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## The Pharmacology of Procaine in the Horse: Relationships Between Plasma and Urinary Concentrations of Procaine

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After intravenous injection of procaine HCl in mares, urinary concentrations of the drug fell slowly ( $t_{1/2} > 3$  hours), in contrast with the relatively short ( $t_{1/2} = 45$  minutes) plasma half-life of the drug in horses. Similarly, after intra-articular injection of procaine HCl, urinary concentrations fell more slowly than plasma concentrations of the drug. After intramuscular injection of procaine HCl or procaine penicillin, plasma and urinary concentrations of the drug fell in parallel and slowly. These experiments suggest that procaine is sequestered in the urinary tract and that its release into urine can be rate limiting for its renal elimination. The nature of this rate limiting step is unknown. After intramuscular administration of procaine HCl or procaine penicillin, the rate of absorption of the drug from the intramuscular site appears to be equivalent to, or slower than, the rate limiting renal process. Under these conditions, plasma and urinary concentrations of the drug declined in parallel. The experiments show that after administration of procaine HCl urinary concentrations of procaine are found long after procaine has become undetectable in plasma. The results suggest that routine racetrack testing be expanded to include plasma procaine determinations and that "calling" of a procaine positive be contingent on determination of plasma levels of the drug. The experiments also suggest that routine urine samples should be cooled to 0°C in the period between collection and analysis.

### Introduction

Under current rules of racing, the presence of procaine in the urine of racing horses is an infraction of the rules and can lead to disciplinary action.<sup>3</sup> This is because the presence of procaine in the urine of a racing animal is taken as evidence that the animal has been raced under the influence of procaine. Since procaine has both local anesthetic and central stimulant actions in the horse,<sup>4,5</sup> the performance of any animal racing "on procaine" may be influenced by this drug.

Simple detection of what may be very small amounts of procaine in the urine of a horse does not, however, mean that the animal was pharmacologically influenced by procaine at the time of racing. Preliminary experiments by Tobin and Blake<sup>6</sup> have shown that urinary concentrations of procaine in the horse decline more slowly than plasma levels of procaine. Therefore, it is possible that under some circumstances a horse might test "positive" for urinary procaine but be analytically free of procaine in its blood stream. Similarly, it is known that urinary concentrations of procaine in horses are found for up to 1 week after dosing with procaine penicillin.<sup>1</sup> This study was undertaken to further investigate the relationship between plasma and urinary procaine levels.

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**Materials and Methods**

Mature Thoroughbred mares of between 400 and 500 kg body weight were used. Housing and general treatment of these animals was as previously described.<sup>1</sup> All urine samples were obtained by catheterization and draining the bladder at the indicated time intervals. A 20-ml aliquot of the total urine sample was taken for analysis. All drugs, injections, and blood samples were as previously described. Plasma and urine samples were stored overnight at -30° C accompanied by appropriate standards for analysis on the following day. Plasma and urine concentrations of procaine were determined by gas chromatography or occasionally colorimetrically (Fig 3) as described by Tobin et al.<sup>8,13</sup> and by Blake et al.<sup>2</sup> In the gas chromatographic method, 1 ml of saturated sodium tetraborate and 2 ml of benzene were added to 4 ml of the biological fluid and the whole rotoracked for 10 minutes. The system was then centrifuged at 5,000 x g for 10 minutes and the benzene layer transferred to a tube containing 50 µl of 5% pyridine in benzene and 50 µl of heptafluorobutyric anhydride (HFBA). The system was allowed to react at room temperature for 3 minutes and 5 ml of saturated sodium tetraborate again added and the system

rotoracked. After centrifugation, the benzene layer was separated and used for gas chromatographic analysis.

Gas chromatography was on a Perkin-Elmer 3920 gas chromatograph equipped with an electron capture detector. Injection temperature was 260° C on a column of 3% OV-101. Detector temperature was 300° C unless otherwise stated, and the carrier nitrogen flow rate was 70 ml/minute. This method was sensitive down to less than 5 nanograms/ml procaine. The results obtained with this method correlated well with those obtained by the colorimetric method. Unless otherwise noted, all experimental points are the means ± SEM of experiments on at least 4 different animals.

