

# Drug Interactions in the Horse: Effects of Chloramphenicol, Quinidine, and Oxyphenbutazone on Phenylbutazone Metabolism

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## SUMMARY

The plasma half-life of phenylbutazone in horses was not increased after pretreatment with chloramphenicol or quinidine, but was increased after oxyphenbutazone. This increased plasma half-life after oxyphenbutazone is consistent with observations in other species and suggests that oxyphenbutazone inhibits the metabolism of phenylbutazone in horses. Lack of inhibition of phenylbutazone metabolism in the horse by chloramphenicol and quinidine is inconsistent with results obtained in other species.

Phenylbutazone (1,2-diphenyl-4-n-butyl-3,5-dioxpyrazolidine) is a nonsteroidal analgesic and anti-inflammatory drug widely used in equine medicine in the treatment of lameness and soft tissue swelling in racing animals.<sup>7</sup> Its use is currently permitted in racing horses in most American states under varying degrees of control. The regulations controlling its use usually require that the racing authority be notified of use of the drug in each horse, that the last dose be given on the day before the race, and that phenylbutazone be detectable in the blood or urine of these animals in pre- or postrace drug tests. Further, to insure that animals are not treated with phenylbutazone on the actual day of the race, some authorities require that urine concentrations of the drug not exceed fixed limits, which are set by the individual authorities.

Racing authorities make these stipulations concerning blood and urine levels of phenylbutazone because they wish to control its use in racing. Thus, Illinois racing authorities hold that urinary levels of phenylbutazone and metabolites greater than 165 µg/ml indicate medication on the day of racing. Conversely, if

phenylbutazone is not detectable in the blood or urine of horses on the day of racing, this fact may be taken by the racing authority as evidence that medication with phenylbutazone has been withheld, and the horse is eliminated.

In interpreting blood and urine levels of phenylbutazone in this way, racing authorities make relatively fine pharmacologic judgments. This is particularly so in view of the fact that other drugs are commonly administered to racing animals along with phenylbutazone. Such drugs administered with phenylbutazone may affect its pharmacokinetics and, thus, urinary levels by actions on (a) protein binding, (b) drug metabolism, (c) rate of renal elimination, or (d) urine volume. The purpose in the present report is to give results of a study of the actions of a number of inhibitors of drug metabolism on the plasma half-life and urine levels of phenylbutazone in Thoroughbred horses.

## Materials and Methods

Mature Thoroughbred mares between 400 and 540 kg weight were used. The animals were maintained in individual loose boxes for the period of the experiment and fed hay and water ad libitum. All experiments were started between 9:00 and 10:00 AM and continued through the following 24 hours. All drugs were introduced by rapid intravenous (iv) injection, and blood samples were obtained by jugular venipuncture into 15-ml heparinized tubes.<sup>a</sup> Blood samples were immediately centrifuged at 5,000 × g for 10 minutes, and the plasma was removed and stored at -30 C until assayed. All drug assays were completed within 48 hours of drawing the blood sample.

Plasma levels of phenylbutazone were determined by extraction into 2:1 hexane:dichloromethane mixture. To 4 ml of plasma or urine sample, 2 ml of 1 M acetate buffer (pH 4.5) and 4 ml of the hexane:dichloromethane mixture were added. The samples then were rotated<sup>b</sup> for 5 minutes and centrifuged at 5,000 × g for 10 minutes. The hexane:dichloromethane mixture (2 ml) was then transferred to a test tube containing 4 ml of 0.1 N NaOH and again rotated for 5 minutes. The tubes were centrifuged, and about 3 ml of the NaOH layer was withdrawn by Pasteur pipette and again centrifuged. Care was taken at this point to minimize transfer of the cloudy interface between the 2 layers. The absorbance of the resulting solution at 265 nm was then determined in a spectrophotometer<sup>c</sup> and the concentration of phenylbutazone was calculated by comparison with appropriate standards. For gas chromatographic determination of phenylbutazone, aliquots of the

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<sup>a</sup> Vacutainer, Becton, Dickinson and Company, Rutherford, NJ.

