

# Plasma and serum concentrations of phenylbutazone and oxyphenbutazone in racing Thoroughbreds 24 hours after treatment with various dosage regimens

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## SUMMARY

The plasma and serum concentrations of phenylbutazone (PBZ) and oxyphenbutazone were measured in 158 Thoroughbred horses after various doses of PBZ were given. All horses were competing or training at racetracks in various parts of the country. All horses used in the study had not been given PBZ 24 hours before they were placed on a specific dosage schedule. Samples were collected 24 hours after the last PBZ administration. Four grams of PBZ were given daily by stomach tube, paste, or tablet for 3 days. On day 4, 24 hours before sample collection, an IV dose of 2 g of PBZ was given, regardless of the dose and method of administration. The 24-hour PBZ plasma concentrations were 3.51, 6.13, and 6.40 µg/ml, respectively. After 2 g of PBZ was administered IV daily for 4 days, the plasma PBZ concentration was 4.16 g/ml; after a single 2-g IV administration, the serum concentration was 0.87 g/ml. Concentrations of oxyphenbutazone were

3.35 (stomach tube), 4.29 (paste), 3.60 (tablet), 3.65 (4-day IV), and 1.11 g/ml (single IV). A significant relationship was not found between the serum and the urinary concentrations at this 24-hour measurement. Split samples sent to various laboratories confirmed the stability of high-performance liquid chromatography as a method of analysis.

lowed by an IV dose (study 2), and daily IV doses (study 3).

**Study 1**—A total of 53 Thoroughbred race horses competing at Gulfstream Race Track, Miami, Fla, were studied. The majority of the horses were not given PBZ for 120 hours before the start of the study, and all the horses had not been given PBZ for 24 hours before the IV dose. Each horse was given 2 g of PBZ IV 24 hours before a race that coincided with the racing schedule of the horse. Urine and blood were collected immediately after the race in the detention facilities. Blood samples were collected in serum separator vacutainer tubes,\* allowed to clot, centrifuged, and the serum was divided into 3 aliquots that were frozen and shipped to the laboratories of the University of Kentucky, Lexington, Ohio State University, Columbus, and of the Illinois Racing Board, Elgin, respectively, for analysis. Urine was analyzed by the laboratory of the Florida Division of Pari-mutuel Wagering, Miami.

Phenylbutazone (PBZ) is a nonsteroidal anti-inflammatory drug with antipyretic and analgesic activity. It has been used extensively in horses for the treatment of bone and joint inflammation, laminitis, and soft tissue inflammation.<sup>1-6</sup>

The 2 identified metabolites of PBZ are oxyphenbutazone (OPBZ) and the  $\gamma$  alcohol metabolite (OHPBZ). The plasma and urinary concentrations and the half-life of PBZ have been reported in experimental horses after administration of various doses.<sup>7-13</sup> However, plasma and serum concentrations in racing and training horses have not been reported. The purpose of the present study was to determine the plasma and serum concentrations of PBZ and its metabolite OPBZ in racing Thoroughbred horses 24 hours after various dosages had been given and to determine whether a relationship exists between blood and urine concentrations of PBZ, OPBZ, and OHPBZ on race day.

**Study 2**—A total of 62 Thoroughbred horses racing and training at Keeneland Training Center, Lexington, Ky, were studied. The horses were given 4 g of PBZ orally for 3 consecutive days followed by 2 g of PBZ IV on the 4th day. Three oral methods of drug administration were used: stomach tube, oral paste, and tablet. For 27 horses, PBZ tablets were crushed, suspended in water, and given via a stomach tube. Twenty-five horses were given the paste orally and 10 were given the compressed tablet using a balling gun. Drugs were administered orally after the morning feeding and training schedules. Blood samples were collected in potassium oxalate-sodium fluoride tubes,\* centrifuged, the plasma was frozen, and aliquots of each sample were shipped to the laboratories at Ohio, Kentucky, and the Pennsylvania Horse Testing Laboratory, West Chester University, for analysis. As in study 1, there was a

## Materials and Methods

Three separate studies were conducted that included a single dose of PBZ administered IV (study 1), daily oral doses fol-

\* Becton, Dickinson & Co. Rutherford, NJ.

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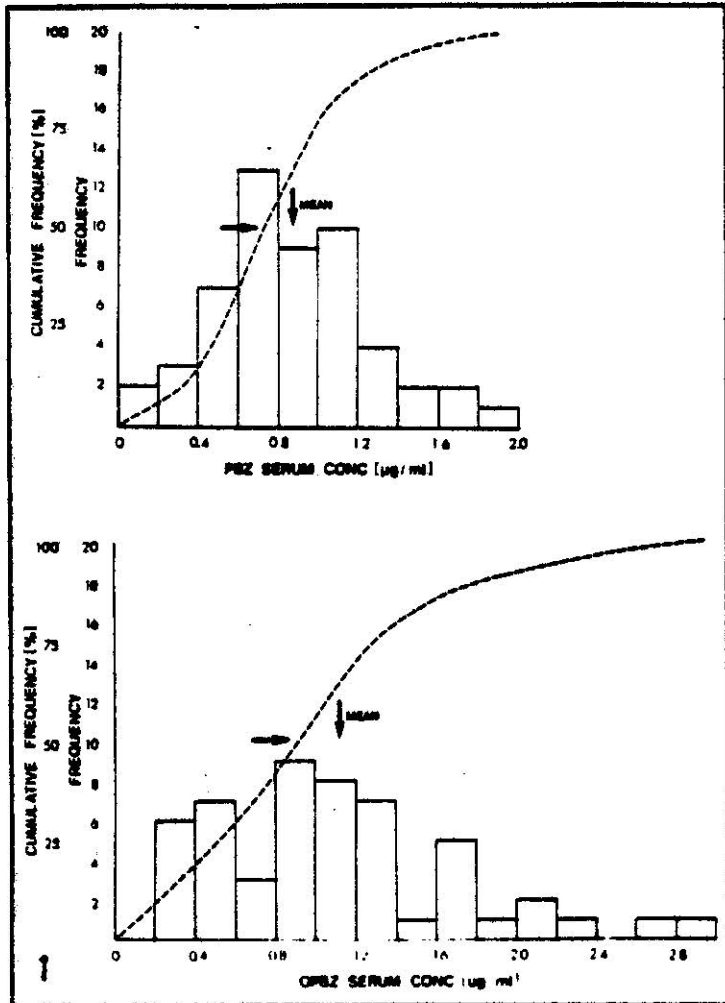


