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PLENARY SESSION 1: Drug Administration

Chairman: D. H. WITHERINGTON

A REVIEW OF THE PHARMACOLOGY, PHARMACOKINETICS AND  
BEHAVIOURAL EFFECTS OF PROCAINE IN THOROUGHBRED HORSES\*

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ABSTRACT

Since procaine has both local anaesthetic and central stimulant actions, its presence in the blood or urine of racing horses is forbidden. After rapid intravenous injection of procaine HCl (2.5 mg/Kg) in Thoroughbred mares plasma levels of this drug fell rapidly ( $t_{1/2\alpha} = 5 \text{ min}$ )\* and then more slowly ( $t_{1/2\beta} = 50.2 \text{ min}$ ). These kinetics were well fitted by a two compartment open model (Model I). This model gave an apparent  $V_{d\beta}$  for procaine in the horse of about 3,500 litres. Since procaine was about 45% bound to equine plasma protein this gives a true  $V_{d\beta}$  for procaine of about 6,500 litres. After subcutaneous injection of procaine HCl (3.3 mg/Kg) plasma levels peaked at about 400 ng/ml and then declined with a half-life of about 75 minutes. These data were well fitted by Model I when this was modified to include simple first order absorption ( $K = 0.048 \text{ min}^{-1}$ ) from the subcutaneous injection site (Model II). After intramuscular injection of procaine penicillin (33,000 I.U./Kg) plasma levels reached a peak at about 170 ng/ml and then declined with a half-life of about 9 hours. These data were approximately fitted by Model II assuming a first order rate constant for absorption of procaine of  $0.0024 \text{ min}^{-1}$ . After intramuscular injection of procaine HCl (10 mg/Kg) plasma levels of procaine peaked rapidly at about 600 ng/ml but thereafter declined slowly ( $t_{1/2} = 2 \text{ hours}$ ). A satisfactory pharmacokinetic model for this intramuscular data could not be developed. An approximation of these data was obtained by assuming the existence of two intramuscular drug compartments, one containing readily absorbable drug and the other poorly absorbable drug (Model III). After intra-articular administration of procaine (0.33 mg/Kg) plasma levels of this drug reached a peak at about 17 ng/ml and then declined with a half-life of about 2 hours. These data were not modelled.

Intravenous infusion of procaine showed that behavioural excitation due to procaine commenced at plasma levels of about 600 ng/ml, with horses becoming uncontrollable at plasma levels of about 1,000 ng/ml. These plasma procaine levels are about one-twentieth of those associated with marked CNS excitation in humans. The horse is thus at least twenty-fold more sensitive to the central stimulant action of procaine than is the human.

Introduction

The pharmacokinetics and behavioural 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 (Meyer-Jones, 1951). The pharmacokinetics of procaine are further complicated by the routes and forms in which procaine may be administered to racehorses. Thus, procaine may be administered subcutaneously or intramuscularly for its local anaesthetic action associated with minor surgery. Alternatively, it may be given intramuscularly in relatively large amounts as procaine penicillin. These uses of procaine are common in equine medicine and surgery and are, *per se*, quite acceptable to racing authorities.

Other possible uses of procaine are regarded with disfavour 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 these techniques permit a horse with joint or tendon problems to improve its performance and may thus 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 levels of procaine are stimulant in nature and may positively affect the courage or performance of racing horses (Meyer-Jones, 1951). No clear-cut experimental evidence is available at present in this area, but this possibility, combined with the well characterized uses of procaine to alleviate lameness, has led racing

Paper # 10 from the Kentucky Equine Drug Research and Testing Programs Department of Veterinary Science, Published as Paper No. 76-4-73 with the permission of the Dean and Director, College of Agriculture. Supported by a grant from the Kentucky Equine Research Fund.

