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Short Communication

Frequency distribution of post race urine pH from Standardbreds compared with Thoroughbreds: research and regulatory significance

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Introduction

The concentration of drugs and drug metabolites in urine samples of racing horses is strongly influenced by urine pH (Tobin 1981), depending on whether the drugs are weak acids or weak bases. Drugs that are weak acids tend to concentrate in basic urine. In contrast, drugs that are weak bases tend to concentrate in acidic urine. These relationships have a well-established theoretical basis (the Henderson-Hasselbalch relationship) and have been demonstrated repeatedly in experimental animals and man (Tobin 1981). More recently, evidence suggests that these relationships also occur with clinically and forensically significant agents in equine urine (Wood *et al.* 1990; Gerken *et al.* 1991).

Urine pH influences the concentrations of many drugs in horse urine (Tobin 1981). Barbiturates and nonsteroidal anti-inflammatory drugs (e.g. phenylbutazone and flunixin) are weak acids; therefore, concentrations of these compounds would be expected to increase as urinary pH increased (Tobin *et al.* 1986). On the other hand, most local anaesthetics, narcotic analgesics, tranquillisers/sedatives, bronchodilators and central nervous system stimulants are weak bases. Included in this group are most of the 'hard' illegal medications (e.g. etorphine, fentanyl, cocaine) detected in racing horses, and the concentrations of the parent or free drug form of these compounds in urine would be expected to increase as urinary pH decreased (Houston *et al.* 1985; Wood *et al.* 1990; Gerken *et al.* 1991).

Therefore, the concentrations of most drugs in equine urine are not directly related to their plasma concentrations. Rather, they are functions of the plasma concentration and the pH of the urine. The effect of pH on urinary drug concentrations has important regulatory consequences. Houston *et al.* (1985) showed the effect of urinary pH on concentrations of phenylbutazone and its metabolites in post race urine samples of racing horses. In that analysis, pH was the major source of the observed variance in urinary concentrations of the drug and metabolites, accounting for more than 50% of the observed variance. More recently, Gerken *et al.* (1991) showed that the urinary concentrations of local anaesthetics were increased and the urinary concentrations of oxyphenbutazone were decreased following acidic urinary pH changes in exercised horses.

Urinary pH is influenced by many factors, including environment, diet, and exercise status of the horse. Horses at rest and at pasture produce alkaline urine with pH values about 8.0–8.5 units. The addition of grain to the diet decreases urine pH by about 1.0 pH unit (Wood *et al.* 1990). However, by far the most important factor determining urinary pH in racing horses is physical activity. Snow (1983) has shown that the pH values of post race urine samples were significantly ($P<0.001$) reduced from prerace values. It appears that the post race urine sample is, in many ways, a physiologically and pharmacologically unique sample (Dyke 1995).

Therefore, urine pH is a critical factor in interpreting the significance of drug detection data generated from post race urine samples. It follows that researchers must be able to reproduce in the laboratory the full range of post race urinary pH values, especially the acidic values, to determine the full range of concentrations of drugs and drug metabolites likely to be found in post race urine samples (Houston *et al.* 1985).

In an attempt to mimic the full range of possible drug concentrations in post race urine samples, researchers have often used Standardbred horses to mimic the drug clearance response of Thoroughbreds. The Agriculture Canada Equine Pharmacology Programme and the Ohio State University programmes have performed substantial portions of their 'detection time' research in exercised Standardbred horses and extrapolated the data to the Thoroughbred. However, as will be pointed out in this communication, it is now clear that urine samples collected from Standardbred horses are unlikely to match the very large drops in urine pH seen in post race urine samples from Thoroughbred horses. This suggests that Standardbreds are an inappropriate model for determining pharmacokinetic patterns and thresholds for therapeutic agents in Thoroughbreds.

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Materials and methods

A total of 419 post race urine samples collected from male and female racing Standardbreds over a 3 month period were analysed by the Official Racing Laboratory of the State of Ohio. The urine samples were collected at 2 parimutuel race track and were obtained from horses finishing first in each race and from other horses selected at random or for other reasons according to the official rules of racing in Ohio.

Urine was obtained in a collection stall by catching urine voided naturally into a cup. The urine was immediately refrigerated at 4°C and was stored for 1–5 days depending on the day of collection. Wood *et al.* (1990) have shown that only minimal pH changes (~0.25 pH units) occur in urine stored for up to 5 days, even urine stored at room temperature. The pH was measured with a calibrated pH meter using a glass electrode.

Results

The pH of the post race urine samples of Standardbred racehorses had a unimodal distribution with 56% of all urine samples between 7.5 and 8.5 pH units (Fig 1). Furthermore, the pH of 90% of all urine samples was between 7 and 9 pH units.