Fig 1—The frequency distribution of serum PBZ and OPBZ concentrations (conc) 24 hours after a single iv dose of PBZ (study 1). The horizontal axis = the range of serum conc, and the vertical axis = frequency of occurrence (No. of horses involved, is shown by the bar graph) and the cumulative frequency (percentage of horses involved, is shown by the dashed line). The horizontal arrow indicated the median, and the vertical arrow is the location of the mean concentration.

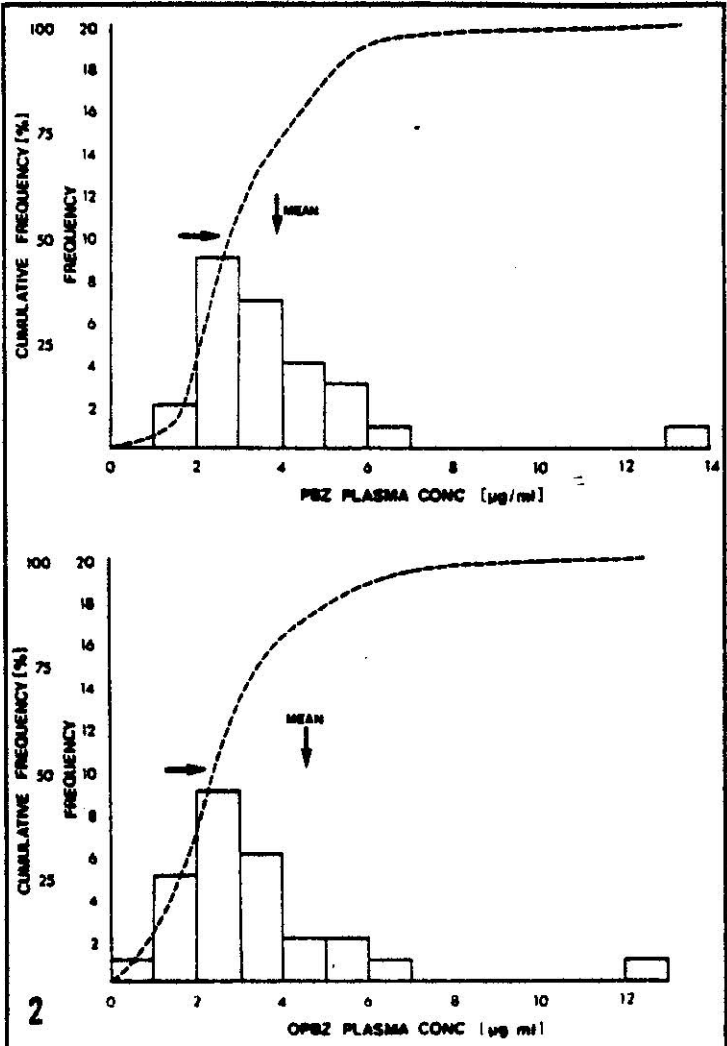


Fig 2—The frequency distribution of plasma PBZ and OPBZ concentrations (conc) after the stomach tube administration of PBZ (study 2). The horizontal axis = the range of plasma concentrations, and the vertical axis = the frequency of occurrences and the cumulative frequency percentage (see Fig 1 for key). The distribution of PBZ is significantly skewed to the right as is the mean concentration.

24-hour period before the oral dose schedule was started.

**Study 3**—A total of 43 Thoroughbred race horses competing and training at Keystone Race Track, Bensalem, Pa, were studied. Each horse was given 2 g of PBZ iv daily for 4 consecutive days. Blood samples were collected in oxalated tubes 24 hours after the last iv administration. As with the other studies, all horses had not been given PBZ at least 24 hours before the study began. Samples were frozen and aliquots of each sample were sent to the Ohio, Kentucky, and West Chester laboratories.