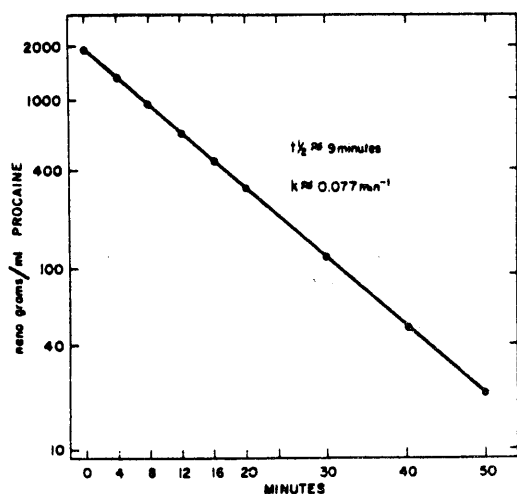
\* $t_{1/2}$  = half life - Ed.

authorities to ban the use of procaine or procaine-containing preparations in horses about to be raced.

Because of these considerations horsemen, veterinarians and racing authorities require information concerning the pharmacology, pharmacokinetics and behavioural effects of procaine in horses. These studies were undertaken in an attempt to provide answers to these questions. The pharmacokinetic analysis was performed as described by Kostenbauder *et al.* (1975) using the SAAM-23 programme (Berman and Weis, 1968). Full reports on the experiments reported here and the methods used are to be published elsewhere (Tobin *et al.* 1975, 1976a, b, c, d).

For detection and quantification of procaine we use Brodie's method, a colour density technique using ethylene diamine. It would not detect para-amino-benzoic acid, as this compound is not extracted at the pH = 8 that we use. Our horses are all in stables, and this produces an alkaline urine.

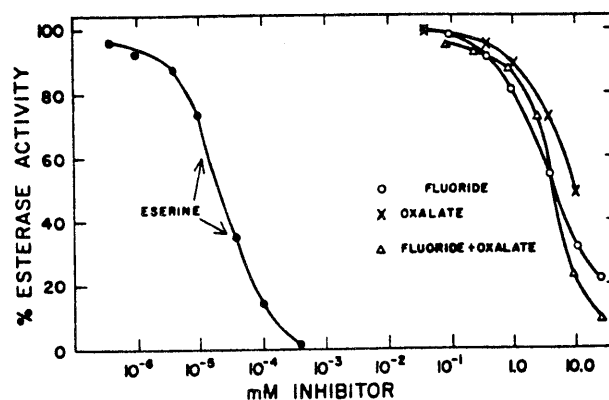
## Results



**Fig. 1** Hydrolysis of Procaine by Equine blood *in vitro*. Freshly drawn whole equine blood was incubated in 15 ml aliquots at 37°C with constant shaking (Tobin *et al.*, 1976b). At indicated zero time the reaction was started by the addition of 3 µg/ml of procaine HCl. The reaction was stopped when required by the addition of arsenite and eserine, the plasma separated and the procaine levels in plasma estimated as previously. All points are the means of three experimental determinations on different blood samples (from Tobin *et al.*, 1976b).

Because of the well known sensitivity of procaine to hydrolysis by human plasma (Brodie *et al.*, 1948), we first studied the hydrolysis of this drug by equine blood. Figure 1 shows the rate of hydrolysis of 2 µg/ml of

procaine added to freshly drawn equine blood *in vitro* at 37°C. Under these conditions the added procaine was hydrolysed with an apparent half-life of about 9 minutes at 37°C. Other experiments (Tobin *et al.*, 1976b) showed that the hydrolytic activity was due to plasma esterases. Though this hydrolysis in equine blood occurs at about one-tenth of the rate observed in human blood (Brodie *et al.*, 1948), this reaction is still sufficiently rapid for it to be an important pathway of procaine metabolism, both *in vivo* and *in vitro*.



**Fig. 2** Inhibition of Equine Plasma Esterases by Fluoride or Oxalate or by these agents combined.

Panel A shows inhibition of equine plasma esterases by the indicated concentrations of eserine (solid circles ●—●), sodium fluoride (open circles ○—○), oxalate (crosses X—X), and fluoride plus oxalate (open triangles △—△). Data points are the means of three individual experiments (from Tobin *et al.*, 1976b).