**Results**

Figure 1 shows typical chromatograms of the procaine HFBA derivative obtained from spiked standards, compared with chromatograms of extracts of urine from treated and untreated horses. A peak with a retention time corresponding to the procaine HFBA peak was seen only in urine from the procaine treated horse. Further, if the column temperature was varied, the retention times of the known procaine HFBA derivative and the putative procaine HFBA derivative varied in parallel. The experiment shows that the peak observed in the urine of procaine treated horses is an HFBA derivative of procaine (Fig 2).

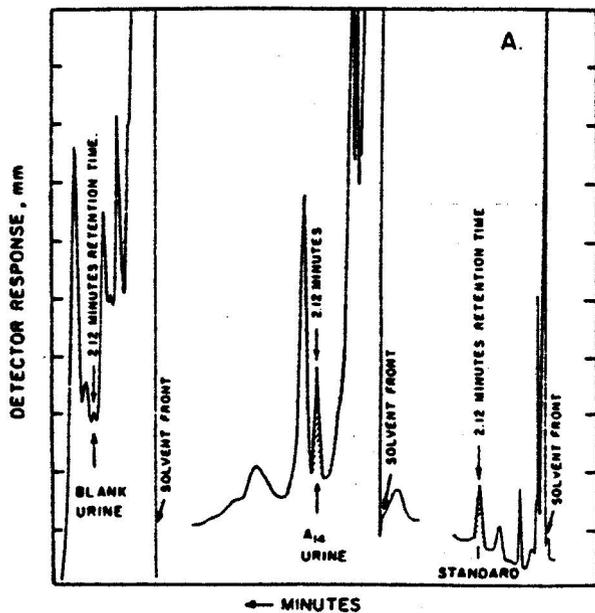


Fig 1 — Gas chromatographic analysis of procaine HFBA derivatives.

The right-hand chromatogram is that of a standard solution of 10 µg/ml of procaine derivatized as outlined in "Methods." The center chromatogram is that of a urine sample from a horse pre-treated with procaine HCl and derivatized as outlined in "Methods." The left-hand chromatogram is that of urine from an untreated animal treated as outlined in "Methods."

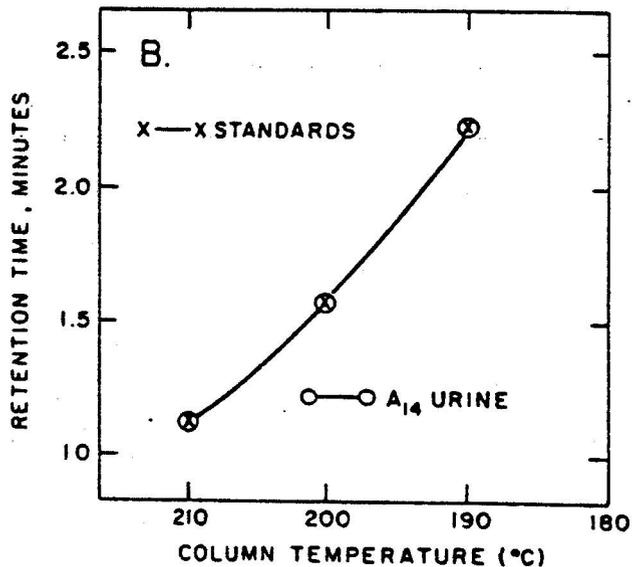


Fig 2 — Comparison of retention times of putative and authentic procaine HFBA.

The crosses (x-x) show retention times for the standard procaine HFBA chromatographed as outlined in "Methods" except that the column temperature was varied as indicated on the horizontal axis. The circles (o-o) show the retention times of the similar peak observed in the urine of procaine treated horses over the same temperature range.

Because of the instability of procaine in equine plasma,<sup>11</sup> we first studied the stability of procaine in equine urine at various temperatures. Figure 3 shows that at 37° C procaine added to equine urine (pH approximately 7.5) had an apparent half-life of about 24 hours.