**Statistical analysis**—The serum or plasma concentrations of PBZ and OPBZ from each laboratory were considered replicates and the concentration for each horse was the mean of the 3 concentrations reported by the 3 laboratories. The mean serum or plasma concentration for each study was the mean of the horses' average concentration. Analysis of var-

iance was used for the comparison of PBZ and OPBZ serum concentrations from the 3 laboratories and the Bonferroni *t* statistic was used for multiple comparisons of means.<sup>14</sup> Comparisons of the difference between various participating laboratories were done in studies 1, 2, and 3. Two laboratories (Ohio and Kentucky) were participants in all analyses. Linear regression analysis using the least-squares method was used to indicate the trend of relationships. Histogram plots of plasma or serum concentrations were analyzed for skewness.<sup>16,18</sup> Concentrations that were not normally distributed were logarithmically transformed (geometric mean), and the probability estimates (95% confidence limits) were calculated from the geometric mean. The use of the SD or SEM to express the normal deviation of each study has little value in data that do not have a normal distribution; thus, for conformity, all plasma and serum concentrations in the tables were expressed as means with the

95% confidence limits. Nonparametric statistics (Wilcoxon 2-sample test) was used to compare the means of the groups in studies 2 and 3 that were not normally distributed. For the comparison of means, the Student's *t* test was used to compare the OHPBZ data that were normally distributed. Values were considered significantly different when *P* < 0.05.

**Analytical methods**—All participating laboratories used high-performance liquid chromatography (HPLC) as the primary analytical method. Each laboratory's technique varied slightly, but followed methods that have been described.<sup>17,b</sup> All samples were analyzed at a spectrophotometric wavelength of 239 to 254 nm. Three laboratories (Ken-

<sup>b</sup> National Association State Racing Commissioners Quality Assurance Program, Schools of Veterinary Medicine, Ohio State University, Columbus (R. Sams), and Cornell University, Ithaca, NY, (G. Maylin).

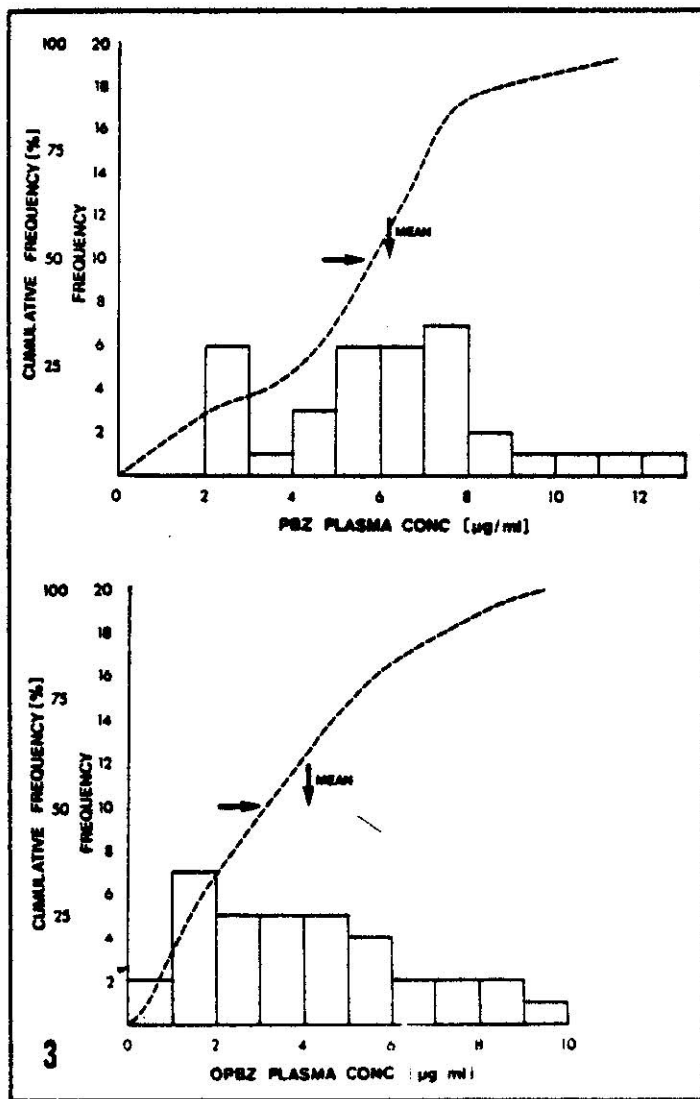


Fig 3—The frequency distribution of plasma PBZ and OPBZ concentrations (conc) after the bolus and paste administration of PBZ (study 2). The distribution is almost flat, indicating the greater variability after this method of administration and the inclusion of 2 means (see Fig 1 for key).

tucky, Ohio, and West Chester, Pa) used an extraction procedure to separate the PBZ and OPBZ from the plasma or serum sample; 1 laboratory (Illinois) precipitated the plasma proteins and, after centrifugation, injected a portion of the supernatant solution. All laboratories used internal standards (hexestrol, cycloprofen, phenolphthalein, or feprazone). All PBZ dosage forms were obtained from the same source,\* with the exception of study 1.<sup>4</sup>

## Results

The 24-hour mean serum and plasma concentrations and the 95% confidence limits of PBZ and OPBZ for all studies were summarized (Tables 1 and 2). The frequency distribu-

\* Jensen-Salsbury Laboratories, Kansas City, Kan.  
<sup>4</sup> Triple Crown Pharmaceuticals, Fort Collins, Colo.