Figure 2 shows inhibition of these equine plasma esterases by eserine and a combination of oxalate and fluoride. These plasma esterases are inhibited rapidly and completely by low concentrations of eserine, but less effectively by oxalate and fluoride. In all the experiments reported in this and other papers from our laboratory (Tobin *et al.*, 1976a, b, c, d) blood samples were drawn into Vacutainer tubes containing 0.5 ml of 1mM eserine to preserve plasma procaine levels. Since Vacutainer tubes containing oxalate and fluoride are commercially available, tubes containing these agents are used for blood sampling in our routine screening programme in Kentucky. When combined with cooling, the combination of oxalate and fluoride gives rise to essentially complete inhibition of these equine plasma esterases (Tobin *et al.*, 1976d).

Since horse to horse variability in these plasma esterases would cause variations in the plasma half-life and thus the pharmacology and pharmacokinetics of procaine in horses, we measured the plasma esterase activities of plasma from a number of Thoroughbred,

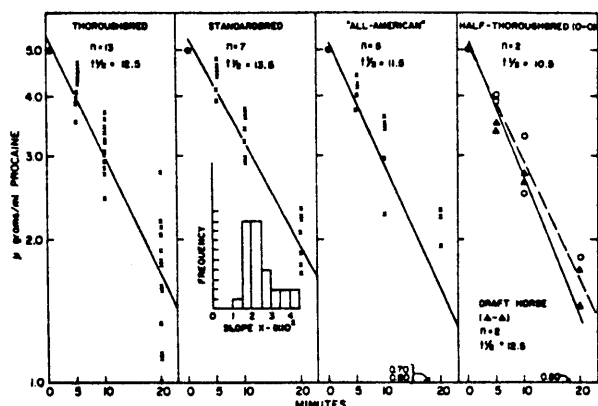


Fig. 3 Rates of Hydrolysis of Procaine by Plasma from different breeds.

Blood was freshly drawn from horses of the indicated breeds, the plasma separated and added to the standard incubation system at 37° (Tobin et al., 1976b). Each panel shows individual data points for the indicated breeds of horse with a least squares regression line fitted to the mean values for each group at each time point. Three data points at 20 minutes plot off the figure and these values are printed just above the horizontal axis. The designation "all American" indicates horses of mixed or unknown background. An *F* test applied to these mean slopes showed that they were not significantly different at the  $P < 0.05$  level. Within each group, however, an *F* test on the least square fit to each individual slope showed that the rates at which individual animals hydrolyzed procaine differed significantly from other members of the group at the  $P < 0.05$  level. The inset panel shows a frequency distribution of the negative slopes of the least squares fit for the hydrolytic rates of each individual plasma tested (from Tobin et al., 1976b).

Standardbred and other horses to determine this variability. The data presented in Fig. 3 shows that the mean rates of hydrolysis among the different groups of horses tested were very similar and that the overall variability encountered was that which might be expected in a single population [see insert, Fig. 3].

Figure 4 shows the blood levels of procaine observed after the rapid intravenous administration of 2.5 mg/kg of procaine to Thoroughbred horses. Plasma levels of procaine declined rapidly at first with an initial or  $\alpha$  phase half-time of about 5 minutes. Thereafter, however, the decline was relatively slow, with a  $\beta$  phase half-time of about 50 minutes. These data are well fitted by the two compartment open model outlined in Fig. 4 and the solid line in Fig. 4 is that obtained from the equations describing this model (Table I). The slow plasma half-life for procaine (50 minutes) was unexpected in view of the observation that procaine is hydrolyzed relatively

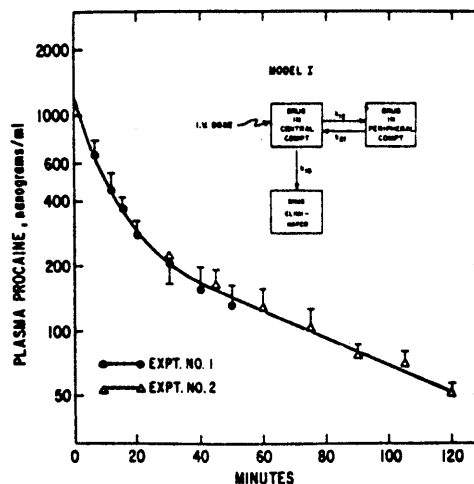


Fig. 4 Plasma Levels of Procaine After Rapid Intravenous Injection of Procaine HCl.

