A LITERATURE REVIEW

An Overview of the Methylxanthines and Their Regulation in the Horse

Caffeine, theophylline and theobromine are naturally occurring members of the methylxanthine family; pentoxifylline, dyphylline and enprofylline are structurally related synthetic pharmaceuticals. Caffeine has predominantly central nervous system effects, theophylline, dyphylline and enprofylline have predominantly bronchodilator effects, while theobromine is associated with diuretic responses. Pentoxifylline is thought to increase red cell deformability and facilitate blood flow through capillary beds. The methylxanthines are not highly potent agents; they are typically administered in gram doses and they tend to have relatively long plasma half-lives. They remain detectable in plasma and urine for relatively long periods. Similarly, traces of the naturally occurring members of this family are not uncommonly identified in forensic samples. In this review we report on the detection, actions, uses and regulatory control of this group of agents in performance horses.

J. Daniel Harkins, DVM, PhD
W. Allan Rees, PhD
Maxwell H. Gluck Equine
Research Center and
The Department of Veterinary
Science
University of Kentucky
Lexington, KY 40506

George D. Mundy, DVM
The Kentucky Racing
Commission
Lexington, KY 40511

Scott D. Stanley, PhD
University of California
CVDLS
Davis, CA 95616

Thomas Tobin, MVB, PhD, DABT
Maxwell H. Gluck Equine
Research Center and
The Department of Veterinary
Science
University of Kentucky
Lexington, KY 40506

Introduction

Caffeine, theophylline, and theobromine are naturally occurring xanthine alkaloids with significant pharmacologic activities. Theobromine occurs widely in certain animal feed materials, while caffeine is sometimes found in certain food supplements. More importantly, caffeine is the most widely used psychoactive agent in humans and is ubiquitous in the environment of most domesticated horses.

The pharmacological effects of these agents include stimulation of the central nervous system (CNS) and cardiovascular system, increased metabolic rate, bronchodilation, and diuresis. Caffeine has the most marked CNS effects, theophylline has the most marked bronchodilator effects, and theobromine is primarily associated with diuretic responses.

The Association of Racing Commissioners International (ARCI) drug classification system lists caffeine as a class 2 agent, theophylline as a class 3 agent, and theobromine as a class 4 agent. As such, caffeine is an agent with "high potential" to affect the outcome of a race, theophylline is an agent with lesser (but significant) potential, and theobromine has the least performance-improving potential of these three agents.

These agents are relatively well absorbed after oral administration, attain significant plasma and urinary concentrations, and are only slowly cleared by the horse. Detection of residues of these agents for a week or longer after clinical doses or after environmental contamination are not uncommon. In general terms, the frequency of identification of these agents depends on the sensitivity of the analytical method used. If highly sensitive (low nanogram/ml limit of detection) ELISA screening methods are used, a substantial fraction of "normal" post-race urine samples may be expected to contain identifiable concentrations of caffeine.

Contamination of equine feed with cocoa husk generated large numbers of theobromine identifications during the 1970's and 1980's in England and Ireland. Research showed that it would be difficult for feed manufacturers to eliminate theobromine from their manufacturing systems. Therefore, a threshold for theobromine in equine urine was set at 2,000 ng/ml to regulate the substance in racing horses in the British Isles. Work in the Far East, where caffeine contamination of feedstuffs is not uncommon, has led to suggested thresholds for caffeine of 10 and 30 ng/ml in plasma and urine, respectively.

Related methylxanthines not found in nature are marketed as pharmaceuticals in human medicine and may also be used in veterinary medicine. Pentoxifylline, a theobromine derivative, is used in the treatment of intermittent claudication in humans. It is thought to increase the flexibility and motility of erythrocytes. In equine medicine, pentoxifylline is used to treat navicular disease and laminitis and has also been suggested as a prophylaxis of exercise-induced pulmonary hemorrhage. Dyphylline is chemically and pharmacologically similar to theophylline and has been used as a bronchodilator in humans. However, it is only one fifth as potent as theophylline. On the other hand, enprofylline is more potent than theophylline as a bronchodilator and is used extensively in human medicine in Europe for the treatment of asthma.

The Methylxanthines: Natural Sources

Caffeine, theophylline, theobromine, and paraxanthine are classified as methylxanthines (Fig. 1). As such,

and amikacin sulfate) for a total of 16 days after admission. After discontinuation of intravenous antimicrobials trimethoprim sulfamethoxazole (30 mg/kg, PO, q 12 h) was administered for an additional 30 days. Phenylbutazone was discontinued 6 days after admission. Hyaluronic acid (40 mg) was injected into the tarsocrural joint at 7 and 14 days after surgery. The foal was trotting soundly 14 days after surgery and experienced no complications related to surgery. Radiographs of the hock were obtained 19 days after surgery. There was no enlargement of the cystic lesion, and sclerotic bone lined the defect. The blood fibrinogen concentration 19 days after surgery was 488 mg/dl and the foal was discharged from the hospital. Follow-up telephone conversation with the referring veterinarian 8 months after hospital discharge was obtained. The colt had no signs of lameness, no tarsocrural joint effusion, and the owners were planning to break the horse for riding.