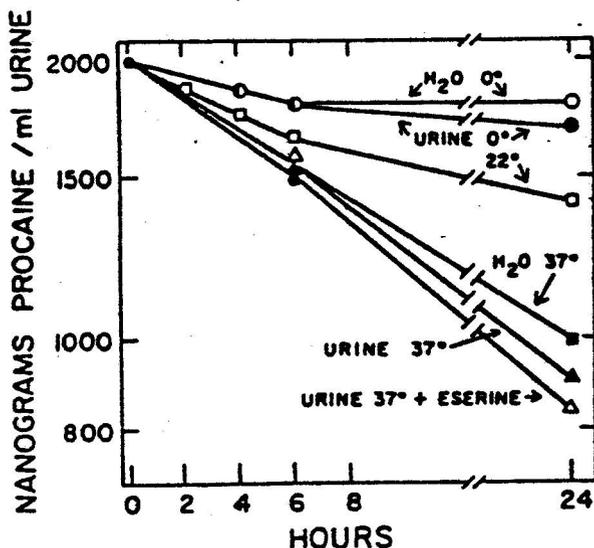


Fig 3 — Hydrolysis of procaine by horse urine.

Two thousand nanograms/ml of procaine HCl were added to 5 ml of freshly drawn horse urine or distilled water buffered to pH 7.4 with 50 mM phosphate buffer. These samples were then incubated at 0°, 22°, or 37° C and aliquots taken at the indicated time periods and assayed for procaine. The open circles (○ - ○) and solid squares (■ - ■) show recoveries of procaine from buffered aqueous solutions at 0° and 37°, respectively. The solid circles (● - ●), open squares (□ - □), and solid triangles (▲ - ▲) show hydrolysis of procaine in urine at 37° in the presence of eserine. All points are means of at least 4 experimental determinations.

This same half-life was observed in eserinated urine and in aqueous solution buffered to pH 7.4. The results, therefore, suggest that the rate of breakdown of procaine in equine urine is about that of the spontaneous breakdown of procaine under similar temperature and pH conditions. Figure 3 also shows that cooling the system to 22° C, or more especially 0° C, protected procaine against this spontaneous hydrolysis.

Figure 4 shows plasma and urinary concentrations of procaine after the rapid intravenous administration of 2.5 mg/kg of procaine HCl. As reported previously, plasma procaine levels initially fell rapidly, then more slowly, with a terminal half-life in the order of about 45 minutes. Urinary concentrations of procaine, however, continued to climb slowly, peaked at about 4 hours, and then declined in a biphasic manner, more rapidly at first with an apparent half-life of about 3 hours, then much more slowly. In this experiment, some individual urinary concentrations of procaine declined with overall half-lives of

8 hours or less.<sup>8</sup> Inspection of Fig 4 thus suggests that procaine is likely to be found in the urine of horses for quite some time after it has become undetectable in plasma.

Figure 5 shows that essentially the same results were observed after intra-articular injection of procaine. In this experiment, plasma concentrations of procaine peaked at about 60 minutes and then declined with an apparent half-life of about 1.5 hours (dotted line in Fig 3 replotted from Fig 9 from reference #9). Urinary concentrations of procaine, however, continued to increase, peaked at about 4.5 hours, and then declined very slowly with an apparent terminal half-life in the order of about 5 hours. The experiment shows that substantial and easily detectable urinary concentrations of procaine were observed after the intra-articular injection of small amounts of procaine and that procaine again continued to be eliminated in urine long after it had become undetectable and presumably pharmacologically inactive in plasma.

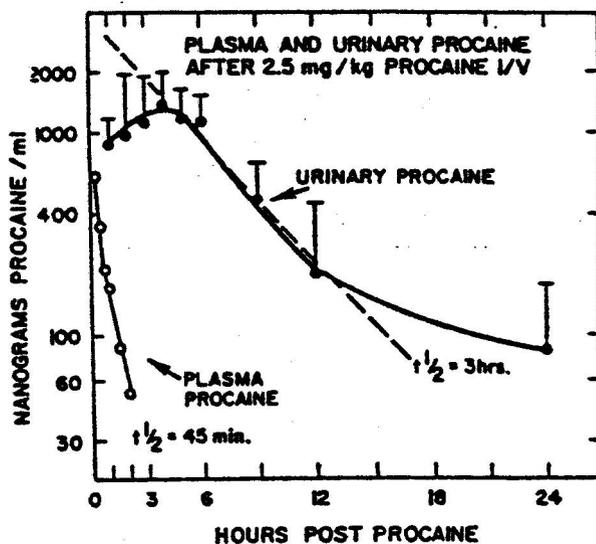


Fig 4 — Plasma and urinary levels of procaine after the rapid intravenous injection of 2.5 mg/kg of procaine HCl.