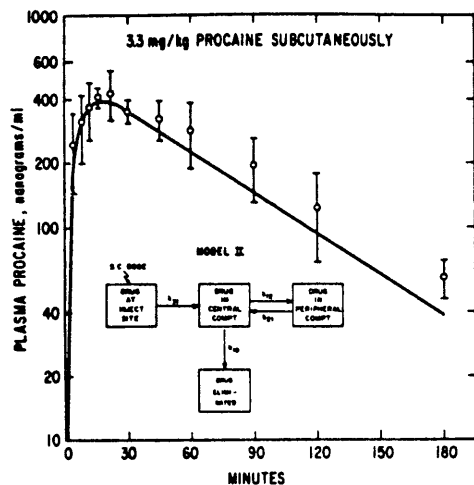
Procaine HCl, 2.5 mg/kg, was administered to horses by rapid intravenous injection. The solid circles (●—●) and open triangles (△—△) show the plasma procaine levels observed in two separate experiments. The solid line is that obtained from a SAAM-23 programme fit of the data to the differential equations describing the two compartment open model (Model I, insert) using a weight of  $1/(\text{std. dev.})^2$  for all data points. The rate constants  $k_{12}$ ,  $k_{21}$  and  $k_{10}$  are all first order rate constants and all were iterated. Values for the various parameters obtained from this analysis are presented in Table I (from Tobin et al., 1976d).

rapidly in equine blood and suggests wide distribution of procaine in the body of the horse. This conclusion is supported by the observation that the calculated volume of distribution ( $V_d\beta$ ) of procaine in these horses is 6.71 litres/kg, i.e. procaine distributes into an apparent volume about 7 times greater than the volume of the horse (Table I).

TABLE I

Pharmacokinetic Parameters for Procaine After Intravenous Administration Calculated from the SAAM-23 Programme (Model I)

$k_{12}$	$0.057 \pm .003 \text{ min}^{-1}$
$k_{21}$	$0.041 \pm .003 \text{ min}^{-1}$
$k_{10}$	$0.044 \pm .044 \text{ min}^{-1}$
$V_c$	1115 Litres or 2.11 Litres/kg
$V_{d\beta}$	3547 Litres or 6.71 Litres/kg
A	905 ngm/ml
B	287 ngm/ml
$\alpha$	$0.1262 \text{ min}^{-1}$
$t_{1/2\alpha}$	5.49 minutes
$\beta$	$0.0138 \text{ min}^{-1}$
$t_{1/2\beta}$	50.2 minutes
Clearance	$1.19 \text{ Litres min}^{-1}$



**Fig. 5** Plasma Levels of Procaine After Subcutaneous Administration of 3.3 mg/kg of Procaine HCl. A 2% solution of procaine hydrochloride (Novocaine<sup>R</sup>) was injected subcutaneously over the superficial pectoral muscles of horses and blood samples drawn at the indicated times. The open circles (○ — ○) show the plasma levels of procaine observed at the indicated times. The solid line is that obtained from a SAAM-23 programme fit of the differential equations describing Model II when  $k_{31}$  was set at  $0.048 \text{ min}^{-1}$ . The rate constants  $k_{12}$ ,  $k_{21}$  and  $k_{10}$  were held constant at the values given in Table I and  $k_{31}$  was iterated. All experimental values are the means  $\pm$  S.E.M. of at least four individual experiments (from Tobin et al., 1976d).

