

Population distributions of phenylbutazone and oxyphenbutazone after oral and i.v. dosing in horses

S. CHAY, W. E. WOODS, T. E. NUGENT, T. WECKMAN,
T. HOUSTON, F. SPRINKLE, J. W. BLAKE, T. TOBIN,
L. R. SOMA,* J. YOCUM† & J. D. SMITH†

Kentucky Equine Drug Research and Testing Programs, Department of
Veterinary Science, University of Kentucky, Lexington, *School of
Veterinary Medicine, University of Pennsylvania, and †Hagyard,
Davidson and McGee, Inc., Lexington, U.S.A.

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Experiments to determine the residual plasma concentrations of phenylbutazone
and its metabolites found in horses racing on a 'no-race day medication' or 24-h
rule were carried out. One dosing schedule (oral-i.v.) consisted of 8.8 mg/kg
(4 g/1000 lbs) orally for 3 days, followed by 4.4 mg/kg (2 g/1000 lbs) intra-
venously on day 4. A second schedule consisted of 4.4 mg/kg i.v. for 4 days.
The experiments were carried out in Thoroughbred and Standardbred horses
at pasture, half-bred horses at pasture, and in Thoroughbred horses in training.

After administering the i.v. schedule for 4 days to Thoroughbred and Standard-
bred horses at pasture, the mean plasma concentrations of phenylbutazone
increased from 0.77 $\mu\text{g/ml}$ on day 2 to 2.5 $\mu\text{g/ml}$ on day 5. The shape of the
frequency distribution of these populations was log-normal. These data are
consistent with one horse in 1,000 yielding a plasma level of 8.07 $\mu\text{g/ml}$ on
day 5.

After administration of the oral-i.v. schedule to Thoroughbred and Standard-
bred horses at pasture, the mean plasma concentrations of phenylbutazone were
3.4 $\mu\text{g/ml}$ on day 2 and 3.5 $\mu\text{g/ml}$ on day 5. The range on day 5 was from 1.4
to 8.98 $\mu\text{g/ml}$ and the frequency distribution was log-normal. These data are
consistent with one horse in 1000 having a plasma level of 15.8 $\mu\text{g/ml}$ on day 5.

In a final experiment, the oral dosing schedule was administered to 62
Thoroughbred horses in training. Plasma concentrations on day 5 in these horses
averaged 5.3 $\mu\text{g/ml}$. The range was from 1.3 to 13.6 $\mu\text{g/ml}$ and the frequency
distribution was log-normal. Statistical projection of these values suggests
that following this oral dosing schedule in racing horses about one horse in
1000 will yield a plasma level of 23.5 $\mu\text{g/ml}$ of phenylbutazone 24 h after the
last dose.

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

INTRODUCTION

Phenylbutazone is a non-steroidal anti-inflammatory drug widely used in the treatment of musculo-skeletal disorders in horses. It is commonly used in the training of performance horses (Tobin, 1981).

Phenylbutazone has a plasma half-life in horses of between 3–11 h (Tobin, 1981). It is metabolized by horses to at least two metabolites, oxyphenbutazone and γ -hydroxyphenylbutazone, better known as the alcohol metabolite. Both phenylbutazone and its metabolites can be detected in equine blood or urine for 7 days or more after therapeutic dosing (Tobin, 1979). Further, calculations suggest that it takes at least 33–38 days for a dose of phenylbutazone to 'clear' or be totally eliminated from a horse (Tobin *et al.*, 1982).

Horses may be treated with phenylbutazone during training and then compete in races or other events. However, the regulators of such events may promulgate rules either prohibiting the presence of phenylbutazone in blood or urine samples, rules specifying that certain amounts not be exceeded, or rules specifying that the horse not be treated with phenylbutazone within a certain period prior to the event (Tobin, 1981).

A common restriction on medication with phenylbutazone is that horses should not be medicated with this agent within 24 h of post time. For example, the National Association of State Racing Commissioners (NASRC) guidelines state that no foreign substance shall be administered to a horse within 24 h prior to the scheduled post time (Tobin, 1981). Some states, however, may have no such restrictions, while still others may restrict administration for 3 days or longer (Tobin, 1981).

In general, proper regulatory control of drugs such as phenylbutazone depends on the analysis of blood levels of these drugs. Urinary concentrations of phenylbutazone and its metabolites are too variable for reliable use (Houston *et al.*, 1983). Based on these considerations, enforcement of a 24-h rule for phenylbutazone depends on the determination of plasma concentrations of phenylbutazone found in racing horses 24 h after the last dose of phenylbutazone.

These experiments were designed to determine blood concentration 'residues' of phenylbutazone and oxyphenbutazone consistent with no medication for 24 h prior to post time. The dosage schedules are based on the recommendation of the American Association of Equine Practitioners that 2 g/1000 lb of phenylbutazone i.v. or 'its oral equivalency' is an appropriate and 'non-abusive' dose of phenylbutazone for a horse (Harvey, 1983).

MATERIALS AND METHODS

Horses

Three groups of horses were used in these experiments. A pool of sixteen Thoroughbred and Standardbred horses from the Kentucky Equine Drug Program were used for most of the experiments. These horses are mares and geldings, between 400 and 500 kg in weight. This group of horses is described as 'Thoroughbred and Standardbred horses'.