The open circles (○ - ○) show plasma levels of procaine after the rapid intravenous injection of 2.5 mg/kg of procaine HCl. The  $\beta$  phase half-life of plasma procaine under these conditions was approximately 45 minutes. The solid circles (● - ●) show urinary levels of procaine in these experiments. The dotted line shows a fit to the fastest portion of the urinary half-life observed with an apparent  $t_{1/2}$  of about 3 hours. All points are the means  $\pm$  SEM of 5 experimental determinations.

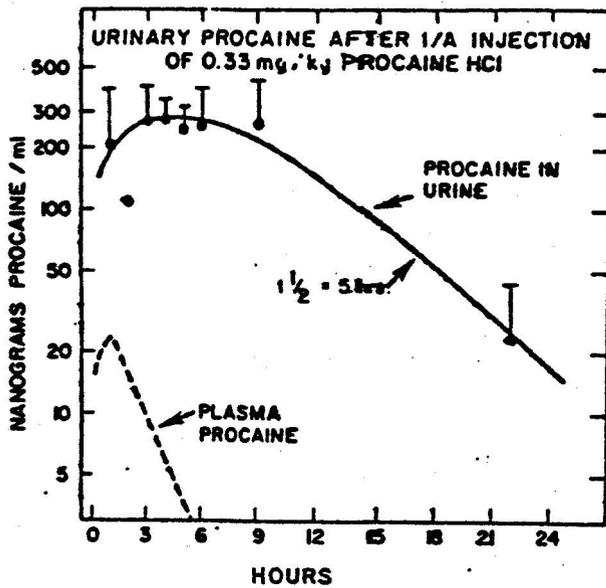


Fig 5 — Urinary levels of procaine after intra-articular injection of procaine HCl.

The solid circles (• - •) show urinary concentrations of procaine after the intra-articular injection of 0.33 mg/kg of procaine HCl as Novocaine®. The dotted line shows plasma levels of procaine after the intra-articular injection of this concentration of procaine, replotted from Fig 9 of reference #9. All points, except that at 2 hours, are the means ± SEM of data obtained on 4 or more animals.

Figure 6 shows that similar results were obtained after the intramuscular injection of procaine HCl (10 mg/kg). As observed previously,<sup>19</sup> plasma concentrations of procaine fell with an apparent half-life of about 3.0 hours after its intramuscular injection. Urinary concentrations of the drug, however, declined at about the same rate, with an apparent half-life on the order of about 5 hours, and urinary concentrations of the drug were again detectable long after plasma levels of the drug had become undetectable.

Figure 7 shows the relationship between plasma and urine concentrations of this drug after administration of procaine penicillin. Plasma concentrations of procaine peaked at about 1 hour and then declined with an apparent initial half-life of about 8 hours. Then, after 24 hours, the apparent plasma half-life of procaine increased to about 24 hours, a rate of decline which was approximately maintained until plasma levels of the drug became undetectable on the sixth day.

After administration of procaine penicillin, urinary concentrations of procaine followed plasma levels much more closely than previously. After peaking at 12 hours postdosing, urinary concentrations of the drug fell rapidly at first, with a half-life apparently paralleling the initial

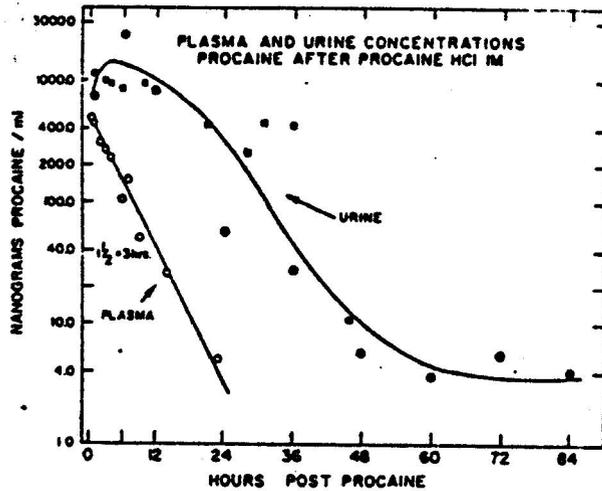


Fig 6 — Urinary levels of procaine after the intramuscular injection of procaine HCl.