Discussion

This is the first report of a subchondral cystic lesion involving the talus in a horse.[3] Subchondral cysts can develop secondary to subchondral bone trauma, chronic osteoarthritis, osteochondrosis, fracture, and sepsis.[4,5] The acute onset of lameness, tarsocrural joint effusion, pyrexia, and hyperfibrinogenemia in this case are suggestive of sepsis as the cause of the cystic lesion.[6,7] Unfortunately, microbial cultures of blood, synovial fluid, and bone were all negative for bacteria growth, and histopathology of the bone removed from the cyst was not performed. However, the foal had been treated with antimicrobials prior to collection of blood, synovial fluid, and bone for microbial culture. The administration of antimicrobials prior to sample collection can inhibit bacterial growth and therefore decrease the likelihood of obtaining a positive culture.[8,9] Despite our inability to confirm sepsis, the long duration of antimicrobial administration was justified because of the abnormal clinical findings supportive of sepsis and osteomyelitis.

Another interesting finding was that a communication between the tarsocrural and proximal intertarsal joints was not identified during arthroscopic examination of the tarsocrural joint.[2] Previously it has been reported that a communication between the tarsocrural and proximal intertarsal joints in newborn foals does not exist although the synovial membrane is thin.[2] In horses greater than 10 months of age a natural opening between the two joints is present and most likely results from mechanical tearing during normal exercise.[2] The proximal intertarsal joint was not sampled separately; therefore, septic arthritis of this joint could not be eliminated. The mild elevations of synovial fluid, nucleated cell count, and total protein within the tarsocrural joint were not compatible with septic arthritis, but were compatible with sympathetic synovial effusion. Sympathetic synovial effusion is the result of sepsis or inflammation adjacent to a synovial structure but without sepsis of the affected joint.[10,11] The inability to identify a natural communication between the tarsocrural and proximal intertarsal joints may explain the sympathetic effusion within the tarsocrural joint without cytological evidence of sepsis.

Arthroscopic examination of the tarsocrural joint via routine arthroscope and instrument portals[12] provided excellent surgical access to the cystic lesion once an iatrogenic communication between the tarsocrural joint and proximal intertarsal joint was established using a motorized arthroplasty system. Debridement of subchondral cystic lesions of the medial femoral condyle, distal phalanx, distal third metacarpus (tarsus), and scapulohumeral joint via

FIG. 1 — Dorsoplantar radiographic view of the hock illustrating the subchondral cystic lesion involving the central aspect of the talus adjacent to the proximal intertarsal joint and distal aspect of the medial trochlear ridge.

arthroscopy has been reported.[3] Surgical debridement is the preferred method of treatment and for many cases provides the best opportunity for regaining soundness.[3] Arthroscopic debridement of the cystic lesion in this case resulted in soundness and overall a successful outcome.

Subchondral cystic lesions of the talus should be included in the differential diagnosis of tarsocrural joint effusion and lameness in the horse, especially in foals. The lesion can be readily identified with conventional radiographic views of the tarsus, and arthroscopic debridement is a feasible and effective treatment option. ⚞

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they are closely related chemically and pharmacologically and are commonly found in many plants world-wide. Tea leaves and coffee beans contain caffeine and small amounts of theobromine and theophylline. Cocoa and chocolate, produced from the seeds of *Theobroma cacao*, contain theobromine and some caffeine. Theobromine comprises about 2.0% of the dry weight of the cacao bean and 0.47% of the cacao husk. The cola nut, a presumed ingredient of Coca-Cola and some other cola drinks, contains considerable amounts of caffeine. More than 60 plant species throughout the world have been identified as containing caffeine.[1] Theophylline occurs in some hollies, and theobromine occurs in species of *Theaceae, Sterculiaceae, Sapindaceae*, and *Aquifroliaceae*.[2]

Methylxanthines are readily absorbed after oral, rectal, and parenteral administration and share several pharmacological actions of importance. They relax smooth muscles, notably bronchial muscles that surround respiratory airways; they stimulate the central nervous system, increasing spontaneous activity;[3] they stimulate cardiac muscle; and they increase urine production by the kidneys.

Caffeine

Ingestion of caffeine (Fig. 2) by humans causes increased alertness, reduced drowsiness, and clearer thought. Although several scientific studies have shown the performance-enhancing effect of caffeine in human athletes,[4-6] the agent impairs coordination and task performance. Higher doses of caffeine increase CNS stimulation, resulting in progressive nervousness, anxiety, insomnia, and convulsions. Caffeine increases the muscle tension generated by submaximal impulses but does not alter the strength of maximal voluntary contractions.[7] Caffeine does not improve short-term or maximal power output in humans, and its effect on endurance performance is equivocal.[8,9]

In horses, caffeine potentiates fentanyl-induced locomotor activity (Fig. 3); however, the effect is short-lived and apparent only after intravenous administration.[3] One study showed improved running performance, as assessed by timed runs, following subcutaneous injection of 2.5 and 5.0 g of caffeine; however, the running speeds in the trials were submaximal. Heart rates during submaximal exercise and recovery are higher after caffeine administration than during control trials.[10]

