

Pharmacology of Procaine in the Horse: Pharmacokinetics and Behavioral Effects

Thomas Tobin, DVM, PhD; J. W. Blake, PhD; L. Sturma, MSc; Steve Arnett; J. Truelove, PhD

SUMMARY

After rapid intravenous injection of procaine HCl (2.5 mg/kg of body weight) in Thoroughbred mares, plasma levels of this drug decreased rapidly ($t_{1/2} = 15.5$ minutes) and then more slowly ($t_{1/2} = 50.2$ minutes). These kinetics were well fitted by a 2-compartment open model (model I). This model gave an apparent V_d for procaine in the horse of about 6.7 L/kg of body weight. Since procaine was about 45% bound to equine plasma protein, this gave a true V_d for procaine of about 12.4 L/kg. After subcutaneous injection of procaine (3.3 mg/kg), plasma levels peaked at 400 ng/ml and then decreased with a half-life of about 65 minutes. These data were well fitted by model I when it was modified to include simple, 1st-order absorption ($k = 0.048 \text{ minutes}^{-1}$) from the subcutaneous injection site (model II). After intramuscular injection of procaine penicillin (33,000 mg/kg), plasma levels peaked at about 270 ng/ml and then decreased with a half-life of about 600 minutes. These data were approximately fitted by model II assuming a 1st-order rate constant for absorption of procaine of $0.0024 \text{ minutes}^{-1}$. After intramuscular injection of procaine HCl (10 mg/kg), plasma levels of procaine peaked rapidly at about 400 ng/ml but decreased slowly (about 60 minutes). A satisfactory pharmacokinetic model for this data could not be developed. An approximation of these data was obtained by assuming the existence of 2 intramuscular drug compartments, one containing readily-absorbable drug and the other poorly-absorbable drug (model III). After intraarticular administration of procaine (0.33 mg/kg), plasma levels of this drug peaked at about 24 ng/ml and then decreased with a half-life of about 97 minutes. These data were not modeled. Absorption of the drug was essentially complete by all routes, since dose-corrected areas under the plasma level curves were all comparable.

Intravenous infusion of procaine showed that behavioral excitation due to procaine was influenced at plasma levels of 600 ng/ml, with horses becoming uncontrollable

at plasma levels of about 1,500 ng/ml. These plasma procaine levels are about one-twentieth of those associated with central nervous system excitation in humans. Horses are thus at least 20-fold more sensitive to the central stimulant action of procaine than are human beings.

The pharmacokinetics and behavioral effects of procaine in the horse are of forensic importance because the presence of procaine in the blood and urine of racing animals is forbidden by most racing authorities.¹⁶ The pharmacokinetics of procaine are complicated further by the routes and forms in which procaine may be administered to racehorses. Procaine may be administered subcutaneously (sc) or intramuscularly (im) for its local anesthetic action associated with minor surgical operation. Alternatively, it may be given im in relatively large amounts as procaine penicillin. These uses of procaine are common in equine medicine and surgical procedures and are, per se, quite acceptable to racing authorities.

Other possible uses of procaine penicillin are regarded with disfavor by racing authorities. These include the use of small amounts of procaine to produce nerve blocks and the direct injection of procaine into inflamed joints. Both of these techniques permit a horse with bone or tendon problems to improve its performance and thus may affect the outcome of a race.

Another less clear-cut aspect of the use of procaine in racing horses is the belief that central effects produced by high concentration levels of procaine are stimulatory and may positively affect the courage or performance of racing horses.⁸ Clear-cut experimental evidence is not presently available in this area, but this possibility, combined with the well-characterized uses of procaine to alleviate lameness, has led racing authorities to ban the use of procaine or procaine-containing preparations in horses about to be raced.

Because of these considerations, horsemen, veterinarians, and equine authorities require information on the pharmacokinetics and behavioral effects of procaine in horses. The veterinarian who administers procaine in any form to racing horses needs to know the plasma concentrations and urinary half-lives of procaine in horses after its administration by various routes so that

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SUMMARY

After rapid intravenous injection of procaine HCl (2.5 mg/kg of body weight) in Thoroughbred mares, plasma levels of this drug decreased rapidly ($t_{1/2\alpha} = 5.5$ minutes) and then more slowly ($t_{1/2\beta} = 50.2$ minutes). These kinetics were well fitted by a 2-compartment open model (model I). This model gave an apparent V_d for procaine in the horse of about 6.7 L/kg of body weight. Since procaine was about 45% bound to equine plasma protein, this gave a true V_d for procaine of about 12.4 L/kg. After subcutaneous injection of procaine (3.3 mg/kg), plasma levels peaked at 400 ng/ml and then decreased with a half-life of about 65 minutes. These data were well fitted by model I when it was modified to include simple, 1st-order absorption ($k = 0.048 \text{ minutes}^{-1}$) from the subcutaneous injection site (model II). After intramuscular injection of procaine penicillin (33,000 IU/kg), plasma levels peaked at about 270 ng/ml and then decreased with a half-life of about 600 minutes. These data were approximately fitted by model II assuming a 1st-order rate constant for absorption of procaine of $0.0024 \text{ minutes}^{-1}$. After intramuscular injection of procaine HCl (10 mg/kg), plasma levels of procaine peaked rapidly at about 600 ng/ml but decreased slowly ($t_{1/2} = 125$ minutes). A satisfactory pharmacokinetic model for this data could not be developed. An approximation of these data was obtained by assuming the existence of 2 intramuscular drug compartments, one containing readily-absorbable drug and the other poorly-absorbable drug (model III). After intraarticular administration of procaine (0.33 mg/kg), plasma levels of this drug peaked at about 24 ng/ml and then decreased with a half-life of about 97 minutes. These data were not modeled. Absorption of the drug was essentially complete by all routes, since dose-corrected areas under the plasma level curves were all comparable.

Intravenous infusion of procaine showed that behavioral excitation due to procaine commenced at plasma levels of 600 ng/ml, with horses becoming uncontrol-

lable at plasma levels of about 1,500 ng/ml. These plasma procaine levels are about one-twentieth of those associated with central nervous system excitation in human beings. Horses are thus at least 20-fold more sensitive to the central stimulant action of procaine than are human beings.

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these animals will not inadvertently be raced with detectable concentrations of procaine in their body fluids. Racing authorities also require this information to enable them to interpret and rule on reports from analytical laboratories. A knowledge of the pharmacokinetics of procaine after administration by different routes may also enable analytical laboratories to identify the route or form in which the drug was administered and thus assist decision making by racing authorities.¹⁶ Finally, evidence is not presently available on blood concentrations of procaine required to produce central nervous system stimulation in horses and any possible relationship between these blood values and clinically used doses of procaine.⁶ The purpose in the present study was to provide answers to these questions.