Another group of fifteen half-bred horses used in reproductive physiology work was made available to us for one experiment. These were all mares of between 386 and 477 kg weight. This group is referred to as 'half-breds', although their breeding is unknown. Both Thoroughbred and Standardbred horses and the half-bred horses were at grass on adjacent pastures on the University of Kentucky farm.

The third group of horses dosed consisted of sixty-two Thoroughbred horses in training at the Kentucky Training Center and Keeneland during July–August 1983. These horses are referred to as 'Thoroughbreds in training'.

Drug administrations and sampling

In the initial experiments phenylbutazone tablets from Butler were used for the oral dosing (Butler Company, Columbus, OH) and Butler injectable phenylbutazone (Butler Company, Columbus, OH) was used for the i.v. administrations. Because of unexpectedly low blood concentrations obtained with use of these preparations, further experiments used Jen-Sal tablets (Jensen-Salsbery Laboratories, Kansas City, MO) and i.v. preparations (Jensen-Salsbery Laboratories,

Kansas City, MO), as in our previous work (Tobin, 1981).

Because individual trainers of the Thoroughbred horses used in these experiments did not wish to have their horses tubed, twenty-five animals in the training experiment were dosed with paste preparations and ten by balling gun.

All blood samples were obtained by jugular venipuncture and drawn into 20 ml green top vacutainer tubes (plasma; Vacutainer, Becton, Dickinson & Co., Rutherford, NJ) or red top tubes (serum; Vacutainer, Becton, Dickinson & Co., Rutherford, NJ). Plasma or serum was separated and stored frozen until ready for assay. All assays were carried out within 2 weeks of sampling.

Analytical methods

A Perkin-Elmer Series 4 microprocessor controlled HPLC equipped with a 6 μ l loop injector and a UV detector (254 nm) was used for all analyses in this study. Separations were done on a C-18, 5- μ m column equipped with a C-18, 30–40- μ m guard column.

Dichloromethane and methanol (Omnisolve, MCB Mfg Chemists, Inc., Cincinnati, OH), acetic acid (A.C.S., J. T. Baker Chemical Company, Phillipsburg, NJ), potassium phosphate (monobasic) (A.C.S., Mallinckrodt, Scientific Products, McGraw Park, IL), and sodium acetate trihydrate (HPLC grade, Fisher Scientific Company, Fairlawn, NJ) were all obtained from commercial sources. The pure standards of phenylbutazone and oxyphenbutazone were gifts from Ciba-Geigy Ltd, while hexestrol (Sigma Chemical Company, St Louis, MO) was obtained from a commercial source.

Eighty milligrams of hexestrol, the internal standard, was added to each 1 ml plasma or serum sample. A standard curve to include our working range was extracted and analysed simultaneously with our samples. Two millilitres of saturated potassium phosphate (monobasic) was added to a screw-cap culture tube containing each sample, adjusting the pH to approximately 4.0. Four millilitres of dichloromethane was added and the tubes mixed at room temperature for 5 min. All samples were then centrifuged at 1150 *g*, 5°C for 30 min. After centrifugation, the aqueous

layer was removed by aspiration and discarded. The organic layer was poured to a clean screw-cap culture tube and the contents reduced to approximately 1 ml by evaporation under a stream of pre-purified nitrogen. Samples were then individually evaporated to dryness, redissolved in 25 μ l of methanol, vortexed for 30 sec and held on ice until analysed.

Six microlitres of the redissolved plasma, serum or standard extract in methanol was chromatographed in the C-18 reverse-phase column using a mobile phase of methanol–0.01 M sodium acetate buffer (pH 4.0) (65:35). Using this solvent system oxyphenbutazone, phenylbutazone and hexestrol eluted as well separated, clearly defined peaks with the following retention times: oxyphenbutazone, 2.6 min; phenylbutazone, 4.56 min; and hexestrol, 6.96 min. Standard curves were expressed as μ g/ml *v.* the ratio of the drug peak area to the hexestrol peak area. The curves were fit with the best fit least squares regression line. The plasma or serum concentration was then extrapolated from this line and expressed as μ g/ml.

Statistical analysis

The frequency distributions of plasma and serum concentrations of phenylbutazone and oxyphenbutazone after oral-i.v. and i.v. administration were analysed for normality by using the Shapiro–Wilk's Statistic (Chay *et al.*, 1983). The best-fit transformation was determined and a distribution curve was estimated using the methods of moments based on the calculated mean and standard deviation. The probability of attaining a given plasma concentration of phenylbutazone and oxyphenbutazone after different dosing schedules was then calculated using this calculated mean and standard deviation.

All data are described as the range and mean of the values reported. For those distributions which could be logarithmically transformed to normal distributions, the standard deviations presented represent the antilog of the standard deviation of the logarithmically transformed data. Where the data did not normalize when log-transformed, the arithmetic standard deviation is presented.