Pharmacological Thresholds

Comparison of the plasma concentrations of caffeine in horses after IV administration with behavioral responses to this agent reported by
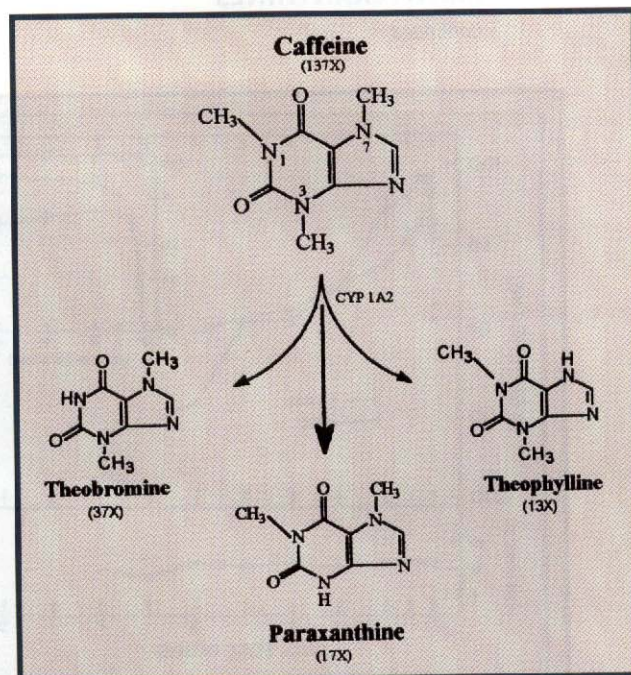

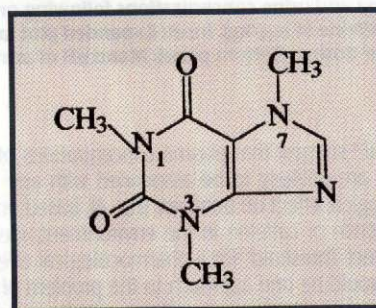FIG. 1 – Structures of the naturally occurring methylxanthines.
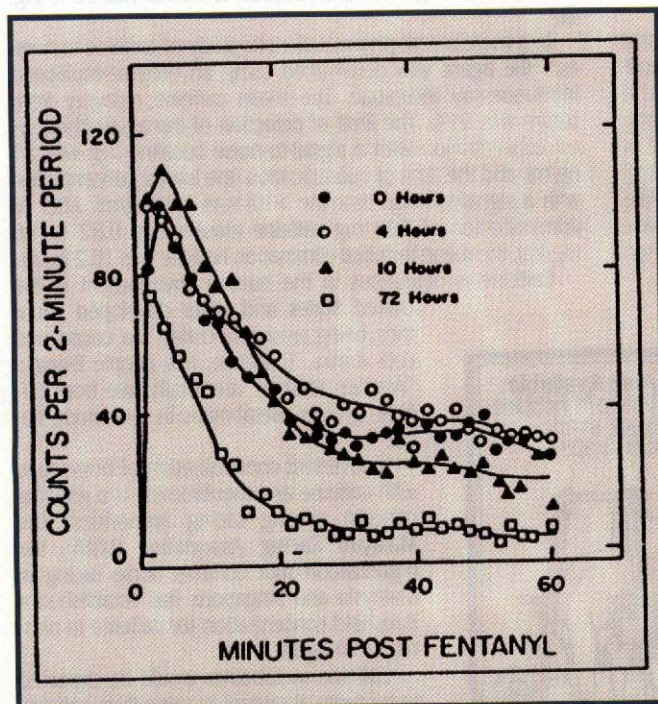

FIG. 2 – Structure of caffeine.

FIG. 3 – Locomotor response in horses (n=4) injected with caffeine (4 mg/kg, IV) and followed by fentanyl administration 0, 4, 10, and 72 hours later.
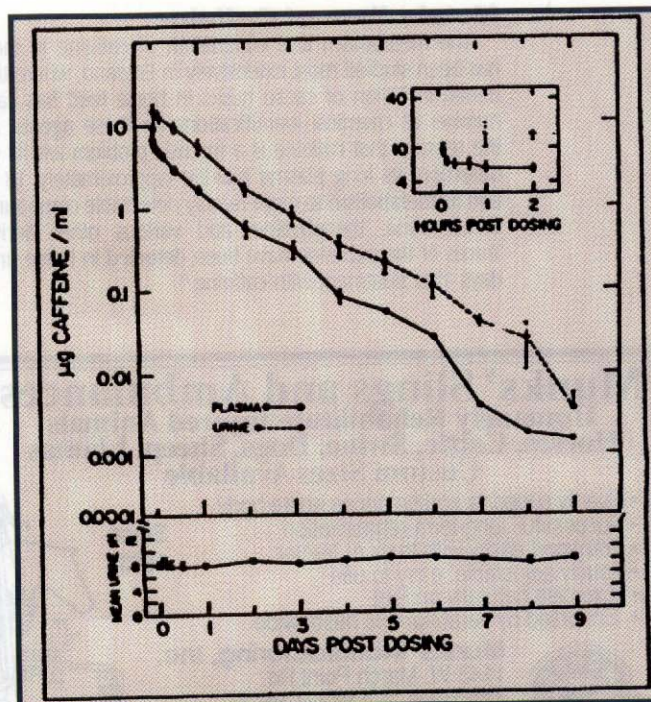

