

RECOVERY OF PROCAINE FROM BIOLOGICAL FLUIDS

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SUMMARY

A published method for the recovery of procaine from human plasma using 5M NaOH gave very poor recoveries. Investigation showed that under the recommended extraction conditions procaine was rapidly hydrolysed. Extraction into benzene of samples buffered to pH 9.0 with borate buffer allowed essentially 100% recovery of procaine from equine plasma and urine.

INTRODUCTION

Because procaine has both local anaesthetic and central stimulant actions (Meyer-Jones, 1951; Green *et al.*, 1974), it may be used to either mask lameness or stimulate performance in racing animals. For these reasons its presence in the blood or urine of racing horses is disallowed by most racing authorities and methods for its estimation in equine plasma and urine are of forensic importance. These methods must be accurate, quantitative, and sufficiently well documented to withstand scrutiny in the courts. Adaption of a recently published method for the measurement of plasma procaine (Green *et al.*, 1974) was found to yield very poor recoveries of procaine. Investigation of this problem showed that procaine is unstable under the strongly alkaline conditions used by these authors but that essentially quantitative recovery from equine biological fluids is possible under milder conditions.

MATERIALS AND METHODS

The colorimetric reaction used for measurement of procaine levels was that described by Brodie *et al.* (1948). Procaine was extracted into (or added directly to) 2.0 ml of 1M HCl and 50% of 0.1% sodium nitrite added. After five minutes 50% of 0.5% ammonium sulfate was added and three minutes later 50% of 0.1% N(1-naphthyl)ethylene diamine dihydrochloride (NEDD). Twenty minutes was allowed for the violet-red color to develop which was then read at 550 nm against a HCl blank. This method depends on the diazotization of the amino group on procaine and its coupling to NEDD to give the resulting dye which absorbs at 550 nm.

Plasma and urine samples were obtained from three Thoroughbred mares, pooled, and stored frozen at -32° until required. Procaine HCl and p-amino benzoic acid (PABA) were obtained from Sigma Chemical Company and ran as single spots on thin layer chromatography (TLC) in methanol or 9:1 chloroform:methanol. The basic method for the extraction of procaine from the plasma and urine samples involved their alkalization with NaOH (as described by Green *et al.*, 1974) or 0.8M borate buffer (pH 9.0), extraction into 10.0 ml of benzene by "rotoracking" for 10 minutes, followed by centrifugation. The procaine was then returned to 2.0 ml of 1.0N HCl by again rotoracking and centrifuging. 1.5 ml of this HCl solution was then removed from the benzene and color reacted for procaine as described above. Thin layer chromatography of the benzene extract was performed where required by spotting the solution onto 250 micron silica gel plates (type GF) and developing in a medium consisting of nine parts chloroform and one part absolute methanol.

RESULTS

When varying concentrations of procaine were added directly to 2.0 ml of HCl and the dye reaction performed, each μg of procaine added to the reaction medium gave an absorbance of about .085 at 550 nm (Fig. 1).