Materials and Methods

Drug Administration and Sample Collection—Mature Thoroughbred mares, weighing between 400 and 540 kg, were used. These animals were housed in individual loose boxes and fed hay and water ad libitum. All drug administration, infusions, behavioral observations, and sample taking were done in the animals' loose boxes. For drug infusion experiments, the horse was tied with a halter and head shank at the door of the loose box. A 0.060 OD polyethylene cannula was placed in the animal's jugular vein and threaded down the vein for about 45.72 cm. Procaine HCl^a (660 mg/ml) then was infused intravenously (iv) at rates of between 0 and 2.0 ml/minute. Since plasma concentrations at which excitement would occur were not known when these experiments were started, drug infusion was usually started at a low rate and later accelerated. When excitement occurred, a certain amount of risk was associated with obtaining blood samples, so the infusion was occasionally switched off for short periods. These experiments usually were terminated when the animal entered into a phase of excitement and backed forcefully away from the restraining rope. To accommodate this response, a triple loop of white twine of about 9 kg breaking strain was included in the tie-up as a weakest link. The jugular catheter pulled out spontaneously on breakaway and sample collection ceased.

Subcutaneous injections of procaine HCl^b were made between the forelimbs over the cranial and caudal superficial pectoral muscles. All *im* injections (procaine HCl^c and procaine penicillin^d) were made deep into the muscles on the side of the neck. Intraarticular injections^b were made into the intercarpal sac of the carpal joint, using aseptic precautions. The knee (carpal) joint was flexed at the time of injection and synovial fluid drawn into the syringe before the injection was completed.

Blood samples were obtained by jugular venipuncture into heparinized tubes (partial vacuum) containing arsenite and physostigmine as described previously.²² Urine samples were collected either by catheterization of the bladder or on natural voiding of urine. All plasma and urine samples were stored overnight at 30 C for analysis the following day. Appropriate plasma and urine with known added standards were frozen with samples to correct for any drug breakdown during the holding. Procaine HCl was prepared (for injection) by dissolving in about 30 ml of double-distilled water and sterilized by drawing it through 45- μ m grid filters. This procaine ran as a single spot on chromatography in

methanol or chloroform:methanol on either silica gel or alumina thin layer plates.²³

Chemical Methods—Two assay methods for procaine were used. One method, a modification²⁴ of the colorimetric method of Brodie et al.,²⁵ was performed as described by Tobin et al.,²⁶ except that the volume of blood or urine extracted was increased to 8 ml and the volume of benzene was decreased to 8 ml. In the transfer into HCl step, the volume of HCl was reduced to 1 ml to increase intensity of the color response. These modifications substantially increased the sensitivity of this colorimetric method and produced minimal changes in the recoveries obtained (Fig 1).

The 2nd assay method for procaine was the derivatization-gas chromatographic procedure described by Blake et al.²⁷ In this method, 1 ml of saturated sodium tetraborate and 2 ml of benzene were added to 4 ml of the biological fluid, and the whole system was shaken for 10 minutes. The system then was centrifuged at 5,000 \times g for 10 minutes, and the benzene layer was transferred to a tube containing 50 μ l of 5% pyridine in benzene and 50 μ l of heptafluorobutyric anhydride (HFBA). The system was allowed to react at room temperature for 3 minutes, 5 ml of saturated sodium tetraborate was added, and the system was shaken. After centrifugation, the benzene layer was separated and used for gas chromatographic analysis.

Gas chromatography was done with a gas chromatograph⁴ equipped with an electron capture detector. Injection temperature was 260 C onto 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 to detect about 5 ng of procaine/ml. The results obtained with this method correlated well with those obtained by the colorimetric method.

Pharmacokinetic analysis of these data was carried out with the assistance of the SAAM-23 program¹ as described by Kostenbauder et al.² The *iv* data (Fig 3) were fitted to a 2-compartment open model (model I) by fixing 1/the apparent volume of distribution of the central compartment (V_c) at 9×10^{-2} ml (obtained graphically) and allowing all other values to float. The line of best fit and the variables calculated from this model are given (Fig 3; Table 1). In subsequent analyses, these variables were fixed at the *iv* values (Table 1), and values for the new rate constants introduced were selected by iteration (i.e., allowed to float).

Unless otherwise noted, all experimental values are the means \pm SEM of determinations on at least 4 experimental Thoroughbred mares.

Results

Because central nervous system stimulation in the horse occurs at much lower plasma concentrations of procaine than in persons, the sensitivity of the usual analytical method had to be improved. The simple modifications (outlined in Materials and Methods) made the colorimetric method¹⁵ effective at concentrations as low as 20 ng/ml, adequate for most aspects of the present study. Recoveries of various concentrations of procaine added to water, plasma, and urine samples are shown (Fig 1); 90% or more of the added procaine was recovered at all concentrations tested.

Because this colorimetric method for procaine was more likely to be susceptible to the presence of interfering compounds than gas chromatographic methods, this method was compared with the HFBA method re-

^a Sigma Chemical Company, St. Louis, Mo.

^b 2% Novocaine, Winthrop, Ltd, New York, NY.

^c Duracillin, Eli Lilly and Company, Indianapolis, In.

⁴ Perkin-Elmer 3920, Perkin-Elmer Corporation, Norwalk, Ct.

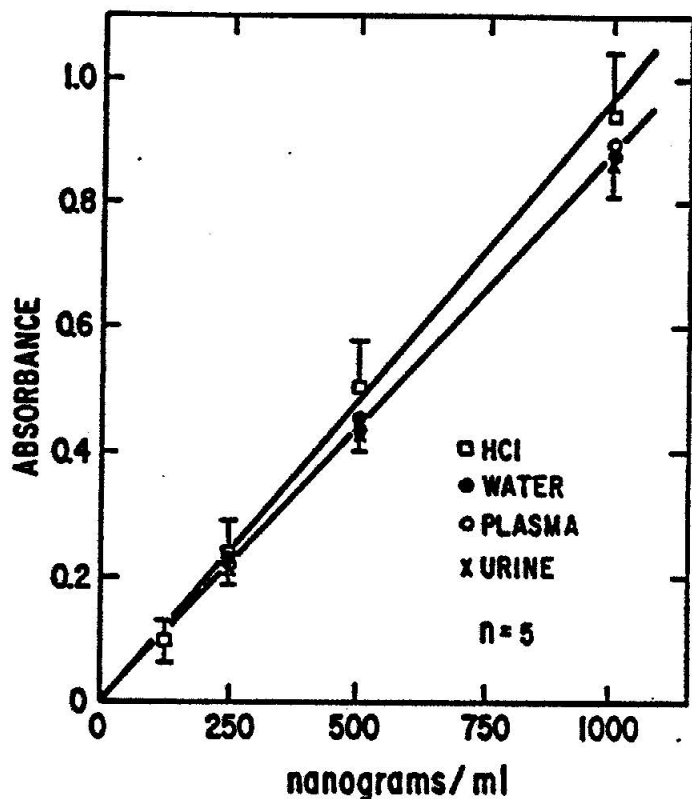


Fig 1—Recovery of procaine from equine plasma and urine—The indicated concentrations of procaine were added to 60 mM phosphate buffer (pH 7.4; ●—●), equine plasma (○—○), or equine urine (x—x). Recovery and estimation of procaine was done and compared with the color reactions obtained when similar quantities of procaine were added directly to HCl (□—□). Each point is the mean of 5 separate determinations. Vertical bars = SEM obtained in the direct addition of HCl and plasma experiments.