The open circles (○ - ○) show plasma levels of procaine after the intramuscular administration of 10 mg/kg of procaine HCl. The solid circles (• - •) and solid squares (■ - ■) show urinary concentrations of procaine in 2 separate experiments. All data points are the means ± SEM of experiments on at least 3 (•) and 5 (■) horses, respectively.

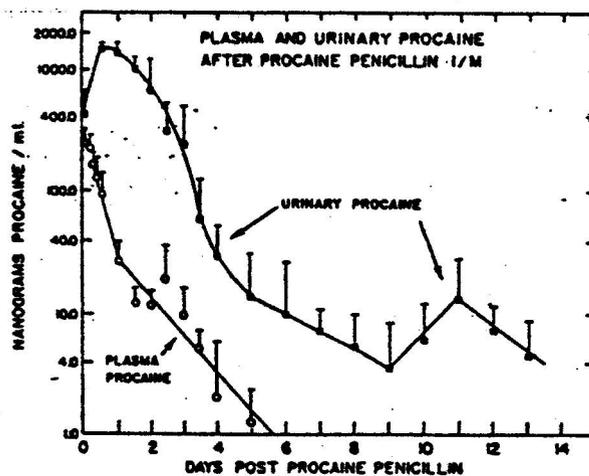


Fig 7 — Plasma and urinary concentrations of procaine after intramuscular administration of procaine penicillin.

The open circles (○ - ○) show plasma concentrations of procaine after the intramuscular administration of 33,000 I.U./kg of procaine penicillin to Thoroughbred horses. The solid squares (■ - ■) show urinary concentrations of procaine in these experiments. All data points are means ± SEM of experiments on 4 different horses.

8-hour plasma half-life of procaine. Then, as the plasma half-life of procaine increased to about 24 hours, the urinary half-life of the drug also increased, though apparently to a somewhat greater value than the apparent 24-hour plasma half-life of the drug. After becoming almost undetectable in urine on the ninth day, the urinary levels of procaine increased to peak at the 11th day and then declined again to the 14th day, when the experiment was concluded.

#### Discussion

Though procaine is relatively rapidly hydrolyzed by equine plasma *in vivo*, substantial concentrations of a material behaving like procaine were found in the urine of horses treated with this drug. This material is procaine because (a) it gave the colorimetric reaction for procaine as described by Tobin et al.,<sup>8-11,14</sup> and procaine concentrations determined by this colorimetric reaction were similar to those determined gas chromatographically; and (b) the HFBA derivative of this material could not be distinguished from the HFBA derivative of authentic procaine over 3 different column temperatures (Fig 2) and under a number of other conditions in our gas chromatographic system. Further, experiments by other workers have demonstrated the presence of procaine in the urine of horses after its administration as either procaine HCl or procaine penicillin. Thus, Brodie<sup>4</sup> showed that after administration of 2 grams of procaine HCl to horses, up to 9  $\mu$  g/ml of procaine were found in the urine of the horses at 5 hours. Similarly, Evans and Lambert<sup>5</sup> have shown that procaine is detectable in the urine of horses in substantial concentration after the administration of either procaine HCl or procaine penicillin. Finally, procaine has been the most commonly reported drug in horse urine by the Association of Official Racing Chemists in 1972,<sup>6</sup> by this group between 1949 and 1962,<sup>7</sup> and also by the equine drug testing laboratory in Kentucky. Based on these considerations, it seems reasonable to conclude that the material found in the urine of these horses which could not be distinguished from procaine is procaine.