FIG. 4 – Mean plasma and urinary concentrations following IV caffeine administration (n=4). Inset: Expanded plot of plasma and urine concentrations during the first 2 hours after dosing. Bottom panel: mean pH of urine after dosing (n=3).
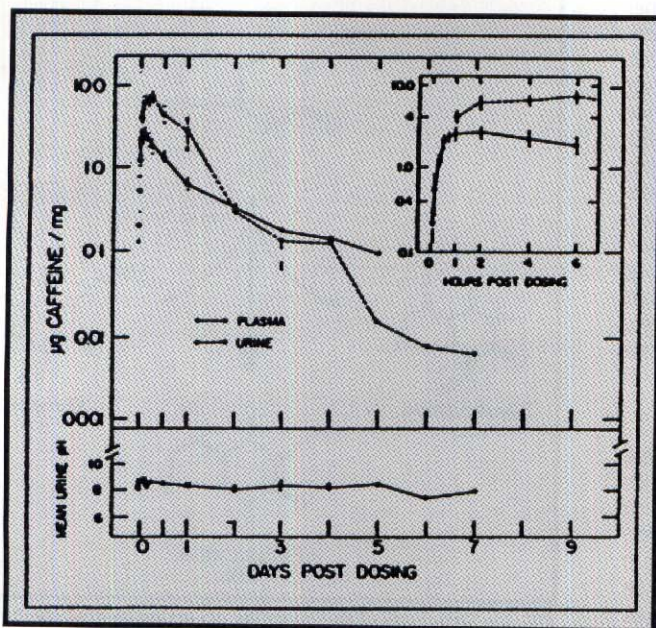
Pharmacology

FIG. 5 — Mean plasma and urine concentrations following oral administration of caffeine (4 mg/kg). Inset: Expanded plot taken during first 6 hours after dosing. Bottom panel: Mean pH of urine (n=4).

Greene et al.[3] suggest that plasma concentrations of less than 300 ng/ml are unlikely to be associated with any measured pharmacological effect on behavior (Fig. 4). Based on the ubiquitous presence of caffeine in the environment, identification of a "No Effect Threshold" for a pharmacological effect on performance would be one approach to the problem of detection of very low concentrations of caffeine and its metabolites in post race plasma and urine samples.

Metabolism of Caffeine

The metabolism and elimination of caffeine in the horse has been studied most extensively in England, where the accidental inclusion of cacao husks in horse feed has caused a number of chemical identifications for these agents. Among the reasons that caffeine is a forensic problem are its ease of detection, its long plasma half-life (approximately 18 hours), and its metabolism to other readily detectable compounds like theobromine, theophylline, and various other derivatives. Traces of theobromine have been detected in horse urine ten days after treatment with caffeine.[11]

Analytical Detection of Caffeine

Caffeine is readily detectable in equine plasma and urine after liquid/liquid extraction with dichloromethane and gas chromatographic analysis, with a sensitivity down to 1.0 ng of caffeine. Using this method, Greene et al.[3] recovered 96% of caffeine from plasma and 88% of caffeine from urine. After IV injection (4 mg/kg), caffeine reached a peak plasma concentration (10,000 ng/ml) within 5 minutes of administration and remained detectable for up to 9 days in plasma. The terminal elimination half-life of caffeine after intravenous administration was 18.2 hours. Peak caffeine concentration in urine (>10,000 ng/ml) was reached at the first sampling (1 hour), and caffeine concentrations were also detectable for up to 9 days in urine. Urine concentrations of caffeine were consistently about three-fold greater than and parallel to plasma caffeine concentrations. The parallel declines of urine and plasma caffeine concentrations indicated that caffeine was being excreted in the urine at a rate similar to its elimination from the plasma.

After oral administration of caffeine (4 mg/kg), peak plasma concentration of caffeine (2,700 ng/ml) was reached 2 hours after administration and decreased until it was no longer detectable by 6 days after dosing. Mean urinary concentration was 4,100 ng/ml at 1 hour and increased to 7,200 ng/ml at 6 hours after dosing. Thereafter, urinary caffeine concentrations decreased until no agent was detected at 8 days after administration. The terminal elimination half-life of orally administered caffeine was 42 hours. In all horses tested (n=4), caffeine remained above the lower limit of detection in the urine for an equal or greater time than it was in plasma.

The relationship between urine and plasma concentrations of caffeine after oral administration was not constant as it was following IV administration. Initially, urine concentration of caffeine was greater (similar to IV administration); however, at 28 hours post-dosing, urine and plasma caffeine concentrations were virtually the same. After 48 hours, urine concentrations of caffeine were less than plasma concentrations and remained less for subsequent samples. The lower plasma concentrations, and the apparently longer plasma half-life of caffeine following oral administration suggest that caffeine may be slowly and incompletely absorbed from the GI tract. The bioavailability of caffeine following oral administration to horses was 39% (Fig. 5).[3]

In a more recent pharmacokinetic study of caffeine in horses,[12] the agent was determined using an enzyme-multiplied immunoassay technique. The mean caffeine recovery from serum was 91%. The limit of detection of the assay (the lowest concentration with a signal-to-noise baseline >3) was 81 ng/ml, and the limit of quantification (the lowest concentration with a signal-to-noise baseline >10) was 270 ng/ml. After IV administration of 500 mg caffeine (dose range 0.82 to 1.8 mg/kg), the mean terminal elimination half-life was 10.2 hours.