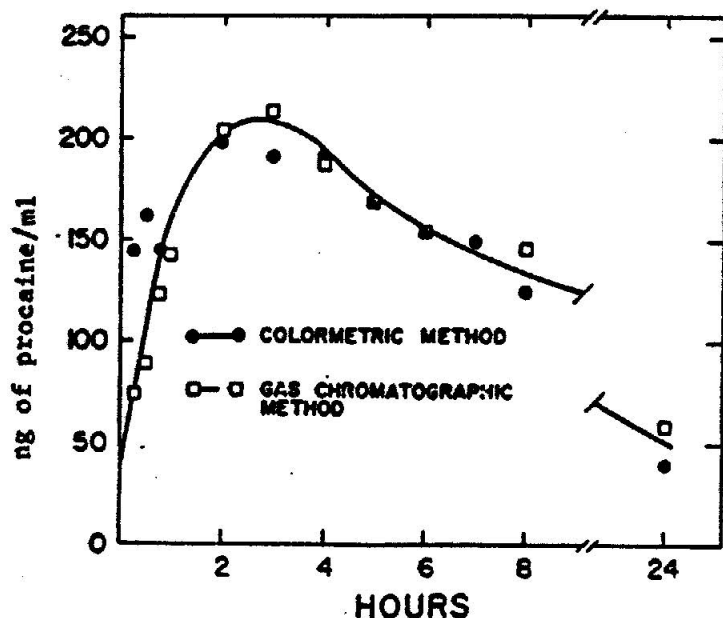


Fig 2—Comparisons of colorimetric and heptafluorobutyric anhydride (HFBA) determinations of plasma procaine concentrations—A horse was administered 33,000 IU of procaine penicillin intramuscularly (IM) at 0 time, and blood samples were collected at the indicated times. Plasma concentrations of procaine (●—●) were determined by the colorimetric method, and plasma concentrations (□—□) by HFBA determination and gas chromatographic determination. All points represent single experimental determinations.

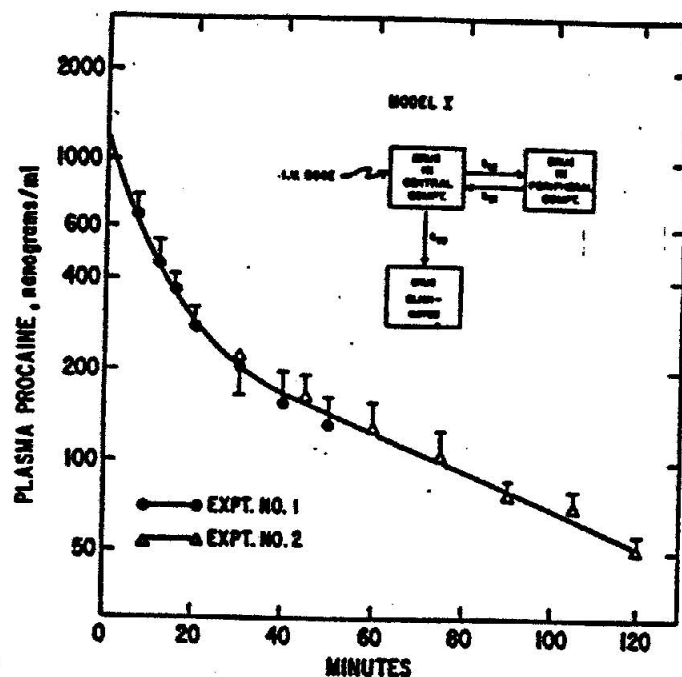


Fig 3—Plasma concentrations of procaine after rapid intravenous (IV) injection of procaine HCl—Procaine HCl (2.5 mg/kg) was administered to horses by rapid IV injection. Plasma procaine concentrations (●—●; △—△) were observed in 2 separate experiments. The solid line is that obtained from an SAAM-23 program fit of the data to the differential equations describing the 2-compartment open model (model I), using a weight of $1/SD^2$ for all data points. The rate constants k_{12} , k_{21} , and k_{10} are all 1st-order rate constants, and all were iterated. Values for variables obtained from this analysis are presented in Table 1.

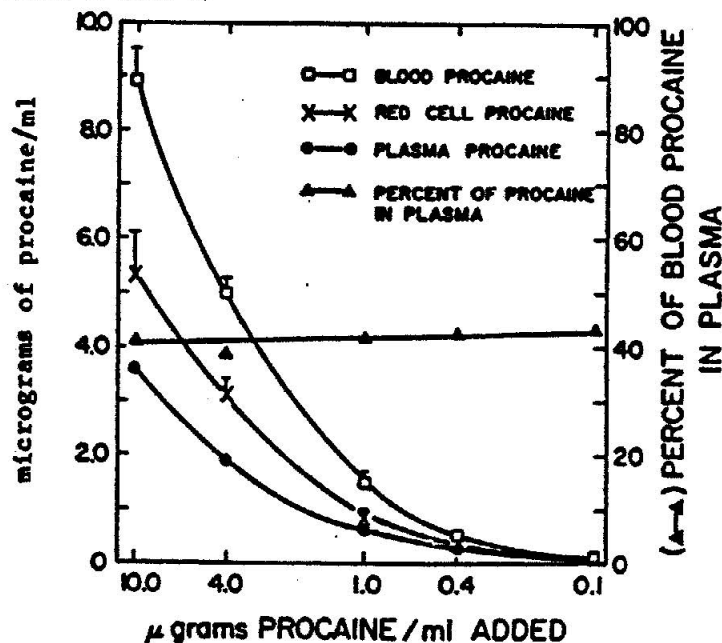


Fig 4—Blood plasma partitioning of procaine—The indicated concentrations of procaine were added to 15 ml of equine blood containing 1×10^{-5} M physostigmine. The system was incubated for 30 minutes at 37°C with shaking to allow equilibration of the drug. The blood then was centrifuged at $5,000 \times g$ for 10 minutes, and portions of the plasma and erythrocyte fractions taken for procaine determination; □—□ = the calculated recovery at each concentration from the erythrocyte and plasma fractions; x—x = the procaine concentration found in the erythrocyte fraction; and ●—● = plasma procaine concentrations; ▲—▲ = the percentage of blood procaine found in the plasma fraction at the various concentrations of procaine added to the system. All experimental points are the means \pm SEM of 4 separate experimental determinations.