RESULTS

In Experiment 1 (Fig. 1) we repeated the 1980 NASRC study, where fifteen horses were dosed with 8.8 mg/kg of phenylbutazone tables orally for 3 days, followed by 4.4 mg/kg i.v. on day 4. To this end, seventeen Thoroughbred and Standardbred horses were administered 8.8 mg/kg of Butler phenylbutazone tablets by balling gun for 3 days, followed by 4.4 mg/kg of Butler phenylbutazone i.v. on day 4. Serum samples were drawn from all horses on day 5, 24 h after the last dose of phenylbutazone. The serum concentrations of phenylbutazone and oxyphenbutazone found in these animals are represented by the hatched bars in Fig. 1. These data show mean blood concentrations of phenylbutazone of 0.65 $\mu\text{g}/\text{ml}$, about one-quarter of the concentrations observed in our experiments of 1980, represented by the dotted line in Fig. 1 (Tobin, 1981). We there-

fore performed Experiment 2, with seventeen Thoroughbred and Standardbred horses, using the same dosage schedule as Experiment 1. However, we elected to exactly duplicate the 1980 protocol in that the Jen-Sal tablets were ground up and administered by stomach tube. To follow the changes in blood levels in these horses, plasma samples were drawn every 24 h after dosing, with the last samples being drawn 24 h after the last drug administration on day 4. The results of Experiment 2 are presented in Figs 2 and 3.

Figure 2 shows the plasma concentrations of phenylbutazone observed in these horses after each day of dosing. By 24 h after the first dose plasma concentrations of phenylbutazone showed a mean concentration of 3.39 $\mu\text{g}/\text{ml}$, with a range of from 1.4 to 6.54 $\mu\text{g}/\text{ml}$. Plasma concentrations of phenylbutazone almost doubled after the next dose, to 6.11 $\mu\text{g}/\text{ml}$, and then showed a smaller increase to 7.36 $\mu\text{g}/\text{ml}$ after the third dose.

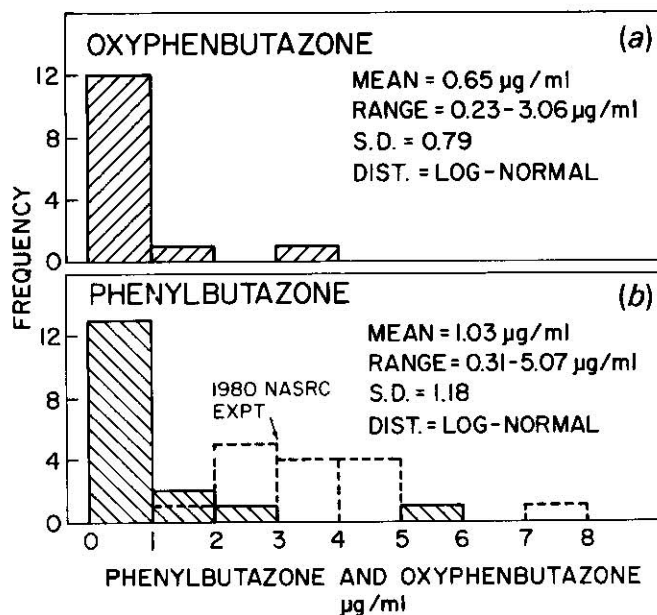


FIG. 1. Serum levels of phenylbutazone and oxyphenbutazone after oral and intravenous dosing with phenylbutazone. Experiment 1: seventeen Thoroughbred and Standardbred horses were dosed by balling gun with 8.8 mg/kg of Butler phenylbutazone tablets for 3 days followed by 4.4 mg/kg of Butler phenylbutazone i.v. on day 4. (a) The hatched bars show serum levels of oxyphenbutazone. (b) The hatched bars show serum levels of phenylbutazone. The dashed lines (---) show the population distribution of plasma levels of phenylbutazone after a similar dosage schedule of Jen-Sal phenylbutazone tablets in an earlier experiment.

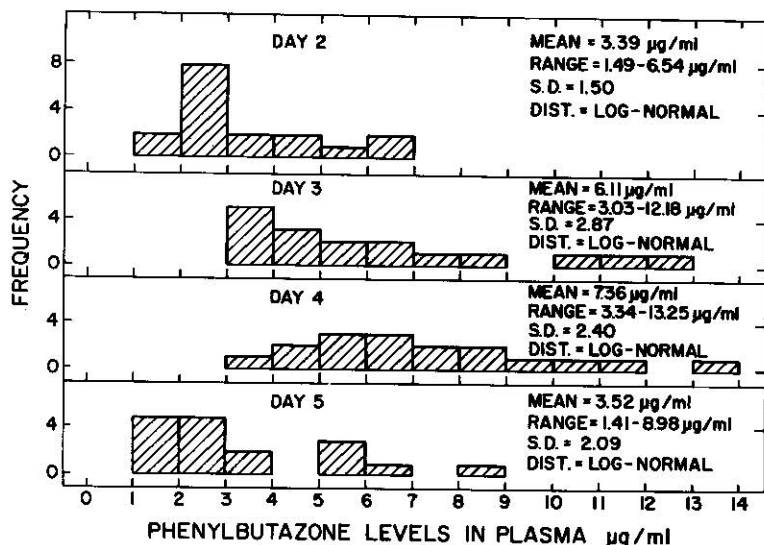


FIG. 2. Frequency distributions of plasma phenylbutazone levels in seventeen horses after oral and intravenous phenylbutazone. Experiment 2: seventeen Thoroughbred and Standardbred horses were dosed with phenylbutazone 8.8 mg/kg orally for 3 days and 4.4 mg/kg i.v. on the fourth day. All phenylbutazone was from Jen-Sal and the tablets which were powdered and administered by stomach tube. The hatched bars show frequency distributions of plasma levels of phenylbutazone at 24 h after each dosing.