Caffeine is ubiquitous in the human environment in the United States and other developed countries, being present in coffee, tea, cocoa, and cola drinks. Therefore, few people living a "Western lifestyle" are totally free from caffeine and its metabolites in their body fluids.[1]

Inadvertent contamination of horse feed with caffeine and theobromine is a growing concern among racing authorities. The Malayan Racing Association (MRA), the organization that controls horse racing in Malaysia and Singapore, has established a threshold concentration for caffeine in plasma at 10 ng/ml.

Caffeine metabolites would be expected to be more abundant in urine than caffeine itself, since the chemical nature of these compounds causes caffeine to be reabsorbed into the blood from the glomerular
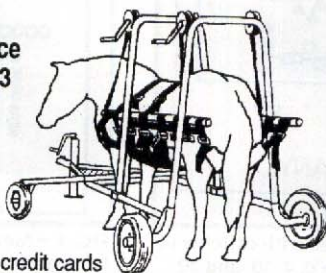
filtrate more readily than the metabolites. In fact, caffeine is only 2 to 5% of the total excretory products following caffeine administration, with the dimethylxanthines (theophylline, theobromine, and paraxanthine), metabolites of caffeine breakdown, representing about 30% of the excretory products.

However, the opposite effect is true in blood, with the concentration of caffeine being much higher than the concentrations of any metabolites. The concentration of caffeine in blood determines the concentration of those agents in saliva and, supposedly, in sweat, although no scientific studies on the concentration of caffeine in sweat have been completed.

Under conditions of moderate to high ambient temperatures, sweating in humans is continuous. Any caffeine in the blood would be continuously transported to the surface of the skin, and the quantity of caffeine on the skin would steadily increase and remain on the skin until washed away. Obviously, the degree of personal hygiene would be a major determinant of the quantity of caffeine present on a person's skin (personal communication, Dr. M.S. Moss) Mild sweating over several hours followed by profuse sweating would produce sweat droplets that contained concentrations of caffeine much higher than the concentration of caffeine in blood (personal communication, Dr. M.S. Moss). Considering the extreme sensitivity of modern detection methods for caffeine, only a few nanograms of contaminant in urine could produce a chemical identification. In 1972 the International Olympic Committee (IOC) removed caffeine from its list of prohibited agents, reasoning that caffeine was a common constituent in the diet of many athletes. However, because of increased use of caffeine by athletes, the IOC has recently barred caffeine at concentrations greater than 15,000 ng/ml in plasma samples. Such a large concentration of caffeine in plasma is thought to be attainable only following caffeine administration by injection or suppository.[5]

Theophylline

Theophylline (Fig. 6) is a potent bronchodilator medication for human asthmatic subjects;[3] however, there is no evidence of a significant bronchodilator effect on human subjects without asthma. There is also no evidence that theophylline administration improves human physical work capacity, maximal oxygen consumption, reaction time, or muscular performance of long bone skeletal muscles (e.g., quadriceps),[4] although recent studies indicate that theophylline does enhance the strength and endurance of respiratory skeletal muscles (diaphragm).[5] Although theophylline has significantly increased heart rate in some studies of human athletes,[6] this is not a consistent finding. Furthermore, an agent that elevates heart rate would not appear to be beneficial to performance.

In horses, theophylline, like caffeine, is also a CNS stimulant and diuretic. In studies using horses, theophylline has been shown to decrease pulmonary resistance and to increase dynamic compliance, respiratory rate, heart rate, and excitement in resting ponies with chronic obstructive pulmonary disease (COPD). All effects were dependent on varying minimal plasma theophylline concentrations, the lowest mean minimal concentration (for decreased pulmonary resistance) being 59 μmol/liter (10.2 mg/l). This value was similar to an early study that determined a minimal plasma theophylline concentration of about 55 μmol/liter (10 mg/l) for bronchodilation in horses. This is in comparison with a plasma concentration of 55 to 111 mmol/liter (10 to 20 mg/l) for optimal management of chronic asthma in humans.[4]

Another study in healthy Standardbreds exercised on a treadmill showed that heart rate and plasma lactate concentration during exercise were significantly elevated when compared to similar values during exercise without theophylline administration.[7] The increased heart rate was attributed to either a direct chronotropic effect of the agent on the myocardium or mediated by an increased catecholamine release from the adrenal medulla. The increased plasma lactate concentration indicated an increased dependency on glycolytic energy metabolism either due to a theophylline-related stimulation of enzymes involved in glycolysis or an insufficient oxygen supply to the exercising muscles. The study also showed a higher respiratory quotient (RQ) during exercise following theophylline administration when compared to control exercise. The higher RQ indicated that less fat and more carbohydrate was being utilized for fuel. This is in contrast to the effect of methylxanthines on the metabolism of humans, where there is increased utilization of fats following administration of caffeine and theophylline.