TABLE 1—Pharmacokinetic Variables for Procaine After Intravenous Administration Calculated from the SAAM-23 Program (Model I)

Kinetic variables	Mean \pm SE
k_{12}	$0.057 \pm 0.003 \text{ minute}^{-1}$
k_{21}	$0.041 \pm 0.003 \text{ minute}^{-1}$
k_{10}	$0.044 \pm 0.004 \text{ minute}^{-1}$
V_c	1.115 L or 2.11 L/kg
$V_{d\beta}$	3.547 L or 6.71 L/kg
A	905 ng/ml
B	287 ng/ml
α	$0.1263 \text{ minute}^{-1}$
$t_{1/2\alpha}$	5.49 minutes
β	$0.0136 \text{ minute}^{-1}$
$t_{1/2\beta}$	50.2 minutes
clearance	$1.19 \text{ L/minute}^{-1}$

TABLE 2—Absorption Variables for Procaine After Intramuscular Administration Calculated from the SAAM-23 Program (Model III)

Kinetic variable	Mean \pm SE
k_{12}	$0.112 \pm 0.55 \text{ minute}^{-1}$
k_{21}	$0.773 \pm 4.5 \text{ minute}^{-1}$
k_{10}	$0.057 \pm 0.008 \text{ minute}^{-1}$

ported by Blake et al.² The data show a good correlation between blood levels of procaine after IM injection of procaine penicillin when measured by either method (Fig 2). Because of simplicity and rapidity of the colorimetric assay, this was the most frequently used method for determination of procaine values in the present study.

Plasma concentrations of procaine after rapid iv injection of 2.5 mg of procaine HCl/kg are given (Fig 3). The 1st series of experiments (solid circles ●-●) showed a rapid decrease in plasma procaine, as reported previously.¹⁰ This decrease was, however, clearly nonlinear. Since this nonlinearity suggested the existence of a slower β phase of drug elimination that had not been identified previously,¹⁰ the present study was extended to examine the time course of the decrease in plasma procaine concentrations for up to 2 hours. These data points (solid triangles ▲-▲) show a clear-cut β portion in the elimination curve. The solid line associated with the data points is that obtained from an SAAM-23 program fit of the data to the differential equations describing the 2-compartment open model (model I; Fig 3) with iv injection, using a weight of $1/(\text{SE})^2$ for all data points. In model I, k_{12} and k_{21} are 1st-order rate constants for intercompartmental drug transfer, and k_{10} is a 1st-order rate constant for drug elimination from the central compartment. The variables k_{12} , k_{21} , and k_{10} were iterated. The apparent volume of distribution of the central compartment (V_c), volume of distribution ($V_{d\beta}$), and half-life ($t_{1/2\beta} = 0.693/\beta$) were calculated from appropriate relationships. Variables obtained from this data analysis are presented (Table 1). The half-life for the α or redistribution phase of this curve is approximately 5 minutes, with the half-life of the β or metabolic phase being 50.2 minutes. The true plasma half-life for procaine after iv injection is longer than had been suspected from preliminary experiments¹⁰ and the data suggest that the pharmacokinetics of procaine in the horse after rapid iv injection are adequately described by a 2-compartment open model.

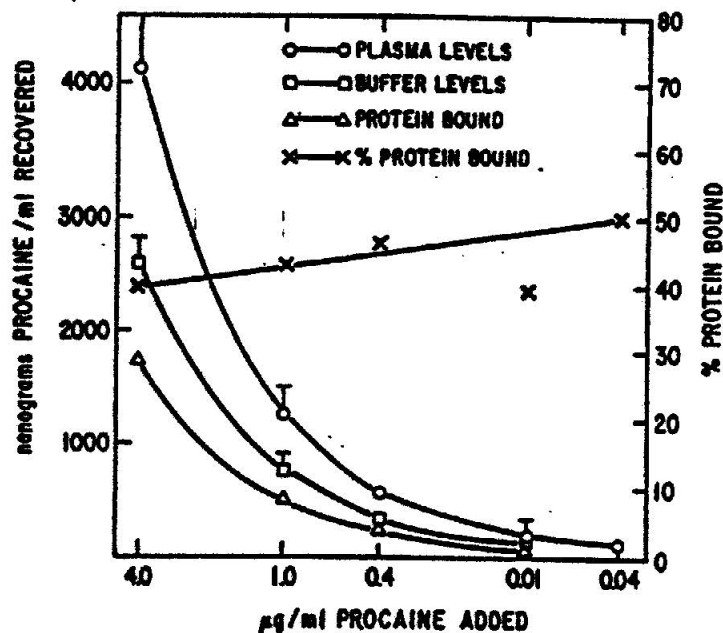


Fig 5—Plasma protein binding of procaine—The indicated concentrations of procaine were added to 45 ml of 50 mM phosphate buffer (pH 7.4, containing 1×10^{-4} M physostigmine) in which 5 ml of equine plasma were suspended in a dialysis bag. The system was incubated at 37 C with vigorous shaking for 16 hours. At the end of this period, 4-ml aliquots of the plasma and buffer were removed, and their procaine contents were measured. ○—○ = Procaine concentrations in plasma; □—□ = procaine concentrations in the phosphate buffer; ▲—▲ = the calculated concentrations of procaine plasma protein bound at each drug concentration; X—X = the percentage of procaine plasma protein bound at each drug concentration. All points are the means \pm SEM of at least 4 separate experimental determinations.

After the rapid iv injection of 2.5 mg of procaine/kg of body weight, signs of central nervous system excitation in the horses appeared within 30 to 40 seconds. The 1st sign of excitation was usually deep, blowing respiration. After about 1 minute, the horses paced unsteadily in their loose boxes; this pacing continued for about 3 to 4 minutes. Some animals were noticeably incoordinated while pacing, whereas others seemed well coordinated. By the end of the 4th minute, the horses had quieted considerably and in some cases had resumed feeding. Further signs of central nervous system stimulation were not seen in these horses or in horses injected with equivalent volumes of saline solution or distilled water.

To calculate the true volume of distribution, a knowledge of the percentage of drug bound to plasma protein under conditions shown (Fig 3) is required. In whole blood, significantly greater concentrations of procaine were found in the red blood cell fraction as compared with plasma, which agrees with results of previous experiments.¹¹ Similarly, in the plasma fraction, a considerable proportion (up to 50%) of the procaine recovered was procaine that had been bound to plasma protein. Therefore, at blood procaine concentrations of 100 ng/ml, only about 40 ng/ml are present in the plasma fraction, and only about 22 ng of this 40 ng are actually free in the plasma (Fig 4 and 5). The concentration of free procaine in the bloodstream of the horse