Discussion

The unimodal distribution of urine pH values in exercised Standardbreds has been shown to be markedly different from the bimodal distribution of urine pH values in post race Thoroughbreds. Moss (1976) reported a bimodal distribution in approximately 5000 urine samples with frequency peaks at pH values of about 5.75 and 7.75 units (Fig 1); Nakajima and Matsumoto (1976) reported bimodal frequency peaks at 4.75 and 7.75 pH units from 10000 samples; and Houston *et al.* (1985) showed a bimodal distribution with frequency peaks at 5.75 and 7.25 pH units from a smaller number of horses. The bimodal distribution is an unusual frequency pattern and appears to be related to the stress of racing and, particularly, that of Thoroughbred racing.

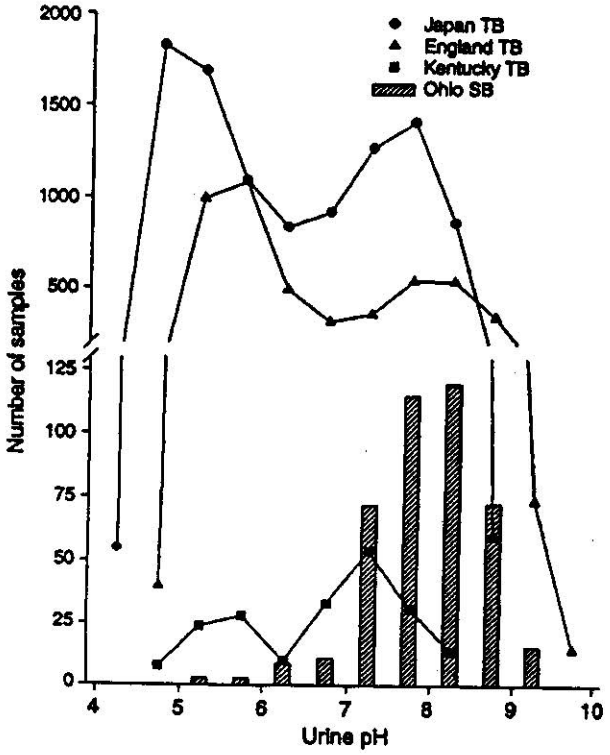

Fig 1: Post race urine pH values of Thoroughbred (TB) and Standardbreds (SB). Line graphs show historical data for Thoroughbreds (Japan-Nakajima and Matsumoto (1976); England-Moss (1976); Kentucky-Houston et al. *(1985), while bar graphs show frequency distribution developed from 419 Standardbreds racing in Ohio in 1995.*

The frequency distribution for urine pH values of trained Thoroughbred horses prior to racing is unimodal, with a mean pH value of about 7.5. As suggested by Snow (1983), the stress of racing apparently produces the shift to a bimodal distribution, with some horses producing urine of very low pH values. When the post race metabolic acidosis is relieved, urinary pH returned to control values. The bimodal frequency distribution seen in post race urine from Thoroughbred horses is probably an indication of the severity of the metabolic acidosis experienced.

However, the frequency distribution of post race urine pH values of Standardbred horses was slightly skewed but essentially unimodal with a peak around 8.2 pH units (Fig 1). In our data, there is no suggestion of the sharply bimodal distribution so characteristic of the post race urine samples from Thoroughbred racehorses, and it appears that post race urine samples from Thoroughbreds are physiologically different when compared to samples from Standardbreds.

These findings suggest that developing pharmacokinetic patterns and detection times for therapeutic medications in exercised Standardbreds may systematically underestimate the true detection times for basic drugs in racing Thoroughbred horses and, possibly, also for racing Quarter 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 increased up to 200-fold as urine pH increased. Similarly, Gerken *et al.* (1991) showed a significant increase in the urinary concentration 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, triggering drug identification long after the 'normal' detection time of the drug had passed.

The different frequency distributions of pH values in post race urine samples between Standardbreds and Thoroughbreds invites speculation about the cause. Thoroughbreds generally race over distances from 1000 to 2400 m 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 to a greater degree 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 1600 m. 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 pH distribution for Standardbreds can 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 Thoroughbreds with a pH value around 5.5 units were collected from sprinters which rely primarily on anaerobic metabolism for fuel.

Conversely, 'stayers' are more dependent on aerobic

metabolism which produces less acidosis, and the urine from these horses would be expected to have a pH closer to prerace values. It is possible that the high number of urine samples from Thoroughbreds with a pH value around 8 were collected from 'stayers'. Since Standardbreds are bred to race at only one distance (1600 m), it would be expected that the capacity for each metabolic pathway would be similar among members of that breed, unlike Thoroughbreds.

These results invite 2 further interesting speculations. First of all, if our hypothesis is correct that 'horses producing the highly acidic urine are the best sprinters', it follows that a pH analysis of post exercise urine might give an indication of the potential sprinting ability of racing horses. On the other hand, and perhaps more realistically, the inability of a horse to produce acidic urine might indicate a lack of potential as a sprinter.

Finally, 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 'hard' or 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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