<sup>b</sup> Fisher Rotorack, Fisher Scientific Company, Silver Spring, Md.

<sup>c</sup> Gilford 240 spectrophotometer, Gilford Instrument Laboratories, Inc. Oberlin, Oh.

hexane:dichloromethane layer were chromatographed on an OV-101 column<sup>4</sup> at 250 C in a gas chromatograph<sup>4</sup> equipped with a flame ionization detector. The retention time of authentic phenylbutazone in this system was approximately 3 minutes.

Commercial injectable phenylbutazone<sup>5</sup> was given to these horses. Authentic phenylbutazone<sup>6</sup> was used to generate standard curves; chloramphenicol<sup>7</sup> was the sodium succinate salt. Quinine<sup>8</sup> and oxyphenbutazone<sup>9</sup> were also used. Because oxyphenbutazone is poorly soluble in water, all oxyphenbutazone injections were administered in 50 ml of absolute ethanol. Since these experiments were directed toward determining the effects of various drugs on the  $\beta$  or metabolic half-life of phenylbutazone in the horse, detailed pharmacokinetic analysis was not made. Unless otherwise noted, all experimental points are the means  $\pm$  SEM of at least 4 separate experimental determinations. All plasma half-lives were determined from unweighted least squares fit to the data, and the criterion for statistical significance was  $P < 0.05$ .

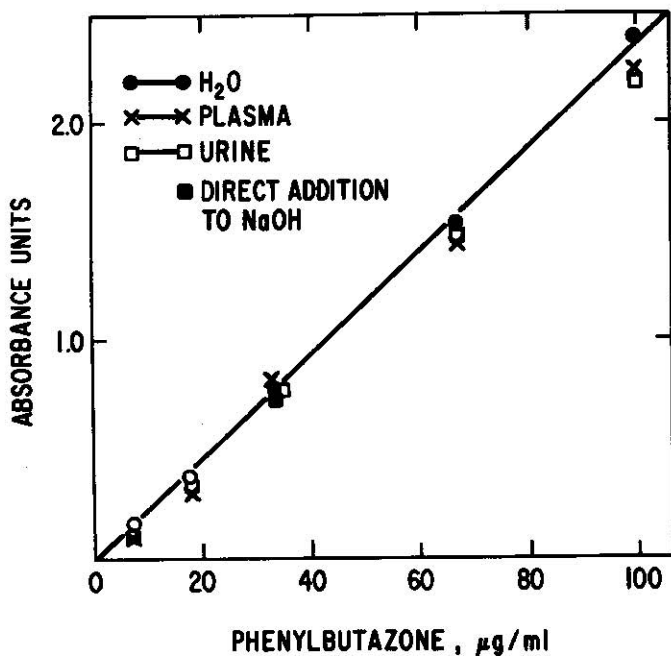


Fig 1—Recovery of phenylbutazone from spiked water, plasma, and urine samples. Samples (4 ml) of freshly drawn equine plasma, urine, or buffered distilled H<sub>2</sub>O were spiked with the indicated concentrations of phenylbutazone; extracted as described in "Methods," and their absorbance at 265 nm in NaOH measured. The solid circles (●—●) show absorbance values received from distilled water; the crosses (x—x) and open squares (□—□) recoveries from plasma and urine. The solid square (■) shows absorbance value obtained when 33 µg/ml of phenylbutazone was added directly to the NaOH. All data points are the means of 4 separate experimental determinations. Standard errors of the mean (SEM) are omitted for the sake of clarity.

## Results

Absorbance at 265 nm of phenylbutazone recovered from 4-ml aliquots of water and equine plasma or urine is shown (Fig 1). Recovery of absorbance was essentially linear over the drug range studied. The method

used appeared to recover all of the phenylbutazone added to the system, in that direct addition of similar quantities of phenylbutazone to 0.1 N NaOH gave similar absorbance readings. In the gas chromatographic method, the detector response was curvilinearly related to the concentration of drug added to the system, so that individual complete standard curves of