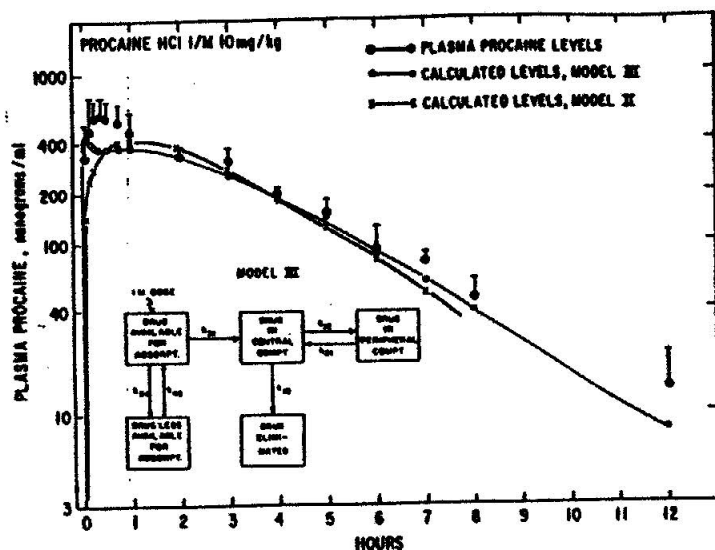


Fig 8—Plasma procaine concentrations after IM administration of 10 mg of procaine HCl/kg—Procaine HCl was administered by IM injection in the side of the neck to 4 horses. ●—● = The plasma concentrations of procaine observed at the indicated times (\pm SEM); X—X shows the best fit to these data calculated from the differential equations describing model II (Fig 7) with k_{12} , k_{21} , and k_{10} held at the values presented in Table 1; ○—○ shows the best fit to these data calculated from the differential equations, describing model III with k_{12} , k_{21} , and k_{10} iterated and the other rate constants held at the values of Table 1. A least squares fit to the plasma values from 22 minutes on gives a half-life time for plasma concentrations of procaine after its IM injection of 2.5 hours.

iterated. The final value of k_{21} was $0.048 \pm 0.005 \text{ min}^{-1}$. The experiment shows relatively rapid absorption of procaine after its sc injection. Consistent with previous data (Fig 6), signs of central nervous system excitation were not seen in these horses which attained plasma procaine levels of only 400 ng/ml.

Plasma concentrations of procaine observed after the IM administration of 10-mg levels of procaine HCl/kg are given (Fig 8). The drug was rapidly absorbed with peak plasma concentrations attained at 20 minutes, followed by an exponential decrease in plasma concentration with apparent half-life of 125 minutes. In this series of experiments, the mean peak plasma levels obtained were about 600 ng/ml, at which signs of central nervous system excitation may be expected. Transient signs of central nervous system excitation were seen in 3 of 9 horses dosed with 10 mg of procaine/kg in the present and related studies. At this point, blood samples were not taken from these excited horses, however, because the laboratory personnel were inexperienced in handling horses excited with procaine. This experiment thus suggests that plasma values greater than 600 ng of procaine/ml are required to produce signs of central nervous system stimulation in horses, which agrees with results of earlier experiments.¹⁰

Inspection of the plasma levels presented in Figure 8 shows that peak concentrations were attained rapidly. Nevertheless, the half-life for drug elimination was much slower than the half-life after sc or iv injection. It was not possible to fit this average (or individual animal data) to a model, assuming simple 1st-order absorption (model II). The dotted line represents the best fit obtained from the SAAM-23 program to model

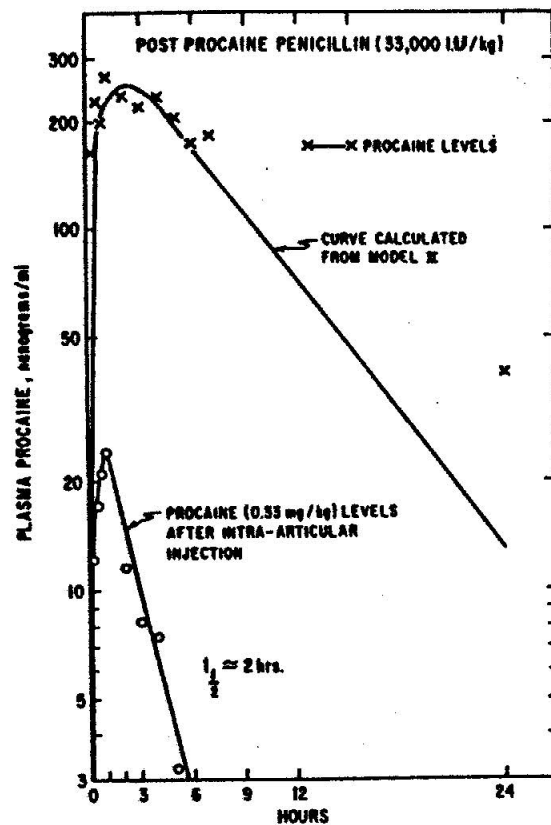


Fig 9—Plasma procaine concentrations after IM injection of procaine penicillin or intraarticular injection of procaine HCl; The X—X show plasma procaine concentrations after deep IM injection of 33,000 IU of procaine penicillin/kg into the side of the neck. (These data are replotted from Fig 3 of Tobin et al.,¹⁰ 1976.) The solid line shows the best fit to these data calculated from the differential equations describing model II, with k_{12} , k_{21} , and k_{10} held at the value presented in Table 1. The ○—○ show plasma procaine concentrations after intraarticular injection of 0.33 mg of 2% procaine/kg. The solid line was fitted by eye. All experimental points are the means of at least 4 individual experimental determinations.

TABLE 3—Absorption Rate Constants and Calculated Areas Under Plasma Concentration Curves

Route of administration	$\int_0^{\infty} \text{Cpdt}$	Rate constant (min ⁻¹)
Intramuscular (IM; set at 1)	122,900	0.067*
Intravenous (IV; X 4)	110,780	
Subcutaneous (SC; X 3)	132,000	0.048
Procaine penicillin (IM; X 0.66)	136,774	0.0024
Intraarticular (X 30)	111,060	

* Slowest or rate limiting rate constant. All areas corrected for dose by arbitrarily setting the IM dose at 1.

II. In this fit, the variables k_{12} , k_{21} , and k_{10} were held constant at the values shown in Table 1, and the additional variable k_{31} (the 1st-order rate constant for absorption of the injected drug) was allowed to float. It is apparent that, while this model can approximately fit the prolonged plasma half-life times noticed, it cannot account for the high initial plasma concentrations observed.

The solid line represents that obtained from a SAAM fit to model III (Fig 8). This model assumes a pre-absorption equilibrium which gives rise to rapidly absorbed and slowly absorbed compartments of drug at the injection site. As with model II, the variables k_{12} ,

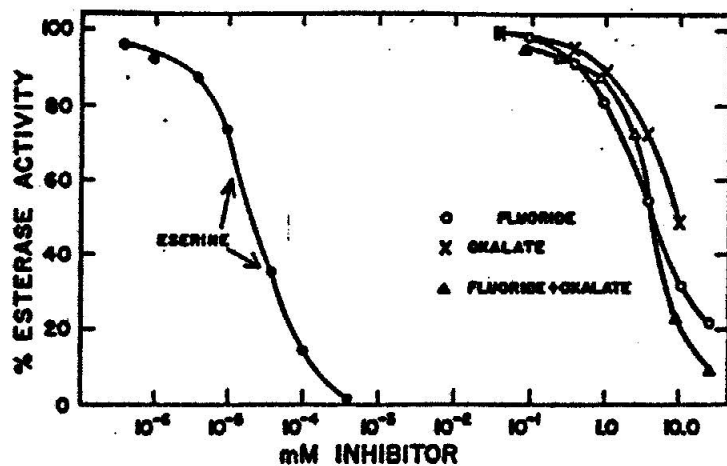


Fig 10—Inhibition of equine plasma esterases by fluoride or oxalate or by these agents combined—Inhibition of equine plasma esterases by the indicated concentrations of physostigmine (●—●), sodium fluoride (○—○), oxalate (×—×), and fluoride plus oxalate (△—△) are shown. Data points are the means of 3 individual experiments.

k_{21} , and k_{10} were held constant at the values shown in Table 1. The additional variables k_{21} , k_{24} , and k_{43} were iterated. The values obtained for these variables are given (Table 2).