In all of the experiments reported here, urinary levels of procaine after administration of procaine HCl fell more slowly than plasma levels of the drug. While this effect showed up clearly after intra-articular administration of the drug, it was not clear from these experiments whether or not the discrepancy in the rates of decline of the urinary levels was a renal phenomenon or simply a reflection of unusual pharmacokinetics of procaine, such as are observed after its intramuscular administration (Figs 8 and 12 in reference #9). Therefore procaine HCl was given intravenously at 2.5 mg/kg to rule out the possibility of tissue binding at the site of injection and show release of the drug as a possible cause for the discrepancy. As shown in Fig 4, procaine levels in the urine again declined

much more slowly than plasma levels of the drug. Based on these observations, it seemed reasonable to conclude that specific factors were acting to delay the elimination of procaine in urine.

Studying urinary elimination of procaine in the human, Brodie<sup>4</sup> reported that about 2% of a 2-gram dose of procaine in man was excreted unchanged in the first 48 hours. In these intravenous experiments, only about 0.15% of the total dose administered was excreted in the first 6 hours which suggests that less than 1% of the total dose will be excreted by this route over a 48-hour period in the horse. The reason for the discrepancy between our results and those of Brodie is not apparent, but a possible source of error is the somewhat less specific colorimetric method used by Brodie.<sup>4</sup>

When drug levels in urine decline more slowly than plasma levels of a drug, the simplest explanation for this phenomenon is that the drug is selectively bound in the urinary tract and only slowly released into urine. In this way, plasma levels of the drug can decline to below measurable levels, but renal levels of the drug remain high and release of the drug into urine (and, similarly, back into the bloodstream) can proceed. This mechanism may provide an explanation for the data of Figs 4 and 5 which show consistently slower renal elimination of the drug than decline of plasma levels.

There is no evidence to suggest the way in which procaine may be sequestered in the urinary tract. Among the possibilities are tissue binding and/or active transport of procaine into renal tissues. While it is possible that procaine is transported into the kidney by the organic base transporting system, this hypothesis runs into the problem of the high lipid solubility of procaine. Thus, though procaine may be readily transported into renal tissue in much the same way as *n*-methylnicotinamide, it should readily diffuse out again by virtue of its lipid solubility and ready movement across cell membranes.<sup>8</sup> The possibility of such rapid movement of procaine in the urinary tract is further attested to by the observations of Evans and Lambert<sup>5</sup> who have shown that urinary levels of procaine are strongly dependent on and vary readily in accordance with urinary pH. Similarly, recent experiments by Tobin and Roberts<sup>12</sup> have shown no effect of furosemide treatment on urinary concentration of procaine in horses, again suggesting rapid equilibration of renal and urinary procaine levels and speaking against transport dependent concentration gradients of procaine in renal tissue.

Another possibility is that procaine is selectively and relatively specifically bound in the equine kidney and only slowly released into the urinary tract. The difficulty with this hypothesis is that, for it to account for the observed data, the procaine-binding site complex would

have to be quite stable. This is because the ease of movement of free procaine into urine<sup>5,12</sup> suggests that the dissociation of procaine from its binding sites would have to be the rate limiting step in the system. However, in support of the hypothesis of "tight" procaine binding in the kidney are the observations of previous experiments which show that similar poorly diffusible pools of procaine were observed after injection of the drug intramuscularly into horses.<sup>9</sup>

In view of the fact that the urinary half-life of procaine in the experiments of Figs 4 and 5 was in the order of about 5 hours or greater, it was of considerable theoretical and practical interest to inject procaine penicillin and study the relationships between plasma and urinary concentrations of the drug. This is because after the injection of procaine penicillin the rate of decline of plasma concentrations of the drug is quite slow, with a  $t_{1/2}$  of about 10 hours,<sup>9</sup> which is slower than the rate of decline of urinary concentrations of the drug. As shown in Fig 7, after intramuscular injection of procaine penicillin in these animals, the rates of decline of plasma levels initially had an apparent half-life in the order of about 8 hours, then later an apparent half-life in the order of about 24 hours or more.

In previous experiments on the pharmacokinetics of procaine in the horse, these authors observed<sup>9</sup> that absorption of procaine penicillin from an intramuscular injection site is a complex process, which starts with a half-time of about 5 hours to account for the plasma concentrations peaking between 1–2 hours but then slows down to a half-time of about 8–10 hours to accommodate the plasma half-life of this drug observed during the 4–24 hour period (Fig 9 in reference #9). Since these rates are all of the same order or slower than the urinary half-lives for procaine observed in previous experiments (Figs 4–6), it appeared possible that for procaine penicillin the rate of absorption from the intramuscular site might be rate limiting and might control the rate of decline of urinary concentrations of the drug.