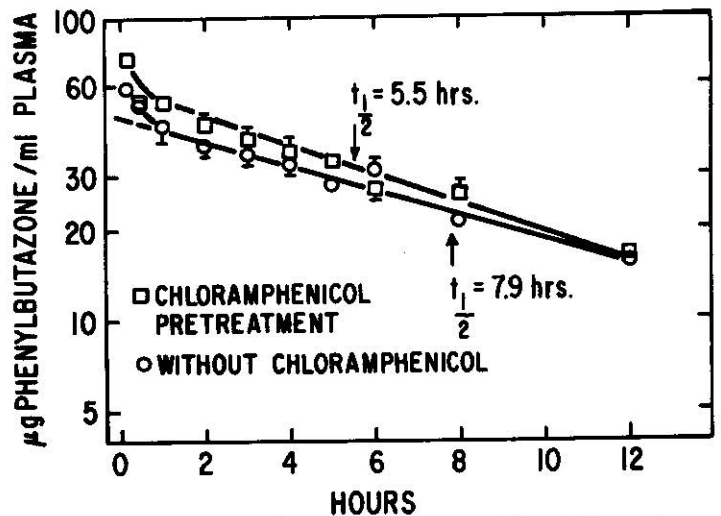


Fig 2—Effect of chloramphenicol pretreatment on the half-life of phenylbutazone in the horse. Chloramphenicol (33 mg/kg) or an equivalent volume of normal saline solution was administered IV to Thoroughbred horses. Forty-five minutes later, phenylbutazone (6.6 mg/kg) was administered by rapid IV injection. The open circles (○—○) show the plasma half-life of phenylbutazone in horses pretreated with saline solution, the open squares (□—□) the plasma half-life in horses pretreated with chloramphenicol. By regression analysis, the slope with chloramphenicol pretreatment was significantly ( $P < 0.01$ ) greater than that without pretreatment. All points are the mean  $\pm$  SEM of experiments on 4 different horses. Plasma levels of phenylbutazone were determined by the spectrophotometric method.

phenylbutazone recovered from plasma and urine were constructed for each experiment, because the ultraviolet method also measures absorbance from small amounts of the metabolites of phenylbutazone, the gas chromatographic method gave slightly lower, but more accurate, results.

Effect of pretreatment with chloramphenicol on the plasma half-life of phenylbutazone in horses is shown (Fig 2). In these experiments, administration of 33 mg of chloramphenicol/kg at 45 minutes before dosing with phenylbutazone produced a significant decrease in the plasma half-life of phenylbutazone from 7.9 hours in the control animals to about 5.5 hours in the chloramphenicol-pretreated animals. At the same time, chloramphenicol increased the initial plasma level of phenylbutazone from 46 µg/ml in the control animals to 61.5 µg/ml in the treated animals. The experiment does not suggest any action of chloramphenicol at this dose level to prolong the metabolism of phenylbutazone in horses.

Because pretreatment with chloramphenicol inhibits microsomal drug metabolism in most mammalian species,<sup>1</sup> this experiment was repeated twice at a larger dose level of chloramphenicol and determined plasma levels of phenylbutazone by gas chromatographic

<sup>4</sup> Perkin-Elmer 900, Perkin-Elmer Corporation, Norwalk, Ct.  
<sup>5</sup> W. A. Butler Company, Columbus, Oh.  
<sup>6</sup> Sigma Chemical Company, Ltd, St. Louis, Mo.  
<sup>7</sup> Provided by Parke Davis Company, Ann Arbor, Mi.  
<sup>8</sup> Geigy Pharmaceuticals, Ciba-Geigy, Ltd, Summit, NJ.