Two other commonly used routes of administration are intraarticularly (for procaine HCl) and IM for procaine penicillin. Blood levels of procaine (X—X) obtained after IM injection of 33,000 IU of procaine penicillin/kg are given (Fig 9). The solid line represents the SAAM-23 program fit to model II. As previously, variables k_{12} , k_{21} , and k_{10} were held at the values of Table 1, and k_{21} was iterated. The final value obtained for k_{21} was 0.0024 ± 0.00018 minutes⁻¹.

Of at least 20 horses administered procaine penicillin IM at doses of 33,000 IU/kg, only 1 horse showed signs of central nervous system excitation. These signs consisted of rapid blowing expirations and were observed briefly about 5 minutes after injection. The plasma procaine concentrations in this horse were atypical, peaking at about 2 minutes and decreasing rapidly thereafter. This pattern was in contrast to the slower peaking and decrease in plasma procaine concentrations usually found in these animals (Fig 9).

Blood plasma levels of procaine observed after intraarticular injection of 0.33 mg of 2% procaine HCl/kg are given (Fig 9). Relatively low peak plasma levels of procaine were attained at about 1 hour; these levels decreased rapidly. Because of the uncertainty of measurement at these low plasma levels, computer fit to these data was not attempted. Nevertheless, the relatively slow peaking of plasma levels and the prolonged apparent half-life of the drug after administration by this route suggest slow absorption of the drug from intraarticular injection sites. Signs of central nervous system excitation were not seen in horses administered procaine intraarticularly.

An important technical problem in pharmacokinetic or forensic work with procaine is the necessity to have an effective inhibitor of procaine esterase present in the test tubes into which blood samples are drawn.¹¹ Although physostigmine or arsenite may readily be added

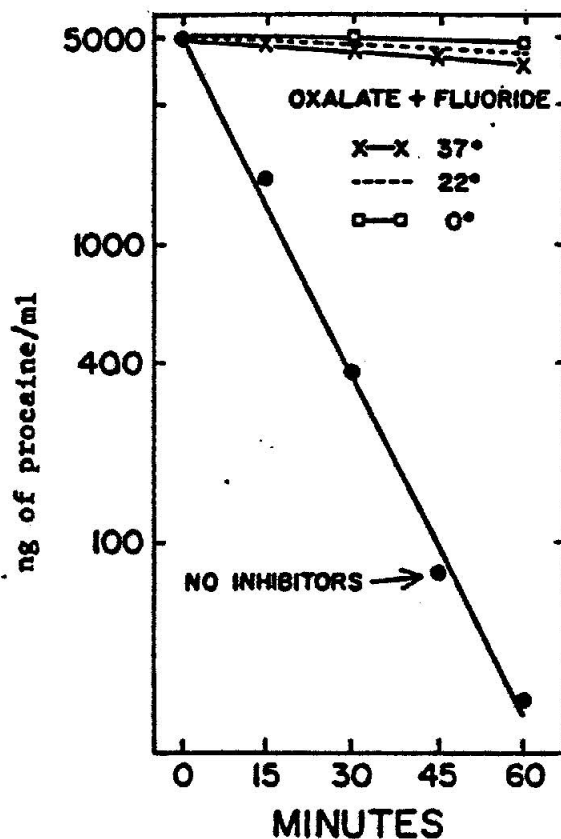


Fig 11—Effect of oxalate, fluoride, and cooling on the rate of hydrolysis of procaine in equine blood; ●—● = the rate of hydrolysis of procaine added to equine plasma at 37°C; ×—× and □—□ = the rate of hydrolysis of blood drawn into tubes containing 12 mM potassium oxalate and 60 mM sodium fluoride and incubated at 37, 22, or 0°C, respectively. All data points are the means of 3 individual experimental determinations.

to the small numbers of vacutainer tubes used in pharmacokinetic work, such additions are too cumbersome for use in connection with routine blood screening of drugs. Since vacuum tubes containing fluoride and oxalate are commercially available, the ability of these agents to inhibit equine plasma esterases was studied. Fluoride and oxalate are relatively poor inhibitors of equine plasma esterases, either alone or in combination (Fig 10). However, when these agents were combined with cooling (Fig 11), the rate of hydrolysis of procaine by whole equine blood was decreased to a rate about 0.01 of that occurring in equine plasma at physiologic temperatures.

Discussion

After rapid IV injection of procaine, plasma concentrations of this drug decreased rapidly at first and then more slowly. These data (Fig 3) are well fitted by a 2-compartment open model (model I), assuming the various rate constants and variables described (Table 1). The apparent volume of distribution V_{dS} of 3,456 L is an underestimate of the actual volume of distribution of the drug, since procaine is about 45% bound to equine plasma proteins at pharmacologic concentrations (Fig 4 and 5). The true volume of distribution of procaine in the horse is therefore about 5,450 L, or about 14 times the volume of these horses. Thus, procaine is extensively tissue-bound in the horse.

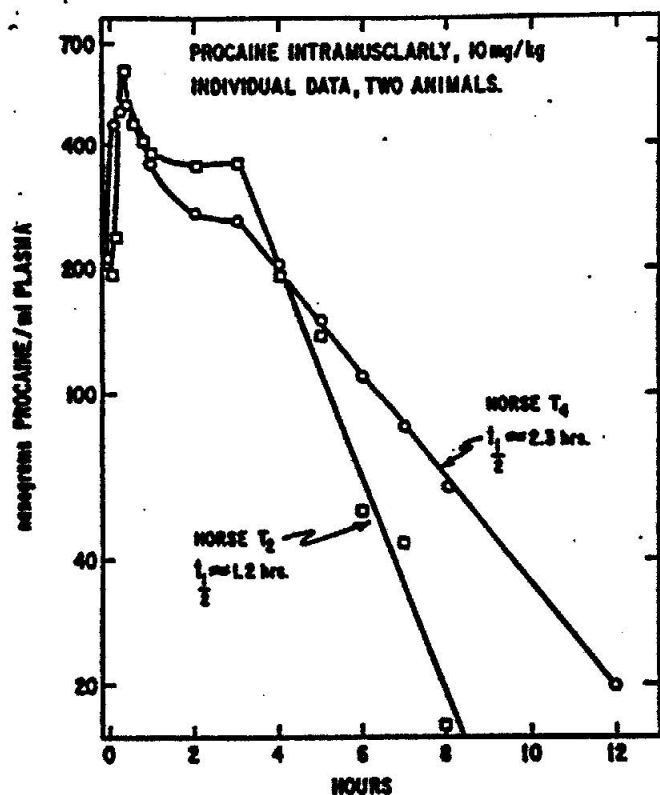


Fig 12—Plasma procaine concentrations after injection in individual horses—The open squares (\square) and circles (\circ) represent plasma concentrations observed in horses T2 and T4, respectively, after IM injection of procaine HCl (10 mg/kg). Data points are individual determinations in animals from the experiment of Figure 8 and the curves were fitted by eye.

