred horses racing in Kentucky (Houston *et al.*, 1983a, b, 1985).

Standard solutions of PB in methanol were added to equine urine to give concentrations of 14.7 µg/ml and 30 µg/ml which are, respectively, the mean concentrations of PB and the highest value observed in 200 urines from Thoroughbred horses racing in Kentucky (Houston *et al.*, 1983a, b, 1985).

Drug extractions

All drug extractions followed the patterns used in the Kentucky Equine Drug Testing Program. To 1 ml of the urine sample were added 4 ml of dichloromethane (DCM) and four drops of concentrated ammonium hydroxide (NH_4OH). The sample was shaken on a rotorack for 5 minutes, centrifuged at

500 ***g*** for 20 minutes and the aqueous fraction removed. The DCM fraction was then evaporated to dryness under a stream of nitrogen and dissolved in two drops of DCM. The entire sample was then spotted on the thin layer plate. For work with spiked samples, 1 μg/ml of the drug in question was added to the initial urine sample. In all initial screening experiments 1 ml of urine was used. In later, more critical experiments (Tables VI and VII) 3 ml of urine were used to allow comparison with routine racetrack testing.

Thin layer chromatography procedures

The thin layer chromatographic techniques used are those current in the Kentucky Equine Drug Testing Program. Whatman HP-KF high performance silica gel plates, 10 × 10 cm, 200 μm layer thickness were used throughout. For spotting, methanol aliquots of the drugs in question were dried under nitrogen and then dissolved in two drops of DCM. These DCM standard solutions were then spotted on the plate using drain-out Pasteur pipettes. Spots were applied in incremental fashion and dried between each movement using a hair dryer. Spots were placed 15 mm above the bottom of the plate and 5 mm apart to give up to 19 spots/plate. Plates were developed by allowing the solvent to rise 75 mm above the bottom of the plate.

Solvent systems and visualization

Three systems were used to develop the plates, corresponding with the screening systems used for basic drugs in the Kentucky Equine Drug Testing Program (Fig. 1). In system no. 1, the 'phenothiazine' (PTZ) system (Table I), plates were developed in a system of 90% chloroform and 10% methanol. Initial visualization was by short-wave ultraviolet (u.v.) (254 nm) in a view box. Subsequently, the plates were sprayed with a 'phenothiazine' location reagent consisting of 250 mg of ferric chloride, 50 ml of ethanol and 50 ml of concentrated sulfuric acid. The plates were then heated at low heat on a hot plate. Thereafter, the plates were sprayed with Dragendorff's reagent (Whatman Co., Clifton, NJ), followed by nitrogen dioxide (NO_2) exposure. NO_2 exposure was achieved by adding concentrated nitric acid (HNO_3) to a beaker

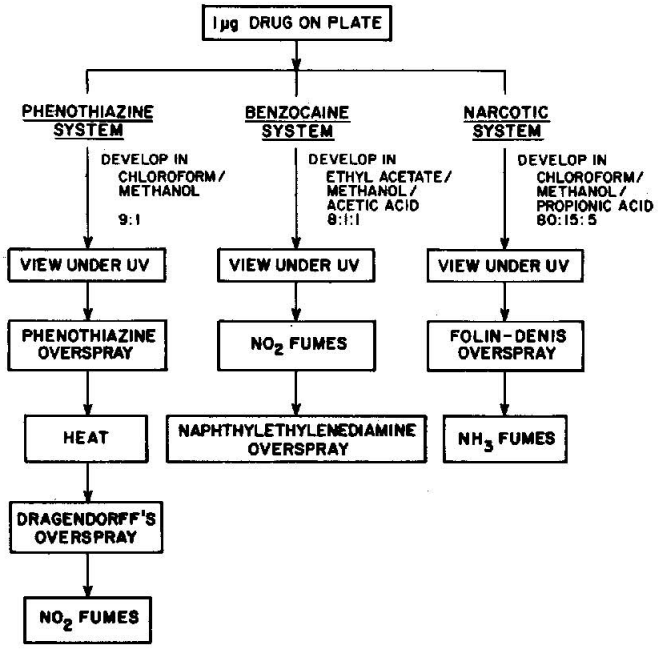

FIG. 1. Flow chart of basic drug detection by HPTLC, including developing systems, oversprays and visualization.