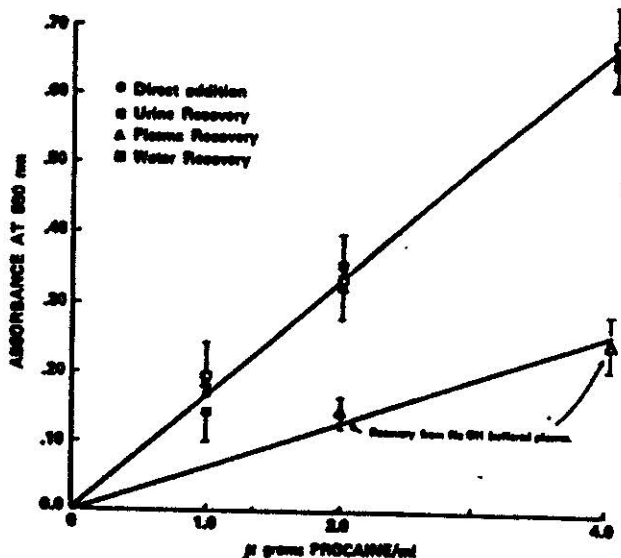


Figure 1. Recovery of procaine from equine plasma and urine. Two, four or eight μg of procaine HCl were added to 2 ml aliquots of pooled equine plasma or urine or 2 ml of distilled H_2O , respectively. These "spiked" samples were then buffered with 0.5 ml of 0.5M borate buffer, 10 ml of benzene added, "rotoracked" for 10 minutes and centrifuged at $4,000 \times g$ for 10 minutes. The benzene layer was then transferred to another test tube, 2 ml of 1N HCl added and the "rotoracking" and centrifugation steps repeated. 1.5 ml of the HCl layer was then transferred to another test tube and the dye reaction described in METHODS performed. The solid circles (●-●) show the absorbance at 550 nm obtained when the indicated quantities of procaine were added directly to the dye system. The solid squares (■-■), solid triangles (▲-▲), and open squares (□-□) show the color reactions obtained if the procaine is added to 2 ml of urine, plasma or water, respectively. The open triangles (△-△) show the color reaction obtained when the plasma was buffered with 5M NaOH and the extraction performed as described above. All points are the means of four or more separate experimental determinations \pm the standard errors of the means.

If, however, the procaine was added to 2.0 ml of plasma and then extracted with 5M NaOH into 10.0 ml of benzene as described by Green *et al.* (1974) and then dye reacted as described above, the absorbance value obtained was about one-third of that obtained by adding the procaine directly to the dye reaction system. Investigation of this problem pointed to the loss occurring during the extraction phase and led to the experiment presented in Fig. 2. Here 20 μ g of procaine were added directly to 0.5 ml of 2.5M NaOH in 2.5 ml of H₂O and the material scanned as soon as possible in a Beckman ACTA MW1 UV recording spectrophotometer. The data show that the absorbance spectrum of the material changed very rapidly, the absorbance maximum shifting from about 285 nm to a final maximum of 263 nm after about 2 hours. On the other hand, procaine suspended in 0.8M borate buffer showed no change in its absorbance spectrum over a two hour period (data not shown), the period during which the procaine would be alkalinized and extracted into benzene during the recovery process. This experiment shows rapid alterations occurring in the procaine molecule when it is exposed to 0.5M NaOH.

If an aqueous solution of procaine was exposed to 0.5M NaOH for one-half hour (i.e. until the reaction of Fig. 2 was essentially complete), no procaine was observed on thin layer chromatography of either acidic or basic extracts of the system (Fig. 2, insert). Further, no material identifiable as p-aminobenzoic acid was recovered either, suggesting that damage to the procaine molecule extends beyond simple hydrolysis of the ester linkage.

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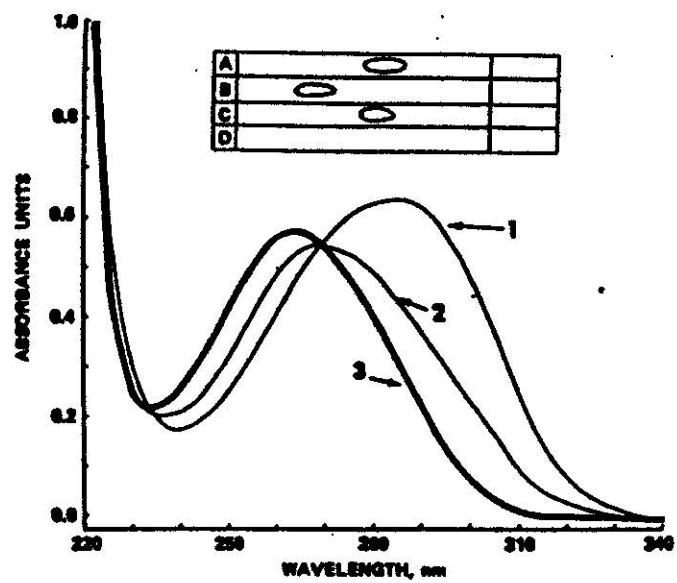