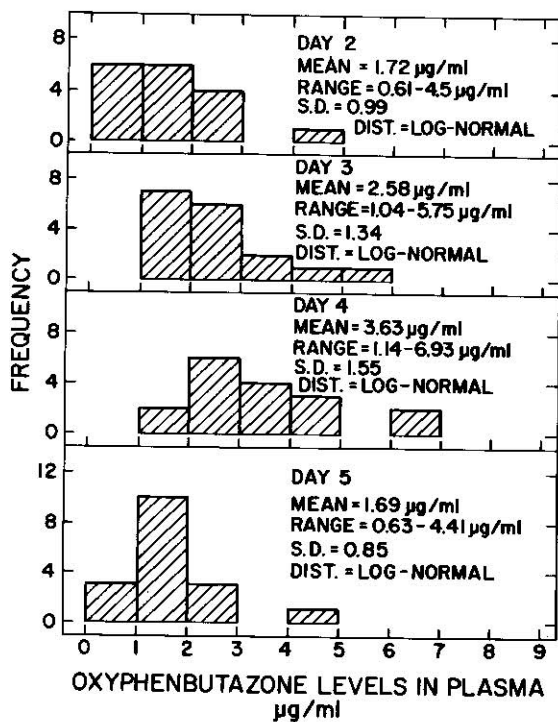


FIG. 3. Frequency distributions of plasma oxyphenbutazone levels in seventeen horses after 8.8 mg/kg orally for 3 days and 4.4 mg/kg i.v. on day 4. The vertical columns show frequency distribution of plasma levels of oxyphenbutazone in the horses of Experiment 2.

Following the i.v. dose on day 4, plasma concentrations of phenylbutazone fell to $3.52 \pm 2.08 \mu\text{g/ml}$. The results for day 5 are in good agreement with data contributed from this laboratory to the 1980 NASRC interlaboratory experiment (dotted line, Fig. 1). As shown in Table I, based on this data one may expect that one in 1000 horses dosed with this schedule of phenylbutazone under these conditions will yield a plasma concentration of phenylbutazone above $15.84 \mu\text{g/ml}$.

In Experiment 3, sixteen Thoroughbred and Standardbred horses were given phenylbutazone at 4.4 mg/kg i.v. for 4 days, with plasma samples taken at 24 h post-dosing on days 2, 3, 4 and 5. This dosage schedule is based on recent reports from the American Association of Equine Practitioners (Harvey, 1983), which suggest 2 g/1000 lb horse i.v. (approximately 4.4 mg/kg) as an approved dosage rate for phenylbutazone in racing horses. The results of these experiments are presented in Figs 4 and 5. As shown in Fig. 4,

TABLE I. Probability of a horse attaining a given plasma level of phenylbutazone after different dosing schedules

Schedule	Experiment no.	Mean*	90%	95%	99%	99.9%	Distribution
Oral/i.v.	2	3.52	6.06	7.35	10.60	15.84	Log-normal
i.v.	3	2.37	3.94	4.55	5.98	8.07	Log-normal
i.v.†	4	0.42	0.72	0.80	0.96	1.14	Normal
Oral/i.v.‡	5	5.32	9.15	11.07	15.84	23.46	Log-normal

*All blood levels, $\mu\text{g/ml}$ of phenylbutazone.

†Half-bred mares at pasture.

‡Thoroughbred horses in training, Keeneland.

Figure 3 shows the plasma concentrations of oxyphenbutazone found in these studies. After the first day the mean plasma concentration of oxyphenbutazone was $1.72 \mu\text{g/ml}$, but one horse, T-7, was an apparent 'outlier' with a plasma level of oxyphenbutazone about twice the mean level. Throughout this experiment, oxyphenbutazone concentrations in T-7 remained consistently high, and on the last day its plasma level of oxyphenbutazone, at about $4.5 \mu\text{g/ml}$, was three times the final mean plasma level of oxyphenbutazone of $1.69 \mu\text{g/ml}$. As shown in Table II, one may expect one horse in 1000 to show plasma concentrations of oxyphenbutazone above $6.55 \mu\text{g/ml}$.

the plasma concentrations of phenylbutazone found in these horses at 24 h after the first dose averaged $0.77 \mu\text{g/ml}$ and ranged from 0.4 to $1.57 \mu\text{g/ml}$. The distribution was log-normal, and the bulk of the values observed were less than $1 \mu\text{g/ml}$. With further dosing, the mean plasma concentrations increased daily from $1.44 \mu\text{g/ml}$ on day 3 to $1.79 \mu\text{g/ml}$ on day 4, and $2.55 \mu\text{g/ml}$ on day 5. After the fourth day of dosing, these samples ranged from 1.01 to $5.09 \mu\text{g/ml}$, and the shape of the distribution was log-normal. These data suggest that after 4 days of dosing one could expect that one in 1000 horses would yield a plasma concentration of $8.07 \mu\text{g/ml}$, as shown in Table I.