Procaine is often administered subcutaneously or intramuscularly in horses for its local anaesthetic action during minor surgery. Figure 5 shows that when given subcutaneously blood levels of procaine rise rapidly and then decline with a half-life of about one hour. Similarly, when procaine was injected intramuscularly (Fig. 6) blood levels of the drug again peaked rapidly and declined somewhat more slowly, with an apparent half-life of about two hours. The experiments show that when given by either of these routes blood levels of procaine peak and then clear relatively rapidly (Table II).

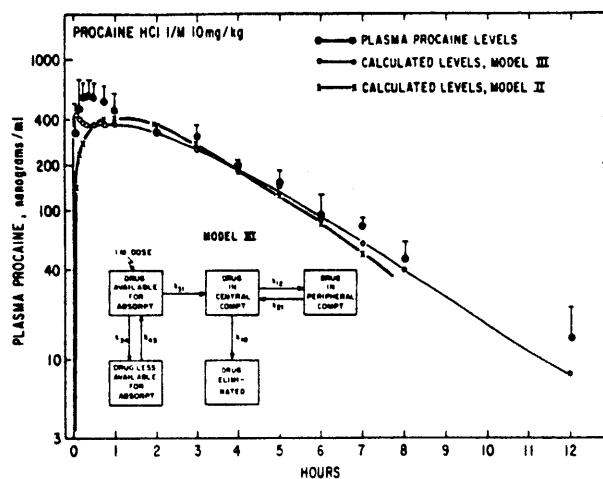
After the intramuscular administration of procaine to horses in the experiments of Fig. 7, signs of central nervous system stimulation were observed in some of these horses. This was rather surprising since Usubiaga et al. (1966) reported that procaine plasma levels of up to 40 ng/ml are required in the human to produce signs of CNS excitation. We therefore infused a number of horses with procaine to determine the relationship between plasma levels of procaine and CNS excitation in the Thoroughbred horse. The data of Fig. 7 show that mild signs of excitement become apparent at plasma procaine

TABLE II

Observed Plasma Half-Lives Compared with those Generated by Kinetic Models

Route of Administration	Model $t_{1/2}$ minutes	Observed $t_{1/2}$ minutes
Intravenous Procaine	50.2	50.2
Subcutaneous Procaine	50.0	65.7
Intra-articular Procaine	—	97.1
Intramuscular Procaine	101.0	125.5
Intramuscular Procaine	300.0	602
Penicillin		

The measured  $t_{1/2}$  values were from least square fits to all plasma values observed after peak plasma levels were attained. The model  $t_{1/2}$  values were taken from the computer generated curves. All plasma half-lives are in minutes.



**Fig. 6** Plasma Procaine Levels After Intramuscular Administration of 10 mg/kg of Procaine HCl. Procaine HCl, 10 mg/kg, was administered by intramuscular injection in the side of the neck to four horses. The solid circles (● — ●) show the plasma levels of procaine observed at the indicated times  $\pm$  S.E.M.'s. The dashed line shows the best fit to these data calculated from the differential equations describing Model II (Fig. 7), with  $k_{21}$ ,  $k_{12}$  and  $k_{10}$  held at the values presented in Table I. The solid line shows the best fit to these data calculated from the differential equations describing Model III with  $k_{31}$ ,  $k_{36}$  and  $k_{43}$  iterated and the other rate constants held at the values of Table I. The best values obtained for these rates were respectively,  $k_{31}$ ,  $0.112 \text{ min}^{-1}$ ;  $k_{34}$ ,  $0.772 \text{ min}^{-1}$ ; and  $k_{43}$ ,  $0.057 \text{ min}^{-1}$ . A least squares fit to the plasma values from 22 minutes on gives a half-life for plasma levels of procaine, 125 minutes after its intramuscular injection (from Tobin et al. 1976d).

levels of about 600 ng/ml, that these signs become marked at about 1,000 ng/ml, and that at concentrations of 1,500 ng/ml or greater the animals go out of control. These experiments show that the Thoroughbred horse is extremely sensitive to the central effects of procaine, being at least 20 times more sensitive to this drug than is the human. These results highlight what the practicing veterinarian probably already knows, i.e. that it is not difficult to produce signs of central excitation in horses by the subcutaneous or intramuscular administration of procaine.