TABLE I. Thin layer chromatography systems

System no.	Solvent mix	Typical Detected Compound
1	Chloroform/methanol 9:1	Phenothiazines (PTZ)
2	Ethyl acetate/acetic acid/methanol 8:1:1	Benzocaine/clenbuterol (Benz)
3	Chloroform/methanol/propionic acid 80:15:5	Narcotic analgesics (Narcotic)

containing copper pellets, in a developing tank.

In system no. 2, the 'benzocaine' (Benz) system (Table I), the plates were developed in 80% ethyl acetate, 10% acetic acid and 10% methanol. Following development and u.v. visualization, the plates were sprayed with N-1-naphthylethylenediamine (NED) location reagent, followed by ninhydrin overspray and heating.

In system no. 3, the 'narcotic' system (Table I), the plates were developed in 80% chloroform, 15% methanol and 5% propionic acid. After u.v. visualization, the plates were sprayed with amodified Folin-Denis reagent. Thereafter, the plates were exposed to ammonia (NH_3) fumes by placing the plates in a tank with a beaker of concentrated NH_4OH (Fig. 1).

Measurement and data recording

All spots visualized in any of the HPTLC systems were circled with a soft lead pencil at each visualization spot, and the plates were photocopied. In addition, the appearance, color and R_f of each spot were observed and recorded.

RESULTS

The R_f values for PB and OPB and any of their breakdown products in each HPTLC system are shown in Table II. In each system, PB and OPB have R_f values of 0.5 or greater. Based on these R_f values, parent drugs with an R_f values of less than 0.5 in these systems cannot have their detection interfered with by PB or OPB.

TABLE II. R_f values of OPB and PB in basic drug high performance thin layer chromatographic systems

System no.	Solvent mix	R_f	n
(a) Oxyphenbutazone			
1	9:1 (PTZ)	0.65 ± 0.05	8
1	9:1 (PTZ)	0.52 ± 0.06*	8
2	8:1:1 (Benz)	0.88 ± 0.05	4
3	80:15:5 (Narcotic)	0.84 ± 0.01	2
(b) Phenylbutazone			
1	9:1 (PTZ)	0.83 ± 0.03	9
1	9:1 (PTZ)	0.76 ± 0.03*	6
2	8:1:1 (Benz)	0.89 ± 0.02	5
3	80:15:5 (Narcotic)	0.97 ± 0.00	6

*Small secondary spot found in the 9:1 system only.

Drugs whose detection in the parent form could not be interfered with by PB or OPB in system no. 1 shown in Table III. Drugs whose detection in system no. 2 would not be interfered with are listed in Table IV. Similarly, Table V shows narcotic analgesics whose detection would not be interfered with on an R_f basis in system no. 3. Fentanyl, which has an R_f value close to that of OPB, is a potential candidate for interference in this thin layer system.

Table VI shows a list of parent compounds whose R_f values in these systems gave rise to potential for masking problems. We therefore determined the amount of OPB which would extract into our basic HPTLC systems under the conditions of our experiments. Assuming 75 μg/ml of OPB in 3-ml urine samples (Houston *et al.*, 1985), we determined that 0.36 μg of OPB actually transferred through the recovery and extraction process to the HPTLC system. When this amount of OPB (0.36 μg) was spotted directly on HPTLC plates and run in each of the solvent systems, no spots were detected during the overspray visualizations. When urine samples containing

TABLE III. Drugs not masked by PB or OPB on an R_f basis in system no. 1

Acepromazine (0.19)	Methocarbamol (0.37)
Acetaminophen (0.28)	Methylphenidate (0.28)
Alphaprodine (0.38)	4-Methyl-5-thiazolethanol (0.40)
Amitriptyline (0.38)	Nefopam (0.35)
Amphetamine (0.12)	Nicotine (0.34)
Chlorobutanol (0.25)	Nicotinamide (0.24)
Chlorpheniramine (0.07)	Oxazepam (0.41)
Chlorpromazine (0.24)	Pemoline (0.27)
Diphenhydramine (0.22)	Phenylboloxamine (0.41)
Dipyrone (0.02)	Promazine (0.21)
Doxylamine (0.04)	Pyrilamine (0.12)
Guiacol glyceryl ether (0.40)	Tetracaine (0.36)
Heptaminol (0.00)	Trichlormethiozide (0.29)
Mazindol (0.08)	Trimethoprim (0.22)
Mephentermine (0.32)	Tripelennamine (0.18)
Methenamine (0.33)	Xylazine (0.28)

TABLE IV. Drugs not masked by PB or OPB on an R_f basis in system no. 2

Clenbuterol (0.33)
Phenylpropanolamine (0.23)
Procaine (0.05)

TABLE V. Drugs not masked by PB on an R_f basis in system no. 3.