TABLE II. Probability of attaining a given plasma level of oxyphenbutazone after different dosing schedules

Schedule	Experiment no.	Mean*	90%	95%	99%	99.9%	Distribution
Oral/i.v.	2	1.52	2.80	3.32	4.59	6.55	Log-normal
i.v./i.v.	3	1.13	1.49	1.62	1.88	2.21	Log-normal
i.v./i.v.†	4	0.17	0.32	0.36	0.45	0.54	Log-normal
Oral/i.v.‡	5	3.20	7.11	8.91	13.64	21.76	Log-normal

*All blood levels, $\mu\text{g/ml}$ of oxyphenbutazone.

†Half-bred mares at pasture.

‡Thoroughbred horses in training, Keeneland.

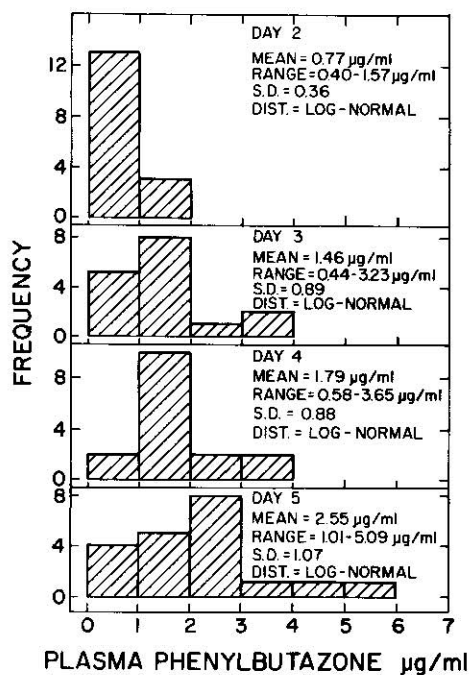


FIG. 4. Plasma levels of phenylbutazone in horses after intravenous dosing, Experiment 3: the vertical bars show plasma levels of phenylbutazone in sixteen horses 24 h after daily dosing with 4.4 mg/kg of phenylbutazone intravenously in Thoroughbred and Standardbred horses.

Plasma concentrations of oxyphenbutazone in these horses were always less than the corresponding levels of phenylbutazone (Fig. 5). After the first day of dosing, plasma concentrations of oxyphenbutazone averaged 0.51 µg/ml, with a range from 0.22 to 1.14 µg/ml. The mean concentrations increased to 1.16 µg/ml on day 5, with a range from 0.75 to 1.57 µg/ml. In only one horse was a plasma concentration of oxyphenbutazone more than 2.0 µg/ml observed, and this plasma concentration was higher (2.69 µg/ml) after the second dose than after the third and fourth doses (Fig. 5).

In an attempt to extend the number of horses in this i.v. study, we performed Experiment 4. In this experiment we dosed half-bred horses at pasture with 4.4 mg/kg of Jen-Sal phenylbutazone i.v. for 2 days. Plasma samples were drawn at 24 h post-dosing on days 2 and 3, and the data are presented in Figs 6 and 7. As shown in these figures, the plasma concentrations of phenylbutazone and oxy-

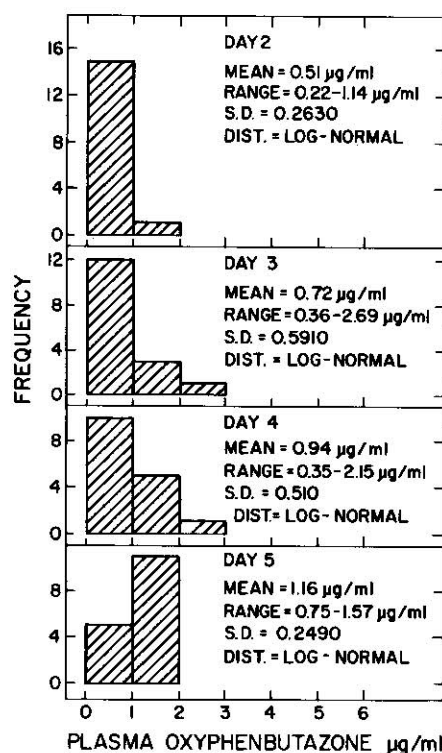


FIG. 5. Plasma levels of oxyphenbutazone after intravenous phenylbutazone. The vertical bars show plasma levels of oxyphenbutazone in sixteen horses 24 h after daily dosing with 4.4 mg/kg of phenylbutazone in Thoroughbred and Standardbred horses in Experiment 3.

phenbutazone observed in these half-bred mares were about one-third the plasma concentrations observed in our Thoroughbred and Standardbred horses. The experiment may suggest differences in the disposition or metabolism of phenylbutazone between half-bred and Thoroughbred or Standardbred horses. The data suggest that any determination of blood concentration residues in horses to be applied to Thoroughbred racing should, properly, be developed in Thoroughbred horses conditioned for racing. We, therefore, performed Experiment 5, in which sixty-two Thoroughbred horses in training were dosed with 4 g/horse of phenylbutazone orally for 3 days, and then followed by 2 g/horse of Jen-Sal phenylbutazone i.v. on day 4. Plasma samples were taken at 24 h after dosing on day 4, and analysed for phenylbutazone and oxyphenbutazone.