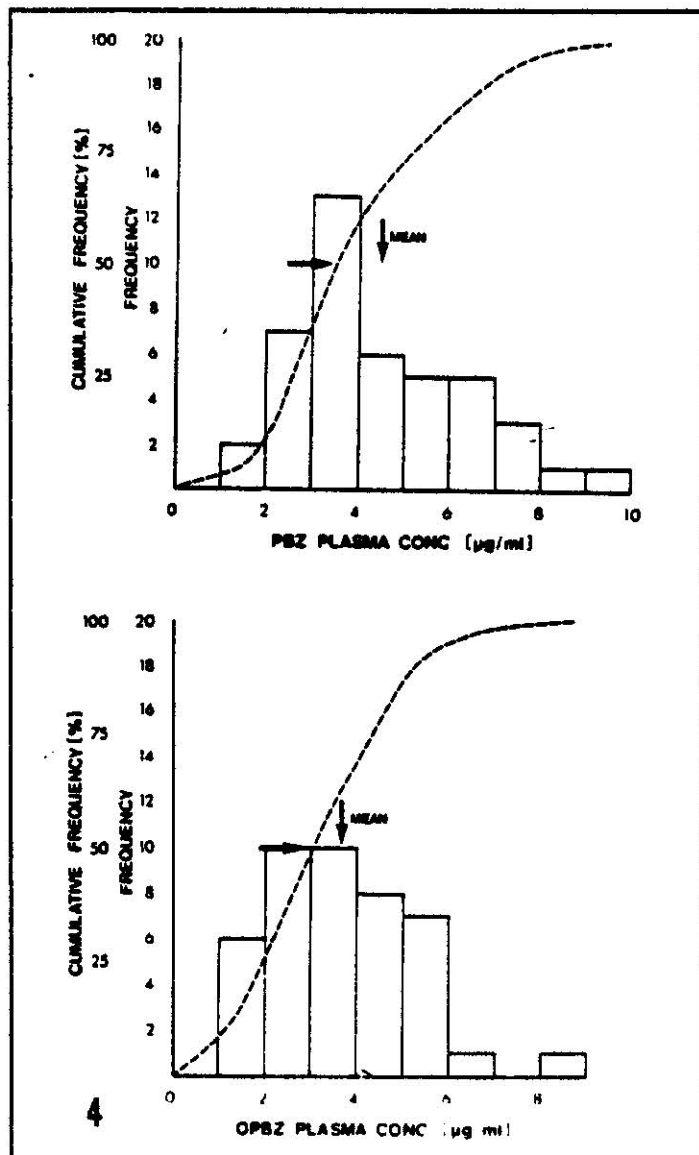


Fig 4—The frequency distribution of the plasma PBZ and OPBZ concentrations (conc) after daily iv administrations of PBZ (study 3). The horizontal axis = the range of plasma concentrations and the vertical axis = the frequency of occurrence and the cumulative frequency percentage (see Fig 1 for key). The distribution of PBZ is significantly skewed to the right as is the mean concentration.

TABLE 1—Phenylbutazone mean concentrations, 95% confidence limits, absolute range, and percentage of variation around the mean after various doses and routes of administration of PBZ

Study location	Study No.	No. of horses	Conc (µg/ml)	95% Conf limits (µg/ml)	Range (µg/ml)	Coefficient of variation (%)	Dose and route of PBZ
Gulfstream	1	53	0.87*	0.82 to 0.92	0.13 to 1.90	44.4	2 g (iv)
Keeneland	2	27	3.51†	2.95 to 4.17	1.58 to 13.20	56.9	4 g (tube, tube, tube) 2 g (iv)
Keeneland	2	25	6.13†	5.05 to 7.20	2.21 to 12.22	42.0	4 g (paste, paste, paste) 2 g (iv)
Keeneland	2	10	6.40	4.52 to 8.27	2.29 to 10.03	39.5	4 g (bolus, bolus, bolus) 2 g (iv)
Keystone	3	43	4.16†	3.67 to 4.74	1.43 to 9.57	42.2	2 g (iv, iv, iv, iv)

\* = Serum samples. † = Logarithmic transformed mean.  
 Conc = Mean PBZ concentrations; Conf = confidence.

tions of PBZ and OPBZ for study 1 were determined (Fig 1). The OPBZ serum concentration for study 1 was 0.44 µg/ml with 95% confidence limits of 0.27 to 0.57.

The method of oral administration

(study 2) influenced the final plasma concentrations of PBZ (Table 1). At 24 hours after the 3 days of oral administration by stomach tube, paste, and baling gun with a single iv dose on day 4, plasma concentra-