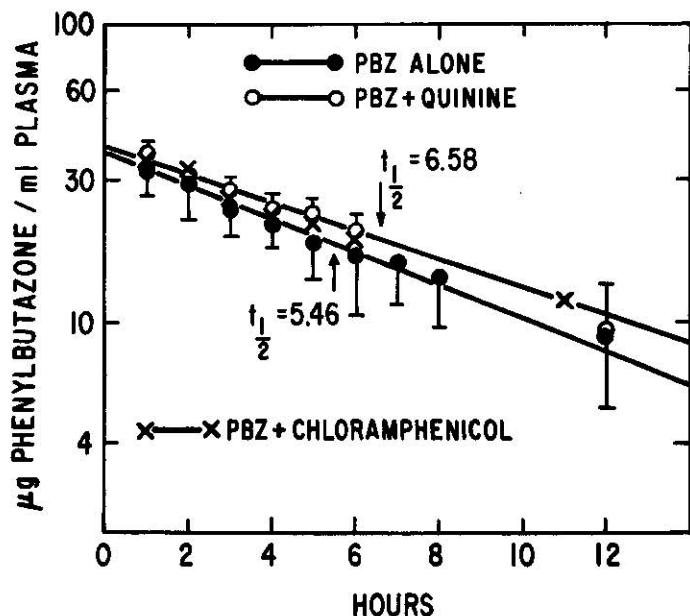


Fig 3—Effects of quinine and chloramphenicol on the plasma half-life of phenylbutazone. Horses were administered quinine (20 mg/kg) or chloramphenicol (66 mg/kg) or an equivalent volume of normal saline solution IV. Forty-five minutes later, phenylbutazone, 6.6 mg/kg, was administered to all horses. The solid circles (●—●) show the half-life of phenylbutazone in animals with no pretreatment, the open circles (○—○) the half-life of phenylbutazone in animals pretreated with quinine, and the crosses (×—×) show the blood levels of phenylbutazone in animals pretreated with chloramphenicol, 66 mg/kg. All data points are the means  $\pm$  SEM of experiments on 4 horses, except for the data points with chloramphenicol which are the means of determinations on 2 different horses. By regression analysis, there were no significant differences between the slopes (half-lives) of the control and treatment groups. Plasma levels of phenylbutazone were determined by the gas chromatographic method.

method. Pretreatment with larger doses of chloramphenicol (66 mg/kg) also had no significant effect on the plasma half-life of phenylbutazone in horses (Fig 3).

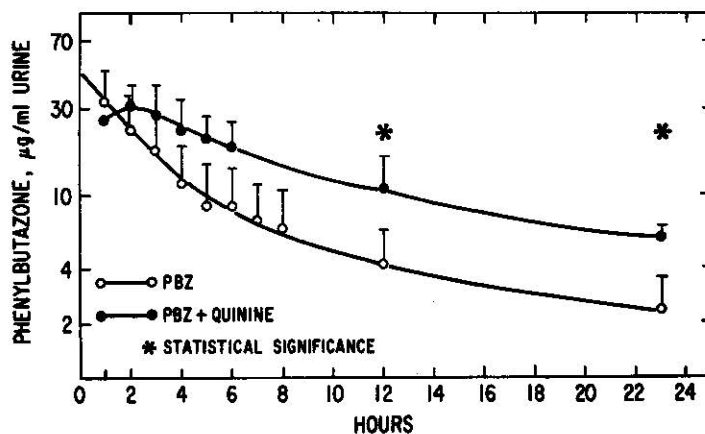


Fig 4—Urinary levels of phenylbutazone in horses pretreated with quinine. The solid circles (●—●) show urinary levels of phenylbutazone in the horses of the experiment of Figure 3 pretreated with quinine, whereas the open circles (○—○) show urinary levels of phenylbutazone in horses without pretreatment. All points are the means  $\pm$  SEM of experiments on 4 horses. Drug levels were determined by gas chromatography. The asterisks (\*) indicate statistically significant differences (*t* test,  $P < 0.05$ ).

Because of this lack of effect of chloramphenicol at dose levels which readily produced effects on both the rates of metabolism and offset of the pharmacologic effects of drugs in other mammalian species,<sup>1</sup> the actions of other drugs known to inhibit drug metabolism were investigated. Quinine, a potential inhibitor of drug metabolism in other species,<sup>3</sup> also had no significant ( $P < 0.05$ ) effect on the rate of metabolism of phenylbutazone in horses (Fig 3). Quinine also did not affect the initial plasma level of phenylbutazone observed. Quinine did, however, affect the urinary concentration of phenylbutazone observed, in that the urinary concentrations observed in quinine-pretreated animals were significantly higher ( $P < 0.05$ ) than those observed in control animals at 12 and 24 hours (Fig 4).