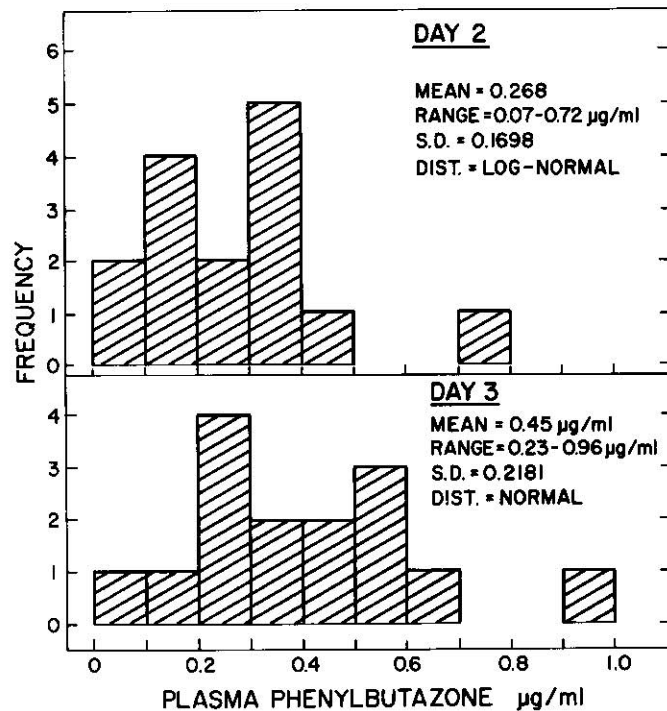


FIG. 6. Plasma levels of phenylbutazone after i.v. dosing in half-bred ponies. Experiment 4: the vertical bars show plasma levels of phenylbutazone in horses dosed with 4.4 mg/kg of phenylbutazone for 2 days.

Three different treatment methods were used because individual trainers placed restrictions on the dosing methods. Twenty-seven horses were tube-dosed, twenty-five horses were treated with paste preparations (Jensen-Salsbery Laboratories, Kansas City, MO) and ten horses were given the tablets by balling gun. (Jensen-Salsbery Laboratories, Kansas City, MO) The results obtained in Experiment 5 are presented in Figs 8 and 9.

In the overall experiment, the mean plasma concentrations observed in these experiments was 5.32 µg/ml with a range from 1.28 to 13.63 µg/ml. The distribution was log-normal and the statistical projections from these data are given in Table I. They show that one in 1000 horses treated with this regimen of phenylbutazone may be expected to have plasma levels of phenylbutazone above 23.5 µg/ml at 24 h after the last dose.

The plasma concentrations of oxyphenbutazone found in these horses are shown in Fig. 9. Overall, the concentrations of oxy-

phenbutazone in these horses at the end of the experiment was 3.85 µg/ml, with a range from 0.83 to 13.72 µg/ml. As with phenylbutazone, the distribution was clearly log-normal. A statistical projection of this data showed that one in 1000 horses may be expected to show a blood level of 21.76 µg/ml (Table II) at 24 h after the last dose.

In parallel with our Experiment 5, Dr Larry Soma and his associates treated horses in training at Keystone Racetrack with 4.4 mg/kg of Jen-Sal phenylbutazone i.v. for 4 days and drew plasma samples from these horses on day 5, 24 h after the last dose of phenylbutazone. Aliquots of these samples were analysed in our laboratory. As analysed in our laboratory, phenylbutazone concentrations in these samples ranged from 1.50 to 9.88 µg/ml with a mean plasma level of 4.75 µg/ml. These plasma values were log-normally distributed.

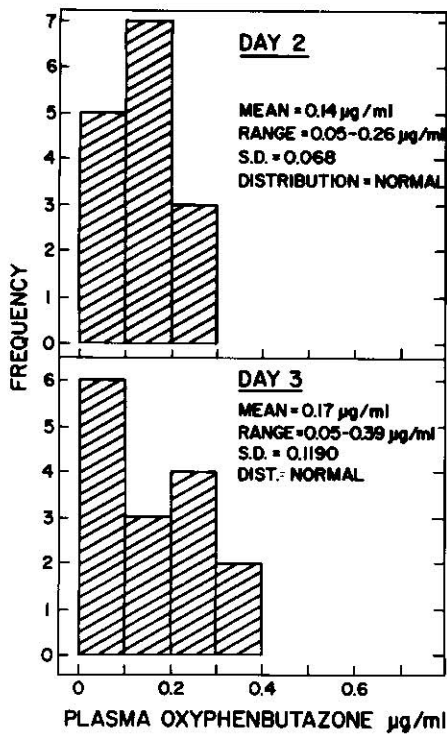


FIG. 7. Plasma levels of oxyphenbutazone in half-bred ponies dosed with i.v. phenylbutazone. Experiment 4: the vertical bars show plasma levels of oxyphenbutazone in half-bred ponies dosed with 4.4 mg/kg of phenylbutazone for 2 days.

DISCUSSION

The objective of these experiments was to determine plasma concentrations or residues of phenylbutazone consistent with a no-race day medication or a 24-h rule. The dosage schedules were those recommended by the American Association of Equine Practitioners in their 1983 Newsletter. This group suggested that a dose of phenylbutazone of '2 g i.v. or its oral equivalency, at 24-h intervals on a daily basis, where necessary, the final administration allowed coming 24 h before the race' (Harvey, 1983) is an appropriate i.v. dose. Similarly, in 1980, members of this organization suggested that 4 g orally for 3 days, followed by 2 g i.v. on day 4, was an appropriate oral-i.v. dosage schedule for the horse (Tobin, 1981).