Apomorphine (0.17)
Butorphanol (0.22)
Levorphanol (0.25)
Morphine (0.15)
Nalbuphine (0.17)
Naloxone (0.51)
Pentazocine (0.49)

TABLE VI. Drugs possibly masked by OPB on an R_f basis

(a) System no. 1
Benzidine (0.58)
Benzphetamine (0.75)
Bupivacaine (0.78)
Diazepam (0.72)
Dimethylsulfoxide (0.48)
Doxapram (0.63)
Lidocaine (0.77)
Mepivicaine (0.52)
Phenothiazine (0.84)
Tetramisole (0.47)
(b) System no. 2
Benzocaine (0.82)
(c) System no. 3
Fentanyl (0.74)

added OPB at 75 μg/ml were extracted and run in system no. 1 and system no. 3, no detectable interfering spots were observed during the overspray sequences. However, when this concentration of OPB was extracted and run in system no. 2, a spot yielding a blue color with an R_f very close to that of benzocaine in this system was observed. While this OPB spot might be confused with benzocaine until oversprayed, it would lead to a false positive and not to a masking effect.

Table VII shows a list of parent compounds which, as judged from their R_f values, would migrate close to PB in our test systems. Assuming 15.0 μg/ml of PB in a 3-ml urine sample (Houston *et al.*, 1984), we determined by HPTLC that 6.99 μg of PB was actually transferred to the HPTLC system. When this amount (6.99 μg) of PB was spotted directly on HPTLC plates and run in each of the solvent systems, spots were seen in all systems under u.v. light at the R_f values for PB in each system. However, during the visualization sequences to develop the presence of drugs detected by each system, these amounts of PB did not give rise to interfering spots in any of these HPTLC systems.

TABLE VII. Drugs possibly masked by PB on an R_f basis

(a) System no. 1
(Phenylbutazone R_f 0.76 and 0.83)
Benzphetamine (0.75)
Bupivacaine (0.78)
Diazepam (0.72)
Lidocaine (0.77)
Phenothiazine (0.84)
(b) System no. 2
(Phenylbutazone 0.83)
Benzocaine (0.82)

When the amount of PB in the samples was increased to 30 μg/ml, as may occur in about one in 200 Kentucky Thoroughbred post-race urine samples, extraction of these samples gave rise to observable PB spots in all three systems. These spots did not interfere in system no. 3, and benzocaine was the only drug possibly interfered with in system no. 2. After treatment with NED, the benzocaine spot yielded a bright purple reaction, compared with the faint yellow spot for PB. In system no. 2, two spots were observed under u.v. light. The spot with the higher R_f (0.85) was PB, and cochromatographed with phenothiazine. This spot, however, did not react with the phenothiazine overspray. Although it did react with Dragendorff's reagent, this spot did not cochromatograph with any of the other drugs listed in Table VII (Fig. 2).

The second PB spot in this system (R_f 0.80) is due to a PB breakdown product. This spot is generally not as distinct as the PB spot and, while this lower spot chromatographed close to the drugs of Table VII, it did not react with Dragendorff's reagent.

DISCUSSION

The phenomenon of 'masking' or interference in drug testing is undefined and undescribed in the scientific literature. Despite its scientific non-recognition, however, this problem has considerable forensic currency (Tobin, 1983). In this paper, we analysed the ability of PB and OPB to mask or interfere with the testing systems used for the detection of basic illegal medications in racing horses in the screening systems used in the Kentucky Equine Drug Testing Program. The drugs against which PB and OPB were tested were selected on the basis of drugs called 'positive' in North American racing in 1981 and later. This paper deals only with interference with detection of the parent drug and does not deal with metabolites.

A 'positive' is an illegal medication detected, identified, confirmed and reported by a racing laboratory in a pre- or post-race blood or urine sample. Basic drugs, which include stimulants, depressants, local anesthetics, narcotic analgesics and tranquilizers, are illegal in all racing jurisdictions. Use of this selection of basic drugs therefore provided a cross-section of drugs currently being detected and 'called' in equine forensic laboratories in North America. If interference by PB or its metabolites with drug testing is indeed a problem, then it might be expected to appear in such a study. From the eighty-five drugs on this list, fifty-five basic drugs were selected for testing against PB and OPB.