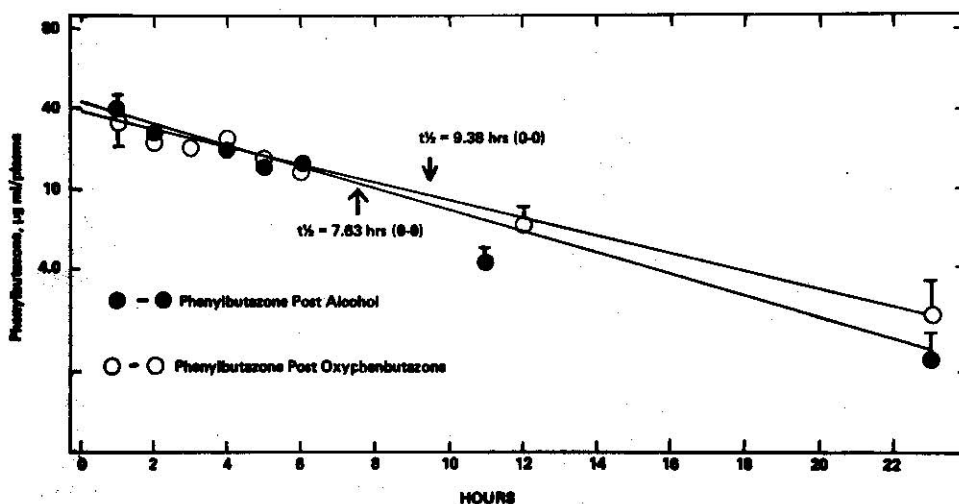


Fig 5—Effect of oxyphenbutazone pretreatment on the plasma half-life of phenylbutazone in horses. Horses were pretreated with oxyphenbutazone, 11.0 mg/kg, in 50 ml of ethanol or an equivalent volume of ethanol. Forty-five minutes later, each horse was given 6.6 mg/kg of phenylbutazone IV. The solid symbols (●—●) show the half-life of phenylbutazone in ethanol-pretreated horses, the open circles (○—○) the half-life of phenylbutazone in horses pretreated with oxyphenbutazone. All points and mean  $\pm$  SEM of determinations on at least 4 separate animals. The solid lines are least squares fit to each group of data with variability between animals deducted out. The slopes of these lines are significantly different (*f* test,  $P < 0.01$ ).



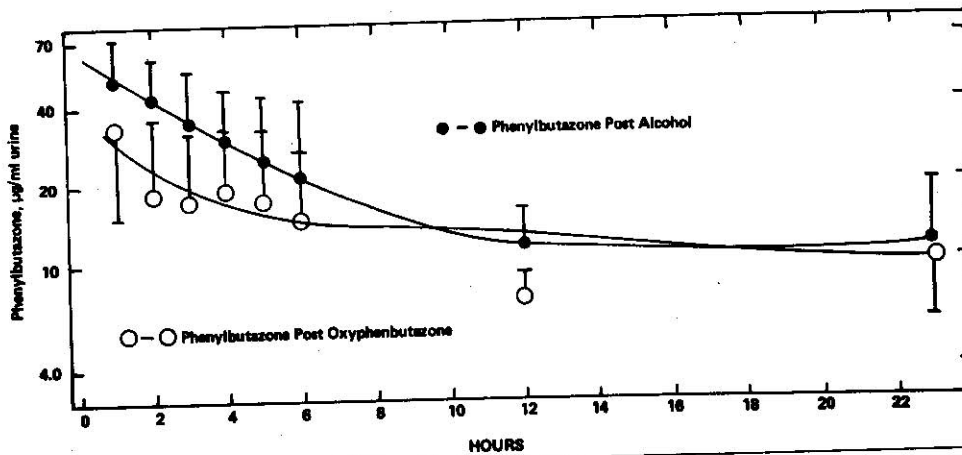


Fig 6—Urinary levels of phenylbutazone in horses pretreated with oxyphenbutazone. The open circles (○—○) show urinary levels of phenylbutazone in oxyphenbutazone-pretreated horses, whereas the solid circles (●—●) show urinary levels of phenylbutazone in alcohol-pretreated animals. Significant differences between the phenylbutazone levels at individual times were not observed. All points are the means of at least 4 separate experimental determinations.