Partial pressure of arterial oxygen (P_aO_2) was unaffected during exercise in horses; however, P_aO_2 was increased following exercise. Similarly, the partial pressure of carbon dioxide (P_aCO_2) was unchanged during exercise; however, before exercise and following exercise P_aCO_2 was significantly lower than baseline values. The altered blood gas values after theophylline administration suggest that pulmonary ventilation was increased at rest, which was consistent with previous studies of humans and animals.[8,19] Additionally, tidal volume was significantly increased by theophylline.

Furthermore, arterial pH was lower during the work after theophylline administration than during exercise without theophylline. The lowered pH was at least partially due to the increase in lactic acid production. The authors[17] concluded that the increased heart rate and the increased lactate production associated with theophylline administration
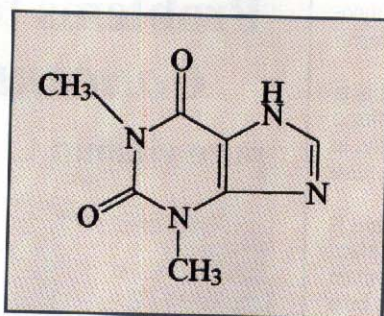
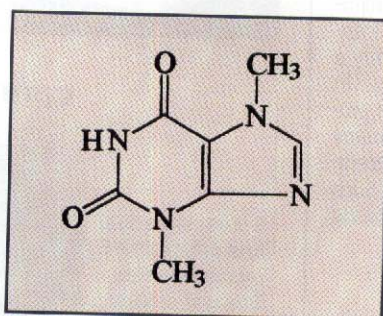**Pharmacology**

FIG. 6 – Structure of theophylline.

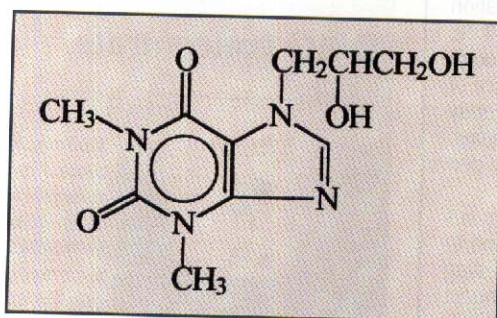FIG. 7 – Structure of theobromine.

FIG. 8 – Structure of dyphylline.
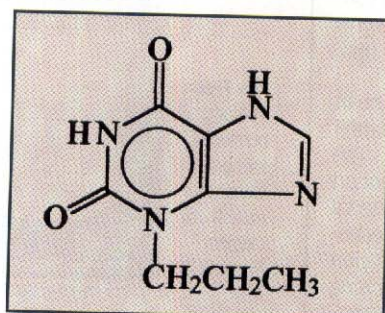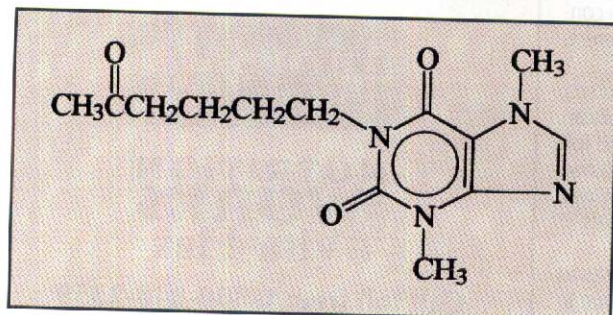

FIG. 9 – Structure of enprofylline.

FIG. 10 – Structure of pentoxifylline.

were negative influences on exercise tolerance in the normal horse.

Theobromine

Theobromine (Fig. 7) is a common contaminant of horse feeds. Over the past 20 years in Great Britain, theobromine has been the most frequently detected prohibited substance in the urine of race horses. The source of this agent has generally been traced to processed animal feed. The results of one study to determine the incidence of theobromine contamination in horse feed showed that about 70% of the feed samples contained theobromine in concentrations up to 1 mg/kg.[20]

Other products that have been identified as sources of theobromine in horses have been chocolate candy bars as treats and feed comprised of bakery waste containing chocolate products. One trainer was disqualified after giving his horse a packet of cocoa-flavored instant breakfast drink.[2] Another unusual source of theobromine has been Doan's Pills, a human back medication containing theobromine and salicylate for back pain.[11]

The effect of theobromine on performance of race horses is unknown. Like other methylxanthines, theobromine is a bronchodilator and a cardiac stimulant.[22] The agent also causes stimulation of the CNS, induces diuresis, and increases basal metabolic rate.

The discovery of trace amounts of theobromine in urine of racehorses prompted the development of an analytical method to detect the agent at low concentrations in horse feed. Taylor[22] refluxed feed samples with distilled water and passed the extracted solution through a glass column filled with a silica gel in chloroform. Theobromine was eluted with a chloroform/propan-2-ol mixture, then the eluate was analyzed using high-performance liquid chromatography (HPLC). Theobromine in animal feeds was determined at concentrations as low as 2 mg/kg.