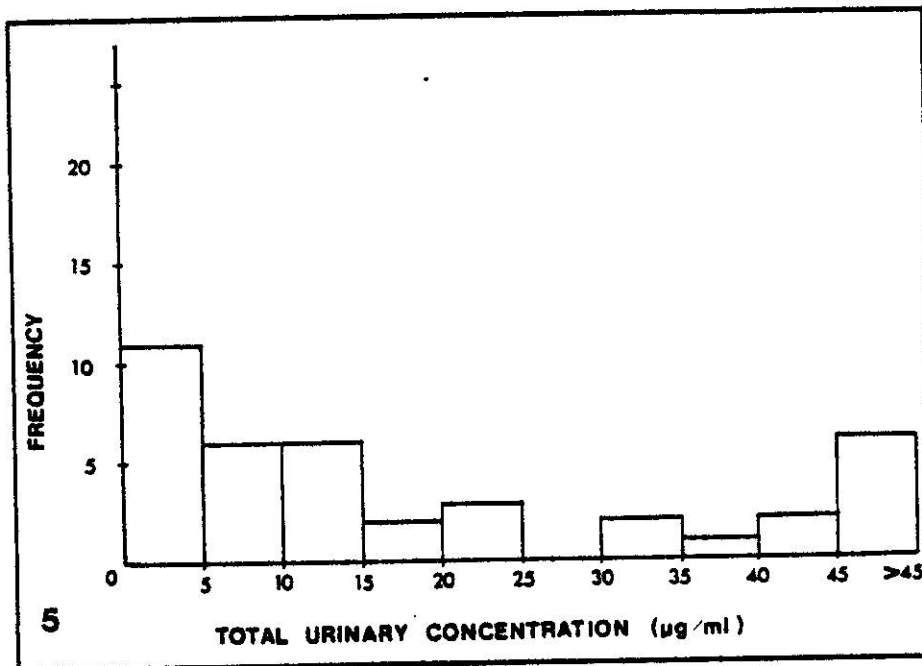


Fig 5—The frequency distribution of total urine concentrations of PBZ, OPBZ, and OHPBZ (study 1). The distribution is flat, indicating the wide range of values measured after a race. The lowest value was 0.8 µg/ml and the highest was 95.8 µg/ml.

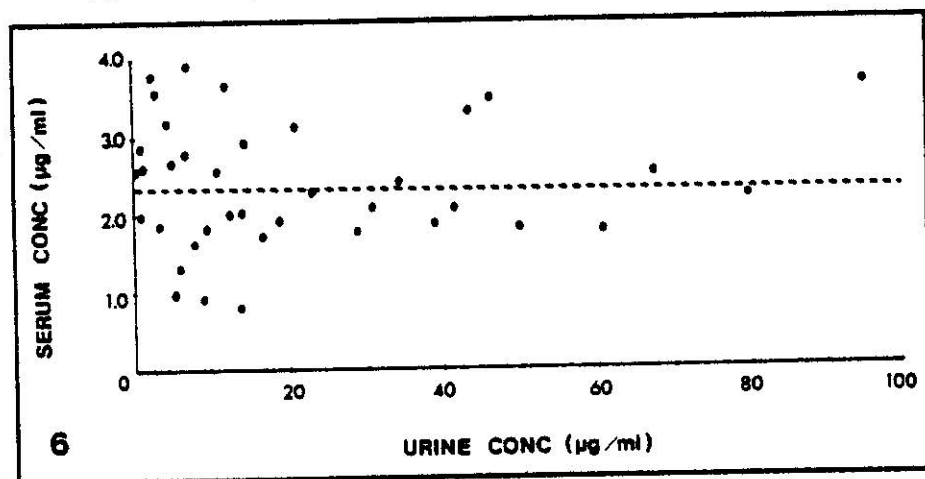


Fig 6—Relationship between the total serum and urinary concentrations (conc) of PBZ, OPBZ, and OHPBZ. The scattergram with a best fit line parallel to the X axis and indicates no relationship between the urine and plasma concentrations when the 2 values are compared 24 hours after a dose of PBZ ( $r = 0.02$ ). The plasma concentration cannot be predicted from the measurement of the concentrations measured in urine.

TABLE 2—Oxyphenbutazone mean concentrations, 95% confidence limits, absolute range, and percentage variation around the mean after various doses and routes of administration of OPBZ

Study location	Study No.	No. of horses	Conc (µg/ml)	95% Conf limits (µg/ml)	Range (µg/ml)	Coefficient of variation (%)	Dose and route of OPBZ
Gulfstream	1	53	1.11*	0.94 to 1.28	0.24 to 2.82	55.2	2 g (iv)
Keeneland	2	27	3.35	2.43 to 4.27	0.87 to 12.56	68.1	4 g (tube, tube, tube) 2 g (iv)
Keeneland	2	25	4.29	3.18 to 5.40	0.98 to 10.00	61.4	4 g (paste, paste, paste) 2 g (iv)
Keeneland	2	10	3.60	2.21 to 4.99	0.96 to 6.83	51.9	4 g (bolus, bolus, bolus) 2 g (iv)
Keystone	3	43	3.65	3.16 to 4.14	1.14 to 8.10	43.1	2 g (iv, iv, iv, iv)

\* = Serum samples.

Conc = mean OPBZ concentrations; Conf = confidence.

tions for the 3 methods of oral administration were 3.51, 6.13, and 6.40 µg/ml, respectively, and the concentrations of OPBZ were 3.35, 4.29, and 3.60 µg/ml, respectively.

Comparison of the plasma PBZ concentrations for the 3 dosing methods indicated significant differences ( $P < 0.001$ ) when tube administration was compared with the

tablet and paste administrations; a significant difference was not observed between the paste and the tablet. The lowest concentrations were found after stomach tube administration and the highest after the tablet regimen. There were no significant differences between the plasma concentrations of OPBZ. The frequency distributions of the plasma PBZ and OPBZ concentrations for tube administration (Fig 2) and for the paste and bolus administration combined (Fig 3) were determined.

The plasma concentrations of PBZ and OPBZ after daily IV-administration of PBZ (study 3) were determined (Table 1). There were no significant differences between the plasma concentration of the tubed horses in study 2 and the IV-dosed horses in study 3, but there was a significant difference ( $P < 0.01$ ) between IV-dosed horses and the paste-and bolus-dosed horses ( $P < 0.01$ ). The frequency distributions of the plasma PBZ and OPBZ concentrations of study 3 were determined (Fig 4).