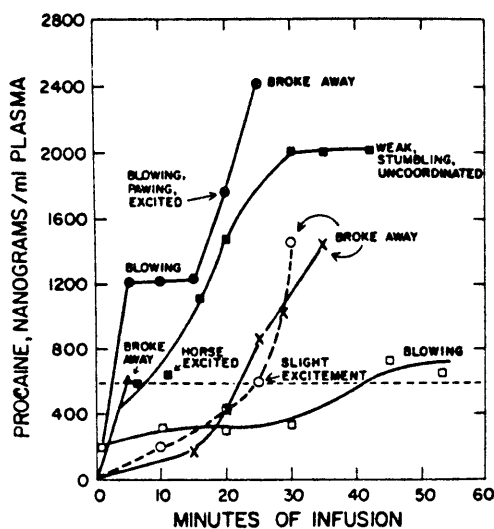


Fig. 7 Relationship Between Plasma Levels of Procaine and Signs of Central Nervous System Excitation.

Thoroughbred mares were infused with solutions of procaine containing 660 mg procaine/ml at rates of between zero and 2.0 ml/minute. Infusion was by means of a jugular cannula and the horses were watched carefully for signs of excitement throughout. Blood samples were taken at 5-minute intervals or whenever the condition of the animal permitted it. Because blood levels required to produce a given level of excitement were not known, infusion rates were varied (usually increased) during the course of the experiment. Occasionally, infusion was stopped for short periods so that the animal could be approached to take a blood sample. The open squares (□ - □) show the first infusion experiment where the rate of infusion was slow. Blood levels in the order of 700 nanograms/ml were obtained at the end of the 1-hour infusion period and only mild signs of excitation (blowing) were observed. In subsequent experiments the rate of infusion was increased. All horses showed signs of excitement once the 700 nanograms/ml level was reached and one that attained this level (solid triangle ▲) broke away. Two horses (crosses, open circles X, ○) broke away at about the 1400 nanogram/ml level and at this level all horses showed obvious signs of excitation. One horse did not break away (solid squares

■ - ■), but on being released, this horse was poorly coordinated, tended to stumble and walked with its head down. All points are single experimental determinations (from Tobin et al., 1976d).

Figure 8 shows blood levels of procaine observed after the intra-articular injection of procaine hydrochloride and the intramuscular injection of procaine penicillin. The experiment shows that after the intra-articular injection of procaine, blood levels of procaine again rose and then declined rapidly. In contrast, however, after procaine penicillin blood levels of procaine reached a peak relatively slowly and then declined very slowly indeed, with a half-life in the order of about 10 hours. These experiments suggest that though it is not possible to distinguish chemically between procaine given as procaine HCl and that given as procaine penicillin, it may be possible to distinguish between these drugs on the basis of their pharmacokinetics. Thus in a horse "called" for procaine the drawing of subsequent blood samples should show slowly decreasing plasma levels of procaine in animals which were treated with procaine penicillin, but more rapidly declining or probably no plasma levels at all in horses which were treated with procaine hydrochloride.