Figure 2. Changing absorbance spectrum of procaine in NaOH buffer. About 20 μ g of procaine was added to 2.0 ml of H_2O plus 0.5 ml of 2.5M NaOH and the solution was immediately introduced into the 10 mm path length flow cell of a Beckman ACTA MVI UV recording spectrophotometer. The changing absorbance spectrum of procaine was followed by repetitively scanning the spectrum from 340 nm to 220 nm at 4 nm/sec. The curve labelled (1) is the spectrum obtained immediately after introduction of the sample into the flow cell; curve (2) is the spectrum recorded five minutes later, while curve (3) shows the spectrum recorded after two hours. The inset shows thin layer chromatograms of (A) authentic procaine and (B) p-aminobenzoic acid. Panel C shows procaine extracted from borate buffered aqueous solution as described in METHODS. Panel D shows that no procaine was recovered into benzene from aqueous solutions buffered with 5M NaOH. In other experiments, PABA was not recovered into benzene after acidification of 5M NaOH treated procaine solutions. The direction of migration is from the origin on the right of the letters to the solvent front on the right of the drug spots.

Figure 1 shows the recovery of procaine obtained from water, plasma and urine using 0.5M borate buffer and benzene in the extraction step, compared with the direct addition of similar concentrations of procaine to the color reaction system. Under these conditions the dye reaction obtained from procaine added to the biological fluid did not significantly differ from that obtained by the direct addition of procaine to the dye reaction system, indicating essentially 100% recovery of procaine.

DISCUSSION

Exposure of procaine to 0.5M NaOH resulted in rapid changes in its absorbance spectrum with a shift in the absorbance maximum of the system from about 285 nm to about 263 nm. This spectral change was associated with the disappearance of procaine from the system as determined by thin layer chromatography of acidic or basic benzene extracts of the system. Further, though the absorbance spectrum of the material formed from procaine in the presence of NaOH was close to that of PABA, acidification of the system with HCl showed its spectrum to be distinct from that of PABA (data not shown). These results show that procaine is unstable in the presence of 0.5M NaOH. Because breakdown occurs very rapidly in 5M NaOH this effect is significant even though the period of exposure to NaOH (extraction phase) is short.

In recovering procaine from samples of human plasma, Green *et al.* (1974) used 5M NaOH, which was added to 2 ml of plasma, to give a final NaOH molarity of 1.6. Though it is difficult to estimate the amount of procaine hydrolysis which occurred in the experiments of Green *et al.* (1974), it must have been substantial. Because Green *et al.* compared their plasma samples with

"spiked" water "standards" they subjected their standards to approximately equivalent hydrolysis. Therefore, their estimated plasma levels of procaine should be approximately correct. However, alkaline hydrolysis of procaine during this extraction procedure introduces a substantial and needless loss of sensitivity into the assay system. A further source of error arises if the period for which the samples are exposed to NaOH varies. This is because the breakdown of procaine is rapid, commencing as soon as NaOH is added and proceeding until the end of the extraction period. Therefore, variations in time of exposure to NaOH caused by manipulations of other samples, small changes in the rotoracking (extraction) times et cetera, will contribute further random errors. Thus the use of concentrated NaOH in the extraction process serves to both reduce the sensitivity and introduces needless error into the estimation of plasma procaine levels.

Though the use of a strong base such as 5N NaOH is theoretically a correct procedure for the extraction of basic drugs, the results presented here show that the absolute recoveries obtained after such treatments should be carefully determined. Simple determinations of recovery relative to "spiked" water standards will not suffice to expose the type of hydrolysis encountered in this instance, and similar difficulties in the recovery of indomethacin from strongly alkaline solutions have also been observed.* For these reasons it is suggested that (a) quantitative recoveries be determined during the development of recovery methods and (b) that the use of excessively acidic or basic recovery conditions be avoided if possible.

*Personal communication: Dr. J. Blake, Racing Chemistry Laboratory, Ohio State University, Columbus.

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