In a personal communication cited in Haywood et al.[20] Dr. Brian Cooke of Dalgety Agriculture Ltd. stated that theobromine can be excluded from manufactured feed at concentrations above 0.8 mg/kg. When feed containing theobromine at concentrations less than 0.8 mg/kg was fed to horses, theobromine was not found at concentrations greater than the limit of detection of the method. Incidentally, European Community (EC) directives allow theobromine to be present in feedstuffs for horses up to a concentration of 300 mg/kg.

In that study, feed containing theobromine at a concentration of 1.2 mg/kg was fed to three horses for 4 days. A maximum theobromine urinary concentration of 550 ng/ml was detected. From this work and to allow a reasonable margin for variations in urinary excretion in individual animals, the Jockey Club of Great Britain and other European racing authorities established a urine threshold concentration for theobromine of 2,000 ng/ml.

In a later study,[23] urinary theobromine concentrations were measured in horses offered feed containing theobromine (19.2 mg/kg) twice a day for 2.5 days. Maximal theobromine concentration in urine (6,020 ng/ml) was measured 4 hours after the last feeding, and theobromine was detectable in urine 6 days after the last feeding in two of five horses. It was concluded that at least 2 days were required after the administration of feed containing EC-permitted concentrations of theobromine for the concentration of theobromine in urine to fall below the threshold value of 2,000 ng/ml. Theobromine concentration in plasma was also determined with a detection limit of 100 ng/ml. In none of the cases could theobromine be detected in plasma. Furthermore, the excretion rate of theobromine was determined to be highly variable, with the time to peak excretion 2 to 12 hours after the final feeding.[23]

The excretion of caffeine, and probably other methylxanthines, is urine-flow rate dependent.[24] Human volunteers were given a caffeine-containing beverage, and half were given a diuretic 2 hours later. There was no significant difference in the urine caffeine concentration between volunteers receiving the diuretic and those that received only the caffeine-containing beverage. Many horses receive furosemide treatment before a race, and diuretics may decrease the concentration of some agents in urine. However, because of the correlation between urine flow and theobromine excretion rate, it appears that furosemide or other diuretics will not artificially reduce theobromine concentrations in urine.

Metabolism of the Methylxanthines

Three major enzymes, cytochrome P450-1A2 (CYP 1A2), N-acetyltransferase, and xanthine oxidase are responsible for generating the wide range of caffeine metabolites in the human[25] and, presumably, in the horse. CYP 1A2 primarily transforms the majority of the parent compound by demethylation and oxidation to paraxanthine (17X) (Fig. 1), which is further converted to the

primary urinary metabolites 1-methyluric acid and 1-methylxanthine. Theobromine (37X) and theophylline (13X) are formed by minor pathways. N-acetyl transferase and xanthine oxidase enzymes metabolize theophylline and give rise to other metabolites in lower concentrations to complete the extensive metabolite profile that has been reported in the horse.[26]

Theophylline, whether produced by caffeine metabolism or administered as a parent compound, is further metabolized by CYP 1A2 or the ethanol-induced CYP 2E1 to 1,3-dimethyluric acid. Theobromine is also subject to the same sequence of enzyme degradation as theophylline to create another metabolite of the xanthine family, 3-methylxanthine.

Moss[26] reported on the metabolism of caffeine specifically as it pertains to the horse. This researcher also discovered many metabolites in equine urine that had previously been detected in human urine. Positively identified metabolites of caffeine included 1,3- and 1,7-dimethyluric acids, 1,3,7-trimethyluric acid, and all three dimethylxanthines as well as caffeine. The 1,3,7-trimethyldihydrouric acid, 1,3-dimethylhydrouric acid, and urea were tentatively confirmed. Also, it is important to note that none of these compounds was conjugated in equine urine, which would support the theory that caffeine metabolism is confined to the phase I cytochrome P450 enzyme system and does not employ any of the phase II (glucuronyl transferase or sulfotransferase) systems.

Dyphylline (Dilor, Lufyllin)

Dyphylline (Fig. 8), 7-(2,3-dihydroxypropyl) theophylline, is chemically similar to theophylline (Fig. 6) and has been used in the past as a bronchodilator in the treatment of asthma, but it appears to be only one fifth as potent.[27] The pharmacokinetics, efficacy, and disposition of dyphylline in the horse have not been extensively investigated. However in one experiment, Ayers et al.[28] compared the efficacy of theophylline and dyphylline in horses and showed that, while theophylline caused significant increases in heart rate, dyphylline did not. Oral preparations are not completely absorbed in the human, and the compound has a relatively short half-life of 2 hours. The literature indicates that dyphylline is excreted unchanged in the urine after administration and is not converted to theophylline.[28]

Enprofylline

Enprofylline (Fig. 9) (3-propylxanthine) is more potent than theophylline as a bronchodilator in the treatment of asthma. In Europe, this agent is used extensively in humans and causes fewer side effects than theophylline, such as impaired CNS and renal functions and gastric disturbances.[27] At least 90% of enprofylline is excreted unchanged in the urine[29] The number of post-race enprofylline identifications in horses during the past 5 years has been limited since the agent is not yet commercially available in the United States.

