FREQUENCY DISTRIBUTIONS OF PH VALUES IN POST RACE URINE SAMPLES FROM STANDARDBRED AND THOROUGHBRED HORSES

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ABSTRACT

Urinary pH has a significant effect on the concentration of medications and their detection in the urine of racehorses. Medications and related agents are commonly weak acids or weak bases; as such, the pH of a urine sample can affect the concentrations found in urine and the post race detection of these agents. Exercise, such as racing, is capable of inducing large shifts in urinary pH levels which can, therefore, cause changes in medication detection. In this regard, post race urine samples from Thoroughbreds show a bimodal pH distribution, with an 'acidic' peak at pH 5.5 and a 'basic' distribution peak at pH 8.0. Post race urine samples taken from Standardbreds, however, show a unimodal distribution with a single peak between pH 7.0 and 9.0. Therefore, these data suggest that results from experiments performed using Standardbred horses cannot be applied generally to predict medication detection capabilities in post race urine samples from Thoroughbred horses.

BACKGROUND

Medication and its detection in equine urine can be affected significantly by urine pH (Tobin 1981; Dyke 1995). Acidic urines tend to concentrate agents that are weak bases, whereas basic urines tend to concentrate agents and their metabolites that are weak acids. For example, non-steroidal anti-inflammatory drugs (eg phenylbutazone and flunixin) and barbiturates are weak acids; therefore, concentrations of these compounds would be expected to increase as urinary pH increases and this has been shown to occur with phenylbutazone and its metabolites (Tobin *et al.* 1986).

On the other hand, most narcotic analgesics, tranquilisers/sedatives, bronchodilators, local anesthetics and central nervous system stimulants are weak bases. Included in this group are most of the 'hard' illegal medications (eg etorphine, fentanyl, cocaine) detected in racehorses, and the concentrations of the parent or free drug form of these compounds in urine would be expected to increase as urinary pH decreases (Houston *et al.* 1985; Wood *et al.* 1990; Gerken *et al.* 1991). Medication concentrations in equine urine must, therefore, be viewed as a function of the pH of the urine as well as the plasma concentration of the agent in question. These relationships have been demonstrated repeatedly in man and animals; more recently, data have been developed showing that these relationships also occur in equine urine.

These findings have a significant impact on the Thoroughbred horseracing industry because Thoroughbred horses show large post race shifts in urinary pH, and the post race urine samples collected from these horses will show variability in the range, type and concentration of the agents that can be detected. Research workers in Japan, England, Kentucky and Hong Kong have shown that post race Thoroughbred urine samples yield biphasic frequency distributions of pH values, with an 'acidic' peak between pH 5.0 and 6.0, and a 'basic' peak at about pH 8.0 (Fig 1). These distributions differ from those observed in horses at rest, whose urinary pH values show a single peak at about pH 8.50.

Work by Snow (1983) in Hong Kong suggests that the shift to a bi-phasic frequency distribution of urinary pH values seen after racing is related to the metabolic acidosis of exercise. Because of the magnitude of this effect in Thoroughbred racehorses, the changes in pH levels are likely to have highly significant effects on the concentrations of medications and their metabolites in post race urine samples. This effect is therefore of considerable forensic significance; a useful laboratory model of this effect would enable

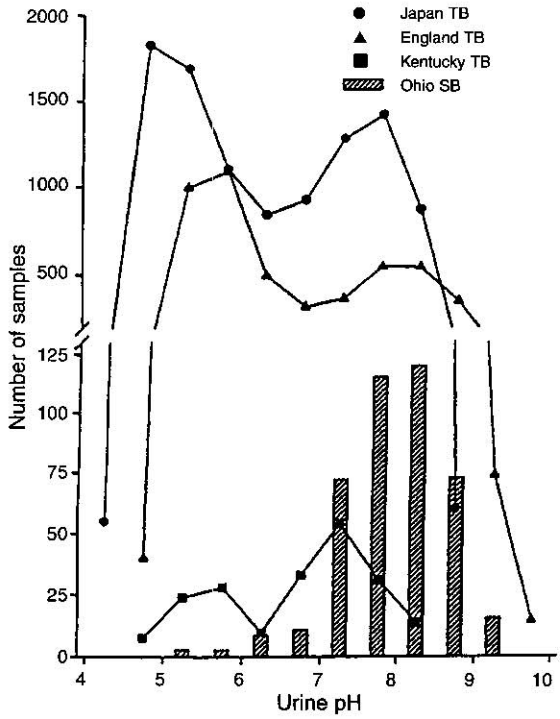

Fig 1: Post race urine pH values of Thoroughbreds (TB) and Standardbreds (SB). Line graphs show historical data for Thoroughbreds (Japan: Nakajima and Matsumoto [1976]; England: Moss [1976]; Kentucky: Houston et al. *[1985]). Bar graphs show frequency distribution developed from 419 Standardbreds racing in Ohio in 1995. Reproduced with permission from: Stanley* et al. *(1995)* Equine vet. J.

researchers to reproduce the full range of post race urinary pH values and to determine the variation in post race drug or drug metabolite concentrations that might be associated with urinary pH changes.