Because distributions of PBZ concentrations were generally skewed to the right, the serum and plasma concentrations were examined for significant skewness. The serum concentrations for study 1 were not skewed significantly, although there were a number of higher values trailing to the right. The multiple IV injections (study 3) and stomach tube administration (study 2) had significant skewness to the right ( $P < 0.05$  and  $P < 0.001$ , respectively), which was not significant after logarithmic transformation. The paste and bolus plasma PBZ concentrations were basically flat. The combination of paste and bolus data produced a distribution that reflected the 2 means (Fig 3).

*Comparisons of serum and urine concentrations on race day*—The collection of serum and urine in the detention barn after a race (study 1) allowed the determination of the relationship between serum and urine concentrations. The total urine concentrations of PBZ, OPBZ, and OHPBZ were  $20.0 \pm 22.8$  µg/ml with a coefficient of variation of 113.6%. The total urine concentrations of PBZ and of the 2 metabolites were 1.4 µg/ml to 95.0 µg/ml (Fig 5). This high coefficient of variation reflected the wide range of concentrations measured.

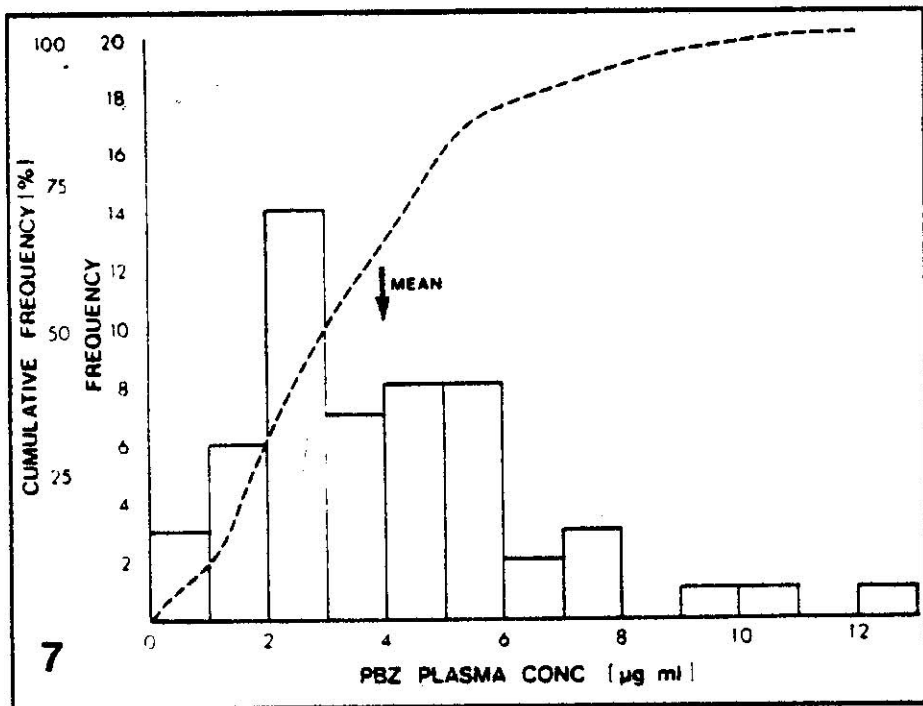


Fig 7—The frequency distribution of the plasma PBZ concentrations (conc) of untrained horses administered PBZ orally for 3 days and iv on the 4th day.\* The mean was similar to the tubed horses (study 2), but with a greater variability (see Fig 1 for key).

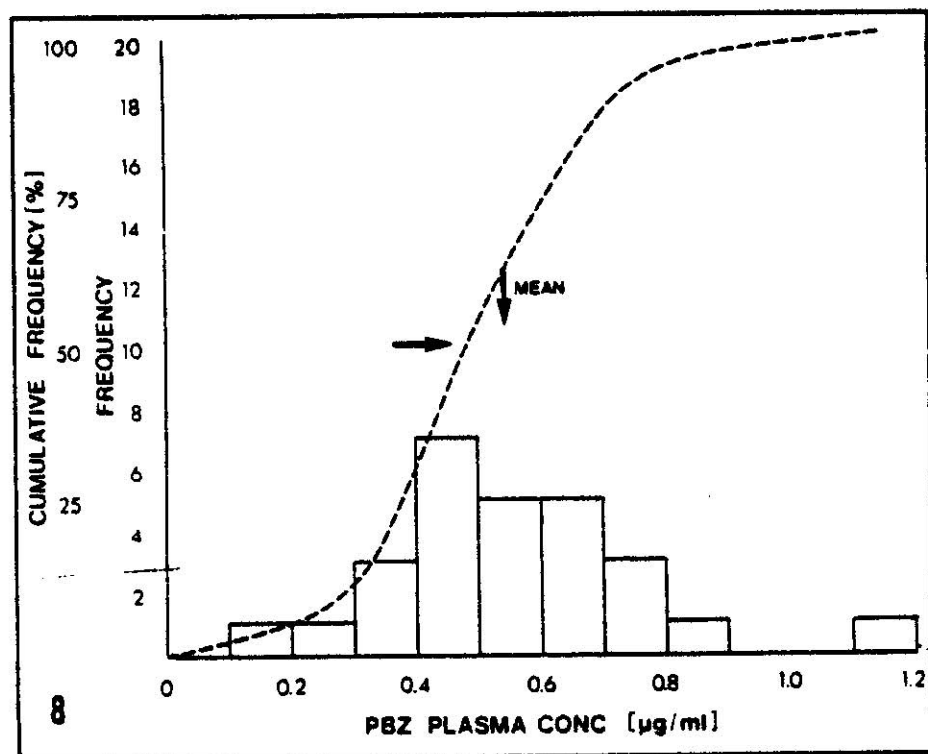


Fig 8—Example of logarithmic transformation (bottom) of the plasma concentrations (conc) from study 2 (tubed), a method which is commonly applied to data which are skewed. The probability estimates are based on this transformed data. The concentration of PBZ is expressed in  $\log_{10}$  (see Fig 1 for key).