Studying the pharmacokinetics of phenylbutazone in horses, Piperno et al<sup>10</sup> noticed that the plasma half-life of phenylbutazone in the horse varied directly with the dose of the drug administered. A possible explanation for this variation comes from the work of Janchen and Levy<sup>8</sup> who have shown that in rats oxyphenbutazone acts to inhibit the metabolism of its parent component, phenylbutazone. Therefore, the ability of oxyphenbutazone to inhibit the metabolism of phenylbutazone in the horse was tested. Pretreatment with oxyphenbutazone, 11 mg/kg, produced a significant increase in the plasma half-life of phenylbutazone ( $P < 0.01$ ) (Fig 5). However, though highly significant statistically, this effect was very small, and significant changes were not seen in urinary levels of phenylbutazone after pretreatment with oxyphenbutazone (Fig 6).

## Discussion

Pretreatment of Thoroughbred horses with chloramphenicol and quinine did not prolong the plasma half-life of phenylbutazone in these animals. These observations are surprising in view of the well-documented actions of chloramphenicol and quinine on both liver microsomal drug metabolism and the plasma half-life of drugs in a number of mammalian species.<sup>1,6</sup> In contrast, pretreatment with oxyphenbutazone produced a small but highly significant increase in the plasma half-life of phenylbutazone. This effect of oxyphenbutazone in the horse is consistent with the results of experiments of Janchen and Levy<sup>8</sup> in the rat.

Investigating the effect of chloramphenicol on hexobarbital sleeping times in mice, Dixon and Fouts<sup>6</sup> showed that relatively small (10 mg/kg) dosage levels of this drug were effective. The effect of chloramphenicol was greatest if the chloramphenicol was given 45 minutes before dosing, the time interval utilized in the present studies. The effect of chloramphenicol on sleeping times was still observable up to 6 hours after dosing.

Various aspects of these initial observations have since been confirmed in other mammalian species.<sup>1</sup> Since phenylbutazone is widely used as a standard to

measure the activity of liver microsomal enzymes in a number of species, there is no reason to think that phenylbutazone metabolism in the horse does not proceed by this route.<sup>2</sup> The chloramphenicol was administered iv as the sodium succinate salt 45 minutes before challenge with phenylbutazone. The succinate salt was also used iv at similar doses and time intervals by Dixon and Fouts.<sup>6</sup> The iv route of administration avoids problems with the absorption of chloramphenicol after the im and oral routes; chloramphenicol is not extensively protein bound in the horse.<sup>11</sup> The dosage rates of chloramphenicol on a milligram-per-kilogram basis are greater than those used in mice and are relatively much greater if calculated on the basis of body surface area, a more accurate guide for relative dosage rates. It, therefore, seems unlikely that adequate levels of chloramphenicol failed to reach the liver in the present experiments.

In a recent paper, Della Bella et al<sup>5</sup> showed that in animals in which drug metabolism is induced, the peak blood levels and pharmacologic actions of chloramphenicol are reduced. Since the horses used in the present experiments had been treated with various other drugs in the period preceding the experiments, it is reasonable to consider drug metabolism in these horses as induced, as indeed metabolism in horses "on the track" is very likely to be. Nevertheless, it is difficult to see how the induction of increased hepatic microsomal activity such as that described by Della Bella et al<sup>5</sup> and Stramentinoli et al<sup>12</sup> could completely eliminate the marked effects of chloramphenicol on drug metabolism which have been demonstrated in other species. It seems reasonable, therefore, to assume that the reason for the lack of effectiveness of chloramphenicol on drug metabolism in the equine animal depends primarily on a lack of responsiveness of equine liver tissue to this drug.

Investigating the actions of quinine on drug metabolism, Boulos et al<sup>8</sup> showed that quinine significantly prolonged pentobarbital sleeping time in rats. Studying the rate of disappearance of pentobarbital from the

plasma of goats, these workers showed that quinine pretreatment doubled its apparent plasma half-life in goats without affecting the apparent specific volume of distribution. These authors concluded that quinine acted by inhibiting drug metabolism at the microsomal level.