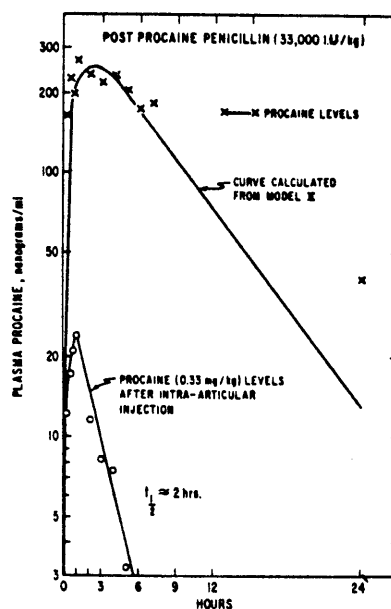
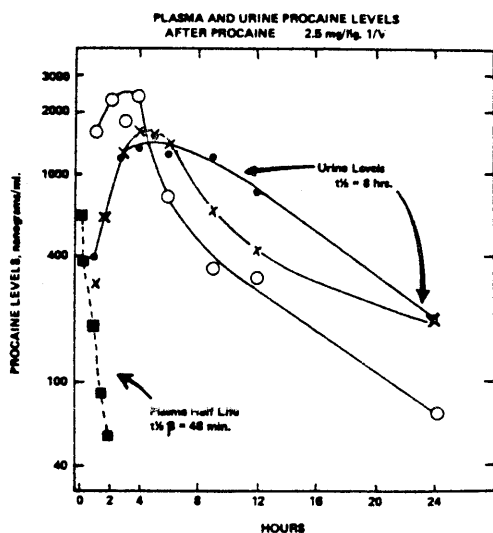


Fig. 8 Plasma Procaine Levels after Intramuscular Injection of Procaine Penicillin, 33,000 I.U./kg or Intra-articular Injection of Procaine HCl, 0.33 mg/kg.

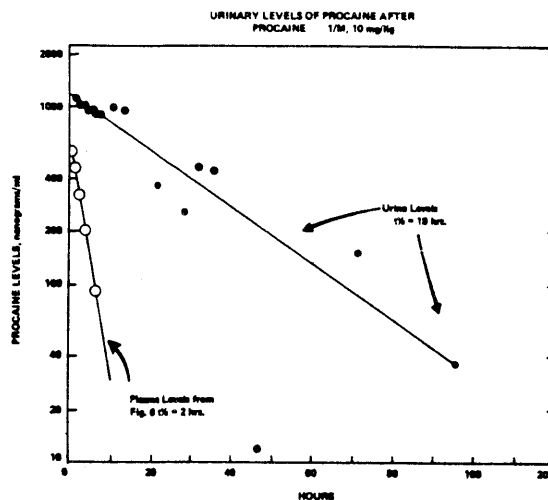
The crosses (X - X) show plasma procaine levels after deep intramuscular injection of 33,000 I.U./kg of procaine penicillin (Duracillin<sup>®</sup>, Eli Lilly and Co.) into the side of the neck. The solid line shows the best fit to these data calculated from the differential equations describing Model II, with  $k_{21}$ ,  $k_{12}$  and  $k_{10}$  held at the

values presented in Table 1, and  $k_{31}$  set at  $0.0024 \text{ min}^{-1}$ . A least squares fit to the post peak values after procaine penicillin gave an apparent plasma half-life for procaine of 602 minutes. The open circles ( $\circ - \circ$ ) show plasma procaine levels after intra-articular injections of  $0.33 \text{ mg/kg}$  of 2% procaine (as Novocaine). The solid line was fitted by eye. All experimental points are the means of at least four individual experimental determinations (from Tobin et al., 1976d).

The relationship between blood and urinary levels of procaine in the horse is complex and, at this time, only partially characterized. Figure 9 shows the blood and urine levels of procaine observed after a single intravenous injection of procaine. While the blood levels of procaine fell rapidly, as described previously, urinary levels of the drug declined very slowly. Figure 10 shows similar results obtained after the intramuscular injection of procaine. In this experiment, blood levels of procaine declined with a half-life of about 2.0 hours, consistent with the experiments of Fig. 6. Urinary levels of the drug, however, declined much more slowly and again



**Fig. 9** Plasma and Urinary Procaine Levels after Intravenous Injection of Procaine HCl, 2.5 mg/kg. The solid squares ( $\blacksquare - \blacksquare$ ) show plasma levels of procaine after the rapid intravenous injection of  $2.5 \text{ mg/kg}$  procaine HCl ( $n = 4$ ). The open circles ( $\circ$ ), crosses ( $\times$ ) and solid circles ( $\bullet$ ) show individual urinary levels of procaine in three animals administered  $2.5 \text{ mg/kg}$  of procaine HCl intramuscularly.