Based on these considerations, we dosed seventeen horses orally with 4 g of Butler phenylbutazone (tablets)/1000 lb for 3 days and followed these doses with 2 g/1000 lb i.v. on the fourth day (Experiment 1). When we analysed the levels obtained after this experiment, we were surprised to find serum levels of phenylbutazone substantially less than those found in earlier experiments from this laboratory. The causes of this substantial

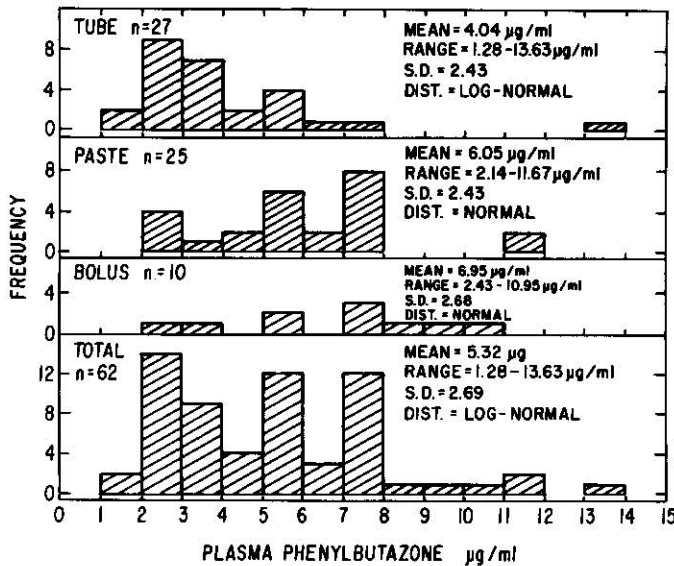


FIG. 8. Frequency distribution of phenylbutazone levels after oral and intravenous dosing. Experiment 5: The vertical bars show plasma levels of phenylbutazone in horses after dosing with 4 g/horse of phenylbutazone orally and 2 g/horse intravenously to Thoroughbred horses in training.

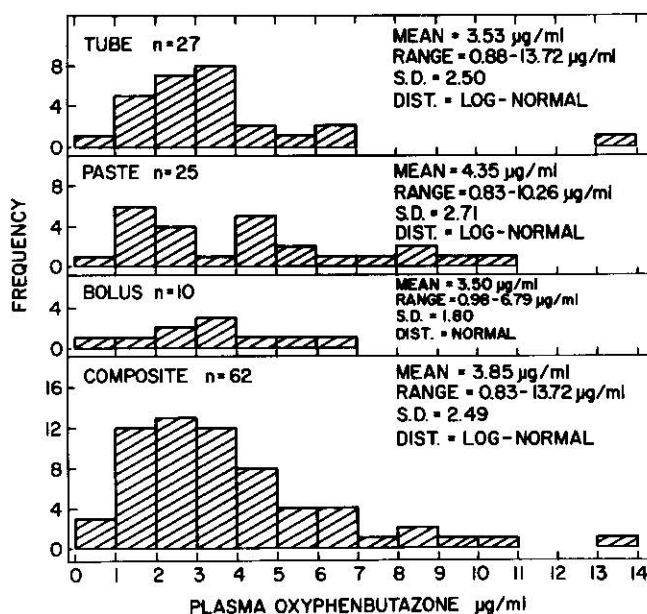


FIG. 9. Frequency distribution of oxyphenbutazone levels after oral and intravenous dosing. The vertical bars show plasma levels of oxyphenbutazone in the horses in Experiment 5.

discrepancy were not immediately apparent to us and we repeated this experiment with the same Jen-Sal phenylbutazone preparations and mode of administration used in our 1980 experiments.

The results of Experiment 2 (Figs 2 and 3) were quite consistent with the earlier work from our laboratory. The mean plasma concentration of phenylbutazone was 3.52 µg/ml, with a range from 1.41 to 8.98. These results are essentially indistinguishable from results reported in our experiments of 3 years ago, in which the mean plasma concentration was 3.50 µg/ml and the range 1.90–7.50 µg/ml.

The source of the difference in the results between Experiments 1 and 2 is unknown but appears unlikely to be found in the experimental animals. Exactly the same horses were used in both the 1983 Butler (Experiment 1) and the 1983 Jen-Sal (Experiment 2) experiments which were carried out within weeks of each other and where large differences were found. In comparison, the 1980 and 1983 Jen-Sal experiments were done 3 years apart and only about eight of the 1980 horses were present in the second herd used in the 1983 experiment. Despite this substantial

change in the horse herd, the results of the two Jen-Sal experiments carried out 3 years apart were almost identical. These observations would appear to favour interpretations that the source of the differences between the Butler experiment and the Jen-Sal experiment more likely lies with differences between the drug preparations used or their modes of administration rather than with the experimental horses.