Despite the clear-cut evidence of Boulos et al.<sup>3</sup> quinine failed to affect the plasma half-life of phenylbutazone in the horse. The doses given (20 mg/kg) were the largest that could be given these horses without running the risk of killing them. Thus, again, it can be concluded that an agent which apparently readily affects drug metabolism in another species is unable to produce similar effects in the horse.

Though urinary concentrations of phenylbutazone were significantly affected by the administration of quinine (Fig 4), it is not possible to conclude from the present observations that quinine affected the metabolism of phenylbutazone. Urinary concentrations of a drug are affected by too many variables for urinary levels alone to be a reliable indicator of rates of drug metabolism.

Studying the pharmacokinetics of phenylbutazone in the horse, Piperno et al.<sup>10</sup> observed that increasing dose levels of phenylbutazone increased the observed plasma half-life of phenylbutazone in these animals. Similarly, the plasma half-life of phenylbutazone is highly dose dependent in dogs, mice, and rats.<sup>8</sup> Investigating this problem, Janchen and Levy<sup>8</sup> showed that in rats the concomitant administration of oxyphenbutazone with phenylbutazone increased both the half-life and apparent volume of distribution of this drug. Further, the rate of plasma clearance of phenylbutazone was markedly decreased, directly indicating reduced metabolism of the drug. Thus, oxyphenbutazone became a likely candidate as an inhibitor of drug metabolism in the horse.

Pretreatment of horses with oxyphenbutazone, 11 mg/kg, produced a small but statistically highly significant increase in the plasma half-life of phenylbutazone (Fig 5). This ability of oxyphenbutazone to inhibit phenylbutazone metabolism thus becomes a possible explanation for the marked dose dependence of the plasma half-life of phenylbutazone reported by Piperno et al.<sup>10</sup> The results reported here may also explain the observations of Maylin<sup>9</sup> and Blake<sup>1</sup> on urinary patterns of phenylbutazone and its metabolites in the horse. Studying the disposition of phenylbutazone in the equine animal, Maylin<sup>9</sup> observed that after a single dose of phenylbutazone the side chain oxidized metabolite (1,2-diphenyl-3,5-dioxo-4-(3-hydroxybutyl)pyrazolidine or  $\gamma$ -hydroxyphenylbutazone) was predominant. However, after repeated dosing, both the proportion of oxyphenbutazone in the urine and the plasma half-life of phenylbutazone in these animals increased. According to Maylin, one possible explanation for these pattern changes could be inhibition of the metabolism of phenylbutazone, either by the drug itself or by a metabolite, as has been reported in rats by Cho et al.<sup>4</sup>

Studying the effects of phenylbutazone on hepatic microsomal demethylase, Cho et al.<sup>4</sup> observed that phenylbutazone pretreatment in rats inhibited the demethylation of ethylmorphine in liver microsomal preparations from these animals. This inhibition was stable, in that it would not be washed out and was kinetically distinguishable from that obtained on adding phenylbutazone in vitro; Cho et al.<sup>4</sup> suggested that this inhibition was due to phenylbutazone itself or one of its metabolites. In view of the observations of Janchen and Levy<sup>8</sup> and those reported herein, oxyphenbutazone becomes a likely possibility for the inhibitory metabolite, both in laboratory animals and the horse.

Reviewing the results obtained in the present study, it is clear that 3 present inhibitors of liver microsomal drug metabolism in other species had no effects which are likely to be of clinical or forensic significance in racing horses. The only statistically significant increase in the plasma half-life of phenylbutazone was observed after pretreatment with oxyphenbutazone, and this effect was extremely small. Similarly, the small differences in the urinary concentrations of phenylbutazone observed in the present experiments are not likely to be of practical significance, since urinary levels of drugs are widely variable, depending on urine volume and pH. Thus, it seems reasonable at this point to rule out inhibition of drug metabolism as a probable mechanism of drug-induced variability in the disposition of forensically important drugs in the horse.

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