**Fig. 10** Urinary Levels of Procaine after Intramuscular Injection of Procaine HCl, 10 mg/kg.

Procaine HCl,  $10 \text{ mg/kg}$ , was injected intramuscularly in the side of the neck in four horses. The solid circles ( $\bullet - \bullet$ ) show urine levels of procaine determined at the indicated times. The open circles ( $\circ - \circ$ ) show plasma levels of procaine replotted from experiments of Fig. 6. All points are the means of determinations in four different animals.

urinary levels of the drug are in evidence long after the drug is no longer detectable in blood. The reasons for this very slow rate of decay of urinary levels of the drug are not clear, but sequestration of the drug in the kidney or urinary tract is presumably involved.

In conclusion, procaine is relatively rapidly hydrolyzed in equine plasma and this hydrolysis is blocked by eserine or cooling plus oxalate and fluoride. Procaine has a metabolic or  $\beta$  phase half-life in the horse of about 50 minutes. This relatively long half-life is due to wide distribution and tissue binding of this drug in the horse. When administered as procaine by any route, its half-life in the bloodstream of the horse is relatively short, usually about 2.0 hours or less. When administered as procaine penicillin its half-life is about 10 hours. This suggests that it may be possible to distinguish pharmacokinetically between procaine given in these forms. For reasons which are currently unclear, procaine is found in the urine of horses long after it has disappeared from their bloodstreams, a finding which may be of some forensic importance.

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MAYNARD: In British Columbia, they also extracted some degradation products of penicillin which were picked up on the GC mass spectrometer. They are investigating it at present.

TOBIN: Model 2 assumes a single site from which absorption of an intra-muscular injection occurs and there is a discrepancy. We can only account for about half the drug absorbed and we assume a single first order rate of absorption.

DEBACKERE: Is it a question of protein-binding?

TOBIN: I see no reason if it is a simple question of protein binding why the simple model should fit subcutaneous but not fit intramuscular administration, it is an event subsequent to absorption, protein-binding occurring in the central compartment. We have to assume binding in the tissue of some sort, this seems to be practised by the drug right through. The calculated half-life is a little slower than we observed for subcutaneous administration, the same, however, was much more marked for intramuscular and the same applied to procaine penicillin and intra-articular injections. There are also discrepancies between the urinary levels and the plasma levels. Some sort of binding seems to be a constant characteristic of the kinetics of this drug.

DEAN: Following a normal therapeutic dose of procaine penicillin injection, for how long could an analytical chemist detect it in the urine?

TOBIN: It would depend on the analytical chemist! We reckon to find the drug for 2 weeks.

BLAKE: The GC derivative method that Tobin was referring to, detects about 1 to 2 picograms injected on the column, depending upon how large blank is.

LAMBERT: With the routine method used in Ireland, we are finding procaine penicillin after about 40 hours, but we wouldn't go on looking for it for 2 weeks.

JAGGARD: We cannot find procaine HCl after 24 hours by our routine procedure. With procaine penicillin we find it for maybe 48 hours, with azomycin, we can find it for 3 or 4 days but that is our limit.

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## EXCRETION AND METABOLISM OF NIKETHAMIDE IN THE HORSE

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### ABSTRACT

It is well known that nikethamide (N,N-diethylnicotinamide, Coramine<sup>R</sup>) is metabolized very rapidly to nicotinamide. Hence, there is difficulty in proving that nikethamide has been used as a doping substance because nicotinamide is a normal physiological metabolite in the organism as well as a vitamin preparation. However, an intermediate metabolite (N-ethylnicotinamide) was found by us in the urine of horses treated with Coramine<sup>R</sup>. This was characterized by gas chromatography/mass spectrometry, and synthesized and identified as being N-ethylnicotinamide.

The excretion and metabolism of nikethamide after intramuscular injection in the horse was followed using quantitative gas chromatography of urinary extracts over a period of several hours and the results of these experiments are reported.

Changes in urinary pH had no significant effect upon either the metabolism or rate of excretion of the drug.

\* *Directeur and Professor.*