Having determined the plasma concentrations found after oral dosing with phenylbutazone, we next determined the plasma concentrations of this agent after dosing with 4.4 mg/kg phenylbutazone i.v. for 4 days (Experiment 3). The results of this experiment are in good general agreement with the data of Fig. 2 and the results of the NASRC Veterinary Chemist's study of phenylbutazone concentrations in horses racing in Florida (Soma *et al.*, 1983). In the Florida study, the mean serum concentrations of phenylbutazone 24 h after 4.4 mg/kg i.v. was 0.79 µg/ml, with no serum concentrations above 2.0 µg/ml. Similarly, in our Experiment 3, (Fig. 4), the mean plasma concentration was 0.77 µg/ml, with no plasma concentrations above 2.0 µg/ml. The agreement between these two sets

of data is good. It must be kept in mind, however, that because the Florida data were based on serum concentrations, they may not accurately reflect the actual plasma concentrations of phenylbutazone present in those horses.

Repeated dosing with phenylbutazone showed a clear tendency for the plasma concentrations of phenylbutazone to increase. After the second dose, the plasma concentrations of phenylbutazone were 1.46 $\mu\text{g/ml}$, almost double those observed after the first dose. Although the apparent increase in the mean concentrations was somewhat less (1.79 $\mu\text{g/ml}$) after the third dose, the mean plasma concentration observed after the fourth dose was about 3.5 times the residual concentration of phenylbutazone after the first dose. The data show a tendency for phenylbutazone to accumulate with repeated dosing at this level for at least 4 days.

When this experiment was repeated in half-bred horses, the plasma concentrations of phenylbutazone observed were only one-third of those observed in the Thoroughbred and Standardbred horses (Fig. 6). One possible interpretation is that phenylbutazone is metabolized more rapidly in half-bred horses than in Thoroughbreds. In view of the fact that phenylbutazone is thought to be more toxic in ponies than in Thoroughbred horses (Soma *et al.*, 1981), this data may suggest that it is a metabolite of phenylbutazone rather than phenylbutazone itself which is the toxic species.

As a final stage in this experiment (Experiment 5), we dosed sixty-two horses in training with the oral/i.v. dosing schedule. These experiments were performed in parallel with experiments being conducted by Dr Larry Soma of the University of Pennsylvania at Keystone Racetrack, where horses in training were dosed with 2 g/1000 lb (4.4 mg/kg) i.v. for 4 days.

It was important to perform these experiments in horses in training. The dosages administered, as g/horse rather than mg/kg, more nearly represent the situation as it occurs on the track. Further, the age, condition and feeding schedule of these horses more closely parallel the actual racing situation. Finally, the number of horses (sixty-two) available to us was much greater than

the number of Thoroughbred horses available in our research programme.

The twenty-seven horses tube-dosed with Jen-Sal tablets showed plasma concentrations essentially indistinguishable from the data obtained in the experiment of Fig. 2 and the earlier NASRC experiment of 1980. The mean plasma concentration was 4.04 $\mu\text{g/ml}$, the range was 1–7 $\mu\text{g/ml}$, with an 'outlier' at about 13.5 $\mu\text{g/ml}$. This outlier turned out to be the highest level of phenylbutazone found in the whole experiment, with a plasma concentration of phenylbutazone about six times that of the median plasma concentration in this experiment.

Both the paste and the bolus groups gave slightly higher mean plasma concentrations than those observed in the tube-dosed horses and the distributions were normally distributed. Although it is likely that the slightly higher plasma residues are due to slower dissolution and absorption of the bolus and paste preparations, the reason for the normal distribution of this data is not clear.

These data compare very well with the data from Dr Soma's Keystone study analysed in our laboratory. After i.v. dosing with 2 g/horse in the Thoroughbreds racing at Keystone Racetrack, analysis of the data showed a log-normal distribution with a mean level of 4.75 $\mu\text{g/ml}$, and a range from 1.50 to 9.88 $\mu\text{g/ml}$. These data, with the exception of the single 'outlier', are actually lower than the data obtained with the twenty-seven tube-dosed horses presented in Fig. 8. The experiment suggests that under racetrack conditions, there is not likely to be any significant difference between the plasma levels observed after oral or i.v. dosing with these schedules. The experiment therefore suggests that the oral schedule used in these experiments is very close to 'oral equivalency' of the i.v. schedule recommended by the AAEP.

The data reported in these studies clearly demonstrate a number of fundamental facts about phenylbutazone kinetics. At these clinically recommended doses, one can expect blood or plasma levels of the drug to accumulate for up to 4 days. Second, in general, the pattern of the population distribution is log-normal, with the apparent spread of the data increasing as the number

of days dosed increased. Finally, the data show that using standard statistical analyses on plasma levels of drugs is incorrect. This is because most standard statistical analyses assumed normal distributions, while plasma and urine level data are most likely distributed log-normally (Chay, 1983; Tobin, 1981).

As a practical matter, therefore, these data show that dosing with the oral dosing schedule used in these experiments gives rise to plasma levels of drugs very close to those found after i.v. administration of half the dose. Although the results of the oral dosing schedule in horses in training are close to the results obtained in stabled Thoroughbreds and Standardbreds, the ranges obtained from the track studies are higher and these differences can have substantial regulatory significance. When these experiments were performed in horses other than Thoroughbreds in training, the differences were substantial. The experiments reported here also suggest that measuring serum concentrations of phenylbutazone may underestimate the actual plasma concentrations of phenylbutazone.

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