The mean urine concentrations ( $\pm$  SD) of PBZ, OPBZ, and OHPEZ were  $0.57 \pm 1.35$ ,  $16.4 \pm 19.2$ , and  $2.95 \pm 2.5$   $\mu\text{g/ml}$ , respectively. Linear regression analysis comparing the concen-

trations of PBZ and OPBZ with the serum PBZ and OPBZ concentrations were not significant ( $r = -0.146$  and  $0.031$ , respectively), indicating no relationship. The total urinary con-

centrations of PBZ, OPBZ, and OHPEZ were compared with the respective total serum concentrations. The differences were not significant ( $r = 0.02$ ; Fig 6).

**Comparison of laboratory values—**In all studies, the serum and plasma concentrations from the participating laboratories were compared using analysis of variance. A significant difference was not observed among the laboratories. The final plasma or serum concentration for each horse was the average of the 3 separate analyses.

## Discussion

The primary purpose of the study was to determine the plasma and serum concentrations of PBZ and OPBZ in a large group of racing Thoroughbreds 24 hours after various dosages of PBZ were administered. Thoroughbred racing regulations in a number of states specify maximum allowable concentrations of PBZ and OPBZ, which, when surpassed, result in penalties. In many cases, a time limit for administration is stated or implied; this time limit is usually no less than 24 hours before race time. Observations in the field have indicated that after various dosages were given plasma PBZ and OPBZ concentrations were higher than would be predicted based on elimination rates established in nonracing horses.<sup>7-13</sup> There are some obvious explanations that would account for the marked variations seen under racing conditions, eg, route of administration, oral dosage form, weight of the horse, time of oral administration relative to feeding and exercise, and inadvertent administration too close to race time. Marked variations were seen in these studies,<sup>7-13</sup> indicating that individual differences in the rate and extent of absorption and the rate of metabolism of the drug may be the major reason for the wide range of plasma and serum concentrations measured in these populations of horses.

A study conducted in 1980\* in experimental horses, revealed a wide variation in plasma concentrations and, as with other multiple administrations, a distribution that was

\* Oak Tree Racing Association and National Association of State Racing Commissioners: Unpublished data, 1980.

skewed to the right (Fig 7). The horses were given 4 g of PBZ orally for 3 days followed by an IV dose of 2 g of PBZ. Twenty-four hours after the IV dose, the mean concentration of PBZ was 3.32  $\mu\text{g/ml}$  with 95% confidence limits of 2.77 to 3.95  $\mu\text{g/ml}$  and an absolute range of 0.5 to 12.9  $\mu\text{g/ml}$ , which was similar to the concentration of the tubed horses (study 2) in the present study. The coefficient of variation around the mean was 61%. This marked variation was not anticipated and was attributed to variability in these horses with respect to breed, age, and general fitness (all horses were experimental horses housed on various campuses). A common characteristic was that none of the horses had been on steady doses of PBZ, all were administered the same dosage regimen, and all were given granular PBZ in the grain, which was consumed immediately. A greater variation in analytical methods, as compared to current HPLC techniques, may also account for the greater variability. The dose and administration in the 1980 study\* were similar to the Keeneland tubed horses as was the mean plasma concentration (Table 1).

The plasma concentrations for stomach tube administration in studies 2 and 3 were not normally distributed, which seems to be a function of the multiple doses. The distribution was skewed to the right, and indicated that a group of horses at the tail of the distribution had higher plasma concentrations. This indicated that the plasma concentrations of PBZ in these horses were not equally distributed around the mean and that (for PBZ) a small sampling may not necessarily represent the population of horses. The samples were more likely to be drawn from the group of horses clustered to the left and not from the horses trailing to the right. For the multiple administrations, all plasma samples were transformed to  $\log_{10}$  because of the skewed distribution. Replotting produced a distribution that was not significantly skewed by minimizing the contribution of the higher and lower valued horses (Fig 8). Transformation for log-normal distribution is commonly used in analyzing a large population.<sup>18</sup>

The administration of PBZ by paste

and tablet produced the highest 24-hour plasma concentrations despite the consistency of a final IV dose of 2 g. The rate of disappearance of PBZ from the plasma after oral paste and tablet administration was probably related to the rate of absorption from the gastrointestinal tract, rather than to the rate of elimination of the drug.