This requirement for experimental work to mimic physiological events in the racehorse is well recognised. The Ohio State University programme and the Agriculture Canada Equine Pharmacology Program have performed substantial portions of their 'detection time' research in exercised Standardbred horses. However, as will be pointed out later, it is now clear that urine samples collected from Standardbred horses are unlikely to match the large drops in urinary pH seen in post race urine samples from Thoroughbred horses.

The effect of urine pH on the detection of basic and acidic drugs is significant. In a study measuring the concentrations of phenylbutazone (a weak acid) and its metabolites (Houston *et al.* 1985), urinary concentrations of phenylbutazone, oxyphenbutazone, and gamma-hydroxyphenylbutazone were found to increase 200-fold as urinary pH increased. Similarly, Gerken *et al.* (1991) showed a significant increase in the urinary concentrations of lidocaine, a basic local anaesthetic, as urinary pH decreased. These observations suggest the possibility that a basic drug could have a very low (ineffective) plasma concentration while being concentrated several hundred-fold in acidic urine, causing drug identification long after the 'normal' detection time of the drug had passed.

CURRENT WORK

Data developed recently by our group suggest that Standardbred horses exercised under conditions approximating those of a Standardbred race are unlikely to yield pH shift data similar to those found following Thoroughbred racing. Figure 1 presents a frequency distribution of post race urinary pH values obtained from 419 Standardbred horses racing in Ohio during 1995. Additionally, for comparative purposes, reanalysed frequency distribution data from Japanese, English and American (Kentucky) post race urine samples from Thoroughbred horses are included.

These data show that post race urine samples from Standardbred horses fail to show the same shift to acidic pH values that have been reported in Thoroughbreds. This suggests that Standardbreds are an inappropriate model for determining pharmacokinetic patterns and thresholds for therapeutic agents for application in Thoroughbred racing, and that developing pharmacokinetic patterns and detection times for therapeutic medications in exercised Standardbred horses may systematically underestimate the true detection times for basic drugs in Thoroughbred racehorses.

These different frequency distributions of pH values in post race urine samples between Standardbreds and Thoroughbreds raise questions about the cause. Thoroughbreds generally race over distances from 5 furlongs to 1.5 miles and are classified as 'sprinters' or 'stayers'. 'Sprinters' depend mainly on anaerobic metabolism to generate the energy needed to compete over the shorter distances, whereas 'stayers' depend more on aerobic metabolism to sustain them over the longer distances. Thoroughbreds selected for breeding, especially males, are the horses that were successful sprinters or stayers. In contrast, American Standardbreds generally race a distance of one mile. Therefore all trotters and pacers in this country are bred to compete over a standard distance, and the horses used to propagate the breed are the ones that were successful at that distance.

The bimodal pH distribution for Thoroughbreds and the unimodal distribution for Standardbreds may be explained by the different distances for

which those horses are bred to compete. Increased lactic acid production is associated with anaerobic metabolism, and it is possible that the large number of urine samples from Thoroughbred horses with a pH value around 5.5 units were collected from sprinters who rely primarily on anaerobic metabolism for fuel.

Conversely, stayers are more dependent on aerobic metabolism, which produces less acidosis, and the urine from those horses would be expected to have a pH closer to pre-race values. It is possible that the high number of urine samples from Thoroughbreds with a pH value around 8 were collected from stayers. Because Standardbreds are bred to race at only one distance (a mile), the capacity for each metabolic pathway would be expeted to be similar among members of that breed, unlike Thoroughbreds.

The findings reported in this paper therefore raise questions concerning the ability to extrapolate from drug detection and 'withdrawal time' data developed in Standardbred horses, for application to the highly acidic post race urines seen in Thoroughbred horses.

With regard to our original interest in this subject, horses producing acidic urine would be more likely to show detectable residues of the parent form of illegal medications in post race urine samples than horses producing neutral or basic urine. This is because the illegal medications are generally basic drugs and are more likely to reach detectable concentrations in post race samples from horses producing acidic urine.

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