The plasma half-life of procaine in the horse ($t_{1/2\beta} \pm 50.2$ minutes) is considerably longer than might be expected in view of the relatively rapid hydrolysis of procaine occurring in equine plasma.¹¹ In preliminary experiments on the pharmacokinetics of procaine in the horse, Tobin et al¹⁰ reported a plasma half-life for procaine in the horse of about 25 minutes and failed to distinguish distinct α or β phases. However, the 1st experiment reported in Figure 3 clearly suggested the presence of a slower β phase of elimination. Increasing the period of this experiment and improving the sensitivity of the detection method demonstrated a slower linear phase of drug elimination. The rates constant for this elimination step were taken as the rate constant for the elimination phase in all subsequent data analyses.

After sc administration of procaine, plasma values increased relatively rapidly to peak at 20 minutes and then decreased exponentially with a half-life of about 65 minutes. This pharmacokinetic pattern is consistent with relatively rapid absorption of procaine from a single subcutaneous compartment by a 1st-order process, with drug distribution and elimination following the patterns determined in model I. Thus, modification of model I by inclusion of a single compartment from which procaine is absorbed by a 1st-order process (model II) at a rate of about 0.048 minutes⁻¹ gave a good fit to the data. This model, however, predicts a terminal plasma half-life for procaine of about 50 minutes, while the actual plasma half-life observed was about 65 minutes. The experiment shows that at least a portion of

the procaine injected sc was only slowly available for entry into the bloodstream.

After IM administration of procaine, the pharmacokinetic pattern described by the pooled data was deceptively simple. Plasma procaine concentrations again peaked rapidly, consistent with relatively rapid absorption of procaine after IM injection (Fig 8). However, despite this evidence for rapid absorption of procaine, the apparent half-life of procaine in plasma after IM injection was about 125.5 minutes. This relatively prolonged plasma half-life of procaine is suggestive of slow absorption of the drug from the IM injection site.

This apparent contradiction is demonstrated quantitatively (Fig 8). The solid line with crosses (X-X) shows the best fit to the data points obtained, using model II, the variables of Table 1, and allowing the 1st-order absorption rate to float. The plotted line shows that, while model II can give a good fit to the terminal half-life portion, it cannot account for the initial high plasma procaine values observed in the present experiments. Similarly, simple zero-order absorption from a single pool fitted to model II did not account for the pharmacokinetic pattern observed.

Inspection of data plots for the individual animals (Fig 12) shows that the pooled data (Fig 8) mask the presence of a distinct discontinuity in the individual pharmacokinetic patterns. Intuitively, this discontinuity appears consistent with the presence of at least 2 separate absorption processes, one proceeding rapidly and giving rise to the high initial plasma levels of the drug, the other proceeding slowly and accounting for the prolonged apparent plasma half-life of the drug. Therefore, the individual data plots (Fig 12) and the pooled data (Fig 8) were fit to model III. This model assumes the existence of 2 compartments at the injection site, one of which contains drug readily available for absorption and the other containing drug poorly available for absorption.⁵ The solid line with the open circles (\circ - \circ) of Figure 8 represents the best fit obtainable with this model to the pooled IM data. The experiment shows that this gives a plot which is qualitatively similar in shape to individual data plots. However, though the qualitative shape of this curve is satisfactory, the quantitative fit to either the pooled data (Fig 8) or to the data points for individual animals (not shown) was less than satisfactory. It is apparent that the pharmacokinetics of procaine after IM administration are determined by unexplained factors resulting in accelerated and delayed absorption of the drug from at least 2 distinct compartments. However, absorption of the drug is nevertheless complete (Table 3).

After IM injection of procaine penicillin, early plasma concentrations of the drug were well accounted for by the simple 1st-order absorption model (model II), assuming a rate constant for absorption of about 0.0024 minutes⁻¹. This absorption rate is about 0.05 of the rate of absorption occurring after the sc injection of procaine, or of the rate-limiting step in the IM model (model III) presented. This slow rate of absorption is consistent with generally accepted explanations for prolonged plasma concentrations of procaine penicillin.¹⁶

TABLE 4—Observed Plasma Half-Lives Compared with Those Generated by Kinetic Models

Route of administration	Model $t_{1/2}$ (minutes)	Observed $t_{1/2}$ (minutes)
iv procaine	50.2	50.2
sc procaine	50.0	65.7
im procaine	101.0	125.5
im procaine penicillin	300.0	602
intramammary	-----	97.1

The measured $t_{1/2}$ values were from least squares fits to all plasma concentrations observed after peak plasma concentrations were attained. The model $t_{1/2}$ values were taken from the computer-generated curves.

It should be noted, however, that the fit afforded by this model at the 24-hour time point is poor. Further, the plasma half-life for procaine predicted by the SAAM fit to the data (Fig 9) is about 5 hours, or half the plasma half-life actually observed (10 hours; Table 4). This discrepancy is consistent with recent experiments in this laboratory which show prolonged tailing of low concentrations (10 ng/ml) of procaine in equine plasma for up to 5 days after im administration of procaine penicillin. These experiments again suggest the existence of a small pool of very slowly absorbed procaine in horses given procaine as procaine penicillin.

After intraarticular injection of procaine, plasma values peaked at 1 hour and decreased thereafter with a half-life of about 97 minutes. Because of the very low plasma concentrations observed and the complex individual pharmacokinetic patterns obtained, attempt was not made to fit these data to a pharmacokinetic model. However, the 60-minute time to peak plasma concentrations by this route and the slow decrease in plasma concentrations of the drug ($t_{1/2} = 100$ minutes) suggest that absorption from the intraarticular site also is hindered compared with absorption from the sc site.

Reviewing these pharmacokinetics, it is apparent from the iv data that procaine crosses cell membranes readily and distributes widely in the body of the horse. These data are consistent with the data of Usubiaga et al¹⁷⁻¹⁹ who showed that procaine entry into the cerebral spinal fluid of dogs has an average half-time of about 5 minutes, which agrees with the 5-minute half-time for the distribution phase (Fig 3). However, despite this evidence for good lipid solubility and easy movement of procaine across cell membranes, procaine injection by any route other than iv showed signs of slower absorption than might be predicted from the iv data. Absorption was apparently complete, however, since the areas under the plasma level curves are all comparable (Table 3). These data all point to delayed absorption of procaine and thus presumably to tissue binding of the drug. The pharmacologic or chemical basis of this binding is not clearly understood. However, recent experiments from this laboratory have also shown evidence suggestive of procaine binding in renal tissues with prolonged release of procaine into urine.