After 4 days of IV administration in study 3 and in the tubed horses in study 2, plasma concentrations were not significantly different. This is consistent with the fact that the administration of the drug in a liquid suspension by stomach tube increases the probability of rapid and more complete absorption. Administration by this method causes maximum plasma concentrations to be attained by 4 hours and 90% of the drug to be absorbed.<sup>12</sup>

The wide range of plasma concentrations measured in the present studies, especially after daily administrations, indicated a greater variability in the rate of drug elimination than was previously indicated. Excluding the dose and the method of administration, the pharmacokinetic determinants that were most likely to influence the 24-hour plasma concentrations were the half-life and the volume of distribution of the drug. Recent studies<sup>10,12</sup> have reported similar half-lives after single and daily administrations of PBZ. These studies<sup>10,12</sup> also reported different volumes of drug distribution and different starting plasma concentrations when comparing the single IV dose with an IV administration after daily oral doses. A higher initial plasma concentration was indicated after an IV administration in horses that had been given daily oral doses. Existing tissue concentrations from previous daily administrations would reduce the rate of distribution of drug from plasma to tissues, thereby producing a higher initial plasma concentrations. In a single IV administration study,<sup>10</sup> initial plasma concentrations were lower when compared with daily administration followed by an IV dose.

The concentration of PBZ in study 1 after a single IV administration of PBZ, was lower than the concentrations of OPBZ. This was reversed after multiple-dose regimens (studies 2 and 3); in these 2 studies, the PBZ concentrations were higher than OPBZ.

**Laboratory comparisons**—Comparison of the serum and plasma concentrations measured by HPLC from the 3 laboratories did not indicate significant differences between laboratories for PBZ and OPBZ, despite some variations in methods. This indicates the stability and reliability of HPLC as a method for the measurement of PBZ and OPBZ.

**Relationship of plasma and urine concentrations**—The total urine concentrations of PBZ, OPBZ, and OHPBZ are being used in a number of states for regulatory purposes. In study 1, the average total urine concentration of PBZ and metabolites was 20.0  $\mu\text{g/ml}$ , with a range of 1.4  $\mu\text{g/ml}$  to 95.0  $\mu\text{g/ml}$ . The high coefficient of variation also reflects the wide range of values that can be expected when urine is used. There was no significant relationship between the total urine concentration and the total plasma concentration of PBZ, OPBZ, and OHPBZ, invalidating the use of urine concentration as a predictor of plasma concentrations on race day. The urinary concentrations of PBZ, OPBZ, and OHPBZ were consistent with values measured in experimental animals<sup>7,12</sup> (low PBZ and OHPBZ concentrations and higher OPBZ concentrations at 24 hours after PBZ administration). This is consistent with the observation that a greater proportion of PBZ is eliminated as the OPBZ metabolite.<sup>7</sup>

## References

1. Gabel AA, Tobin T, Ray RS, et al: Phenylbutazone in horses: A review. *J Equine Med Surg* 1:221-225, 1977.
2. Ebert EF: Clinical use of phenylbutazone in large animals. *Vet Med* 57:33-35, 1962.
3. Dunn PS: A clinician's view on the use and misuses of phenylbutazone. *Equine Vet J* 4:63-65, 1972.
4. Davidson AH, Franks WC: Anti-inflammatory agents in equine surgery. *Mod Vet Pract* 27:46-49, 1966.
5. Barragry TB: Phenylbutazone in equine practice: A review. *Irish Vet J* 27:7-11, 1973.
6. Oehme FW: Phenylbutazone in the treatment of soft tissue reactions of large animals. *Vet Med* 57:229-231, 1962.
7. Maylin GA: Disposition of phenylbutazone in the horse. *Proc Am Assoc Equine Pract* 20:243-248, 1974.
8. Piperno E, Ellis DJ, Getty SM, et al: Plasma and urine levels of phenylbutazone in the horse. *J Am Vet Med Assoc* 153:195-198, 1968.
9. Gandal CP, Dayton P, Weiner M, et al: Studies with phenylbutazone, oxyphenbuta-

zone and para-dichlorophenylbutazone in horses. *Cornell Vet* 59:577-580, 1969.

10. Jenney E, Steinijans VW, Seifert P: Pharmacokinetic interactions of isopropylaminophanazone and phenylbutazone in the horse. *J Vet Pharmacol Ther* 2:101-108, 1979.

11. Tobin T, Blake JW, Valentine R: Drug interactions in the horse: Effects of chloramphenicol, quinidine, and oxyphenbutazone in phenylbutazone metabolism. *Am J Vet Res* 38:123-127, 1977.

12. Soma LR, Gallis DE, Davis WL, et al: Phenylbutazone kinetics and metabolite concentrations in the horse after five days of administration. *Am J Vet Res* 44:2104-2109, 1983.

13. Duer WC, Calvo PP, Valdez M, et al: Concentrations of phenylbutazone and its metabolites in blood and urine of equine, in *Proceedings, Convention Assoc Off Racing Chem* 36:187-192, 1982.

14. Miller RG: *Simultaneous Statistical In-*

*ference*. New York, McGraw-Hill Book Co, 1966, pp 67-68.

15. Sokal RR, Rohlf FJ: Estimation and hypothesis testing. *Biometry*, ed 2. San Francisco, WH Freeman Co, 1981, pp 174-175.

16. Periman G: Data analysis program for the UNIX operating system. *Behav Res Methods Instr* 12:554-558, 1980.

17. Marunaka T, Shibata T, Minami Y, et al: Simultaneous determination of phenylbutazone and its metabolites and urine by high performance liquid chromatography. *J Chromatogr* 183:331-338, 1980.