The second piece of information that one needs to study in the masking or interference

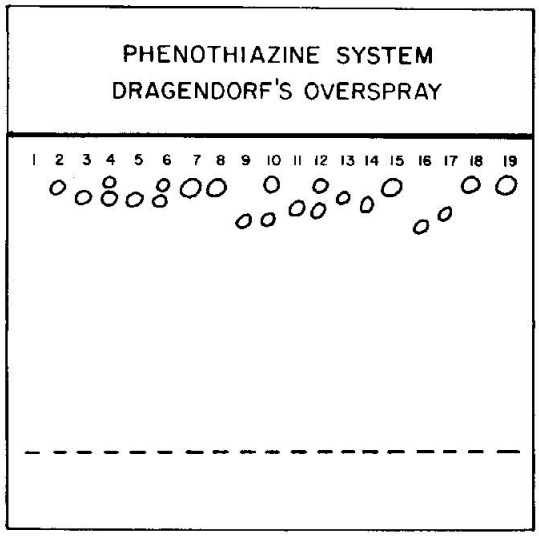

FIG. 2. Thin layer chromatogram of PB and basic high R_f drugs after Dragendorff's overspray. Urine samples were spiked with the indicated drugs, extracted as outlined in the Materials and Methods, and run in the 'phenothiazine' system. The sample numbers correpond with: (1) blank urine; (2) PB spike, 30 μg/ml; (3) diazepam spike, 4 μg; (4) PB + diazepam spike; (5) lidocaine spike, 4 μg; (6) PB + lidocaine spike; (7) phenothiazine spike, 4 μg; (8) PB + phenothiazine spike; (9) benzphetamine spike, 4 μg; (10) PB + benzphetamine spike; (11) bupivacaine spike, 4 μg; (12) PB + bupivacaine spike; (13) diazepam, 4 μg direct; (14) lidocaine, 4 μg direct; (15) phenothiazine, 4 μg direct; (16) benzphetamine, 4 μg direct; (17) bupivacaine, 4 μg direct; (18) PB, 4.78 μg direct; (19) PB, 30 μg direct. The plates were then viewed under u.v., sprayed with the phenothiazine overspray, heated, visualized and sprayed with the Dragendorff's overspray. After exposure to NO_2 fumes as indicated in Fig. 1, the positions of the spots were noted by marking the plate with a soft lead pencil. With the exception of the phenothiazine spot, none of the drug spots cochromatographed with phenylbutazone.

problem is information on the concentrations of PB and its metabolites found in the urine of horses racing in the jurisdiction in question. This information was provided by the work of Houston *et al.* (1985) who measured the concentrations of PB and its metabolites in post-race urines of about 200 horses racing in Kentucky in the Spring of 1983. This study showed that the highest concentrations of PB and OPB likely to be found in post-race urines in Kentucky are about 30 and 75 μg/ml, respectively.

These concentrations of PB and OPB are sufficiently high that they may coextract with basic drugs, comigrate with these agents in HPTLC systems and interfere with, or 'mask', their detection. For masking to be a problem, however, two conditions must be obtained. First, sufficient PB and OPB would have to extract under basic conditions to form a visible spot on the HPTLC plate. Second, these spots would have to coincide with and obscure a spot caused by an illegal medication in our test system.

For OPB, the problem of masking does not arise in our basic drug screening systems because OPB does not give rise to detectable spots in our basic HPTLC drug screening system. On the other hand, for PB the possibility of masking does occur, since at the highest concentrations of this drug found in post-race equine urines it gives rise to observable spots in our HPTLC test systems. This is due to the fact that at this basic pH (9.5), PB is predominately un-ionized and hence somewhat extractable, whereas the phenolic hydroxyl group of OPB is ionized and OPB is not as readily extractable. Because of this, the potential for masking by PB in these test systems was carefully evaluated.

In a survey of the amounts of PB found in the urine of horses racing in Kentucky, Houston and her coworkers (1984) found a range of urinary PB concentrations of 0.1–

30.50 μg/ml. While the 30 μg/ml concentrations were rare, and primarily dependent on the pH of the urine sample, they may occur in a small proportion of basic post-race urine samples in Kentucky. We therefore evaluated the effects of 15 and 30 μg/ml concentrations of PB on the detection of basic drugs in our experiments.