The data of Fig 7 are in good agreement with this hypothesis, showing that urinary concentrations of the drug at first fell rapidly with an apparent half-life of about 8 hours, paralleling the initial rate of decline of the plasma concentrations. From about 4 days on, however, the rate of decline of the urinary concentrations slowed, in parallel with the slower decline in plasma concentrations of the drug. Toward the end of the experiment, the rate of decline of the urinary concentrations appeared slower than the rate of decline of the plasma concentrations. It is, however, important to note that the rate limiting step in the whole process, i.e., the rate at which procaine was absorbed from its intramuscular site, decreased over the whole time during which it was followed and that it is

likely to continue to decrease. Thus, the apparently very slow terminal half-life for urinary procaine in Fig 7 is probably consistent with a similar slowing in the no longer measurable plasma half-life of procaine.

The increase in urinary levels of procaine between 9 and 11 days is likely due to a change in urinary pH, and similar sharp changes in procaine levels associated with urinary pH changes have been reported by Evans and Lambert.<sup>8</sup> Urinary pH values were not, however, monitored throughout these experiments.

A hypothesis has been presented<sup>6</sup> which suggests that after administration of procaine penicillin, procaine exists in the blood or urine of horses as a "procaine-penicillin" complex. Examining this hypothesis, Tobin et al.<sup>8-11,14</sup> could find no experimental evidence in vitro or in vivo in support of this hypothesis. In good agreement with this conclusion are the experimental data of Evans and Lambert<sup>8</sup> showing similar patterns of variation in urinary procaine concentrations whether or not the procaine was administered as procaine HCl or procaine penicillin. These observations therefore offer further support for the concept that the procaine found in equine urine after dosing with procaine penicillin exists as free procaine.

The practical significance of these results is clear. The experiments confirm earlier reports which suggest that clearance of procaine from the urine of horses is relatively slow. While the actual periods will depend on the sensitivity of the analytical techniques used by the testing laboratory, the data suggest that after administration of small amounts of procaine (i.e., 8 ml of a 2% solution) intravenously, subcutaneously, or intra-articularly, at least 48 hours should be allowed for the urine to clear. After administration of more substantial doses (gram quantities) into muscle or subcutaneous tissues, it is probably prudent to allow at least 6 days. After procaine penicillin, low concentrations of procaine were still detectable in equine urine for up to 2 weeks. Longer than this should obviously be allowed, but how much longer is difficult to say. If a horse is put on an intensive course of procaine penicillin for medical reasons it is possible that months would be required for the animal to clear procaine from its system.

The current practice of most racing authorities of taking only a single postrace urine sample maximizes the potential for penalizing innocent transgressors of the procaine rule. The taking of blood samples with appropriate precautions to prevent hydrolysis of procaine<sup>11</sup> as close to race time as possible, in association with urine samples, would help considerably. Thus, absence of a plasma concentration of procaine in the presence of a urinary concentration would rule out the possibility that the ani-

mal was raced under the pharmacological influence of procaine. In the presence of a plasma concentration of procaine, the problem becomes a determination of whether the plasma concentration is due to a nerve or joint block with procaine HCl or is due to procaine penicillin. To make this determination, access to the animal is necessary. If the animal shows a rapidly declining plasma concentration of procaine ( $t_{1/2}$  approximately 2 hours or less) and becomes lame, the evidence is consistent with a procaine HCl induced nerve or joint block. If the plasma concentration of procaine declines slowly ( $t_{1/2} > 8$  hours) and the animal stays sound, the picture is consistent with administration of procaine penicillin to a

sound animal. A long procaine half-life ( $t_{1/2} > 8$  hours) and an animal rapidly becoming lame is consistent with (a) procaine nerve block and a masking dose of procaine penicillin, or (b) innocent administration of procaine penicillin with lameness developing during or after the race.

In the unlikely event that plasma concentrations of procaine were used in an attempt to improve performance of an animal by virtue of its central stimulant effect,<sup>11</sup> the high plasma and urinary concentrations of the drug required would make this pattern of abuse readily identifiable.

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