When plasma levels of procaine of more than 600 ng/ml were attained, signs of central nervous system excitation became apparent. The 1st signs were usually deep blowing expirations. If the plasma levels of procaine continued to increase, pacing and signs of agitation commenced. In restrained horses, instead of pac-

ing, there was rapid shifting from leg to leg and occasional pawing with the forefeet. In unrestrained horses, pacing took the form of either to and fro weaving movements or circling. Pacing and circling were well coordinated in some animals, but poorly coordinated to the point of staggering in others. All animals with plasma procaine concentrations greater than 600 ng/ml showed an accentuated startle response and fine muscle twitching along their backs and haunches. In restrained horses, this startle response was followed by their backing away and usually termination of the experiment. Though often incoordinated, no horses went down after any dose was given. In 1 preliminary experiment, an animal administered 4 g of procaine iv went down about 120 seconds later and had severe difficulty respiring and exhibited tonic-clonic convulsions for about 4 minutes. After 10 minutes, it regained its feet and its behavior was essentially normal at 30 minutes.

The plasma concentrations of procaine associated with central nervous system stimulation in the horse are low in comparison with those reported in other species.⁷ Studying the passage of procaine across the human maternal-fetal barrier, Usubiaga et al¹⁷ observed maternal plasma values of procaine of up to 16 μ g/ml, while the only signs of symptoms in these patients were transient shivering, apprehension, and mental confusion. The latter experiments suggest that human patients just approaching the threshold of excitation had plasma levels of procaine about 20 times greater than those of horses approaching the threshold of excitation. Similarly, Usubiaga et al^{18,19} studying the onset of convulsions in unpremedicated human beings, reported mean plasma concentrations of about 38 μ g/ml associated with the onset of convulsions. These concentrations are also about 20 times the plasma concentrations at which the horses in the present study became uncontrollable and broke away.

These observations agree with those of Green et al⁴ who reported blood values of procaine of between 3.6 and 11.0 μ g/ml in patients immediately after im administration of large doses of procaine penicillin. In Green's study,⁴ only 3 patients showed transient behavioral changes which were attributed to these plasma values of procaine.

In other experiments, Kunde and Frey⁶ studied plasma concentrations and behavioral effects after the subcutaneous administration of 5 mg of procaine/kg to horses. These authors observed plasma concentrations of procaine in the order of about 600 ng/ml, which agree with the plasma concentrations reported in the present study. These authors saw no signs of central nervous system excitation in the horses, consistent with the fact that 600 ng/ml is the highest plasma concentration at which no signs of central nervous system excitation were observed in the present study. Kunde and Frey,⁶ however, further suggested that no central nervous system effects might be expected from plasma concentrations of this magnitude, an argument presumably based on plasma concentration response data obtained in other species. The results presented here, however, which emphasize the sensitivity of the horse

to low plasma procaine concentration, suggest that Kunde and Frey were much closer to obtaining signs of central nervous system stimulation in horses than they may have suspected.

Both Green et al⁴ and Kunde and Frey⁵ used high concentrations of NaOH in their recovery procedures for procaine. Since the ester bond of procaine is hydrolyzed rapidly in NaOH,¹⁵ the possibility of significant error in plasma concentrations of procaine reported by the 2 groups of workers should be kept in mind.

The reasons for the high sensitivity of horses to the central nervous system effects of procaine are not clearly understood. One possibility is that procaine is much more tightly bound to human than to equine plasma proteins. Thus, at the plasma procaine concentrations associated with excitement in man (20 µg/ml) the levels of free procaine might be similar to about 400 ng/ml free in equine plasma when central nervous system excitation becomes apparent in this species. Investigating this possibility, an attempt was made to measure the in vitro binding of procaine to human plasma under the conditions of Figure 5. These experiments were not successful in demonstrating any binding of procaine to equine plasma, apparently because of the high procaine esterase activity associated with human plasma.³ Another possible explanation for the higher sensitivity of the horse to procaine might be a higher apparent affinity of central nervous system receptors in the horse for procaine. Studying the apparent affinity of the cardiotonic steroid binding site on Na⁺ + K⁺ - ATPase for cardiac glycosides, Tobin et al^{9,12-14} showed that the affinity of this receptor for cardiac glycosides and erythrophleum alkaloids varied between species and that this variability accounted for species-dependent differences in sensitivity to the cardiac glycosides. While such explanations are theoretically possible in the case of procaine, they must be considered speculative at this point, since pharmacologic receptors for local anesthetics have yet to be identified.

As a practical matter, the present results bear directly on several aspects of the horse-doping problem. The efficacy of procaine alone as a stimulant dope in horses appears marginal. While some of the horses in the present and other experiments, in which central nervous system excitation was observed, paced well and appeared likely candidates for improved racing performance, many were poorly coordinated and liable to exaggerated startle and escape responses. This variability in response to procaine, in combination with the brief period for which plasma concentrations of the drug were maintained after sc and im injection, make procaine itself an unlikely candidate for use as a doping drug.

An unexpected result of the present study was the ease with which procaine penicillin may give rise to plasma concentrations of this drug which are associated with central nervous system excitation in horses. Further, after administration of procaine penicillin, plasma procaine concentrations are maintained for longer periods than by any other method of administration. Thus, doubling the dose of procaine penicillin administered to these horses should give rise to plasma pro-

caine values of about 500 ng/ml, close to those associated with central nervous system excitation. At this point, the possibility that such plasma concentrations of procaine may give rise to subtle central nervous system stimulant effects and thus improve the racing performance of horses cannot be excluded. However, since dosing with procaine penicillin at these doses produces easily detectable urinary levels of procaine, doping in this manner is readily controllable by present forensic techniques.

The present experiment also showed that small amounts of procaine administered intraarticularly gave rise to low, but detectable, plasma procaine concentrations. There is no reason to suppose that the equivalent small doses used for nerve blocks do not give rise to similar pharmacokinetic patterns. Thus, the plasma concentrations of procaine after the administration of small doses appear to be low and transient. Fortunately for the forensic chemist, however, more substantial urinary levels are found.

When procaine is found in the blood or urine of a racing horse, those responsible for the animal occasionally claim that the horse was administered procaine penicillin therapeutically and that this treatment accounts for the urinary procaine. An ability on the part of forensic laboratories to distinguish between procaine and procaine penicillin is thus of importance and could greatly assist racing authorities.¹⁶ The results of the present study show that procaine is cleared rapidly from the body when administered alone ($t_{1/2} \leq 2$ hours), but much more slowly when administered as procaine penicillin ($t_{1/2} = 10$ hours). Thus, one possible approach to this problem would be to take sequential blood samples from the horse in question and determine the half-life of procaine in the plasma of the animal. A half-life of 10 hours or longer would be consistent with administration of procaine penicillin, while a half-life of 2 hours or less would suggest administration of procaine alone, presumably for either its local anesthetic or central nervous system stimulant actions.

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