Because most of the drugs in this survey did not cochromatograph with PB, a large proportion of these agents could be eliminated from consideration. Thus, in system no. 2, only benzocaine was likely to give rise to detection problems. However, the color reaction of benzocaine in the NED test system used in its detection is sufficiently distinct that PB at any of the concentrations found in urine samples in Kentucky is not likely to give rise to detection problems.

In system no. 1, PB gives rise to two spots: one with an R_f of 0.83 is due to PB itself; the other, with an R_f of 0.76, is due to a breakdown product of PB. These spots may have the potential to interfere with the five drugs listed in Table VIIa on the basis of their ability to chromatograph in the same general region as PB.

However, the color reactions to location reagents for PB are different from those of the agents listed in Table VII. The spot with the higher R_f value does not react with the phenothiazine location reagent, and thus does not interfere with the compound phenothiazine. Beyond this, phenothiazine is an anthelmintic agent and not the type of agent likely to be used to affect the performance of horses or, indeed, to be a prohibited medication in the U.S.A. Further, it is from a family of agents which are likely to be detected as metabolites. Since metabolites commonly have smaller R_f values than parent drugs, they are thus even less likely to be interfered with.

The other four agents in Table V may cochromatograph with the unidentified breakdown product of PB. These agents are bupivacaine, lidocaine, diazepam and benzphetamine. Of these four drugs, only benzphetamine is likely to be detected as the parent drug, the others usually being detected as metabolites. However, these spots are sufficiently distinct to be distinguishable from these agents on the R_f basis. Beyond this, the breakdown product of PB does not react with Dragendorff's reagent, and thus does not interfere with the color test for these drugs.

In summary therefore, OPB, in the concentrations found in the post-race urines of racing horses in Kentucky, did not extract into the basic HPTLC testing systems in use in Kentucky in sufficiently high concentrations to give rise to observable thin layer spots. Because of this, OPB is unable to give rise to interference with routine thin layer testing for any basic drug under the test conditions reported here.

PB, in the concentrations found in post-race urines in Kentucky, will extract into the basic thin layer testing systems in concentrations yielding observable spots in the HPTLC test systems. While these spots chromatographed in the same areas as spots from five drugs from the test list of drugs, these spots were always distinguishable from PB on either an R_f basis or by virtue of their reactions with oversprays. Therefore, in these test systems and under the conditions used in these tests, there was no evidence of an ability of PB to interfere with testing for basic drugs.

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REFERENCES

Girod, E., Delley, R. & Häfliger, F. (1957) Über derivate des phenylbutazons. III. Die struktur der reduktionsprodukte des γ-keto-phenylbutazons. *Helvetica Chimica Acta*, **40**, 408–418.

Houston, T.E., Chay, S., Woods, W.E., Combs, G., Kamerling, S., Blake, J.W., Edmundson, A.G., Vessiney, R. & Tobin, T. (1985) Phenylbutazone and its metabolites in plasma and urine of thoroughbred horses: population distributions and effects of urinary pH. *Journal of Veterinary Pharmacology and Therapeutics*, **8**, 136–149.

Houston, T.E., Tobin, T. & Blake, J.W. (1983a) Effect of urine pH on urine levels of oxyphenbutazone in racing horses. *Drug Metabolism and Disposition*, **11**, 617–619.

Houston, T.E., Tobin, T. & Blake, J.W. (1983b) Effect of urine pH on urine levels of phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone in racing horses. In *Proceedings of the 5th International Conference on the Use of Drugs in Racehorses*. Toronto, Canada.

Johnston, G.H. (ed) (1982) *Proceedings of the 36th Meeting of the Association of Official Racing Chemists*. Fort Lauderdale, Florida.

Takade, D.Y. & Vassilaros, D.C. (1982) Evaluation of the scientific literature concerning the effect of administration of furosemide and phenylbutazone on screening tests for drugs affecting the performance of horses. *Midwest Research Institute*, Project no. 7551-C.

Tobin, T. (1979) Pharmacology review: the non-steroidal anti-inflammatory drugs. I. Phenylbutazone. *Journal of Equine Medicine and Surgery*, **6**, 253–258.

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

Tobin, T. (1983) Testimony on phenylbutazone, furosemide, and drug testing before the House Subcommittee on Criminal Justice. *Horseman's Journal*, **34(7)**, 142–149.

Tobin, T., Combie, J. & Nugent, T.E. (1982) 'Detection times' and 'clearance times' for drugs in horses and other animals: a reappraisal. *Journal of Veterinary Pharmacology and Therapeutics*, **5**, 195–197.