Pentoxifylline (Trental)

A derivative of theobromine, pentoxifylline (Fig. 10) (1-[5-oxo-hexyl]-3,7-dimethylxanthine) is currently used as a treatment for human patients with intermittent claudication due to chronic

TABLE 1
REPORTED CLINICAL DOSE, MINIMAL EFFECTIVE PLASMA CONCENTRATION, PLASMA HALF-LIFE, AND DETECTION TIMES FOR METHYLXANTHINES IN A 500 KG HORSE

	CLINICAL DOSE	ESTIMATED EFFECTIVE PLASMA CONC.	HALF-LIFE	REPORTED DETECTION TIME
CAFFEINE	2.0 g	2,000 ng/ml	18.2 hr (IV) 42 hr (Oral)	9 days
THEOPHYLLINE	1.5 - 6.0 g	10,000 - 20,000 ng/ml	9.7 - 19.3 hr	4 days*
THEOBROMINE	N/A	N/A	N/A	10 days
DYPHYLLINE	5.0 g	10,000 - 20,000 ng/ml	1.9-2.9 hr	4 days*
ENPROFYLLINE	N/A	N/A	< 2.0 hr (human $t_{1/2}$)	N/A
PENTOXIFYLLINE	2.0 - 4.0 g*	N/A	N/A	2 days*

*Canadian data; N/A = not available

TABLE 2
CLINICAL AND FORENSIC CORRELATES OF SELECTED METHYLXANTHINES IN THE HORSE

	PLASMA THRESHOLD (NG/ML)	URINE THRESHOLD (NG/ML)	ARCI CLASSIFICATION	LIKELY INADVERTENT SOURCES
CAFFEINE	10 Far East	30 Far East	2	Contaminant from human sources
THEOPHYLLINE	—	—	3	Medication residue
THEOBROMINE	—	2,000 (Urine) International	4	Feed stuff contaminant
DYPHYLLINE	—	—	3	—
ENPROFYLLINE	—	—	Not Classified	—
PENTOXIFYLLINE	—	—	3	—

TABLE 3
NUMBER OF IDENTIFICATIONS OF XANTHINE AGENTS REPORTED BY THE ASSOCIATION OF OFFICIAL RACING CHEMISTS (AORC) LABORATORIES FOR 1993 TO 1995.

	1993	1994	1995
CAFFEINE	34	67	50
THEOPHYLLINE	19	11	25
THEOBROMINE	19	11	20
DYPHYLLINE	0	6	0
ENPROFYLLINE	0	0	0
PENTOXIFYLLINE	1	1	0

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occlusive arterial disease.[27] Intermittent claudication is characterized by increased blood and plasma viscosities, decreased erythrocyte deformability or flexibility, and decreased capillary blood flow to distal limbs resulting in reduced tissue perfusion. Long term treatment with pentoxifylline improves the clinical responsiveness in patients by increasing the flexibility and motility of erythrocytes, reducing blood viscosity, decreasing platelet aggregation, decreasing fibrinogen levels, and inhibiting the action of inflammatory cytokines.

In horses, pentoxifylline is used to treat the signs of navicular disease. One of the proposed causes of navicular disease is the formation of thrombi in the navicular arteries, which reduces blood supply to the navicular bones.[30] Ischemia leads to ulceration and erosion of bony tissue causing lameness. Although no scientific studies have shown beneficial effects from pentoxifylline treatment in horses with navicular disease, an agent that increases microcirculatory blood flow could halt or even reverse the effects of navicular disease,[3] assuming navicular disease is a result of decreased blood flow.

Pentoxifylline is extensively metabolized in humans with seven metabolites recovered from urine and/or serum. The carboxylic acid metabolites are most prevalent in urine of humans with demethylated and dihydroxy metabolites present in minimal amounts.

The relative amounts of metabolites detected in horse urine samples after pentoxifylline administration are different from those found in human urine. The most abundant metabolite is the demethylated metabolite, 3-methyl-1-(5-oxohexyl)-xanthene (MOX), which accounts for 80% of the total metabolites/parent material collected. The large amount of this metabolite indicates that the biotransformation is primarily due to the xanthine moiety rather than its side chain. This suggests a metabolic similarity to other xanthines in humans such as theophylline, theobromine, and caffeine.[3]

In metabolic studies of pentoxifylline in humans, 60% of the total administered dose was excreted in the urine as parent material and its metabolites. In contrast, less than 10% of the administered pentoxifylline was excreted in urine of horses. As an explanation for this, the presence of feed concomitant with pentoxifylline administration may have reduced the rate and the amount of agent absorption. Furthermore, the faster gastric emptying rate for horses may have decreased absorption. It is also possible that the bulk of the agent may be distributed to the bile and subsequently eliminated in the feces.

The information contained in this review is summarized in Tables 1-3. Table 1 contains the range of normal clinical doses of the methylxanthines, the estimated plasma concentration necessary for a pharmacological effect, the plasma half lives of the agents, and the reported time for which each agent can be detected following administration. Table 2 contains the ARCI classifications for each methylxanthine, likely inadvertent sources of contamination, and the plasma and urine thresholds of each agent where such thresholds have been accepted. Table 3 shows the number of times an identification was reported and/or regulatory action was taken for these agents during 1993 to 1995.

Figures 3, 5, and 9 are reprinted with permission from: Greene et al: "Pharmacology, Pharmacokinetics, and Behavioral Effects of Caffeine in Horses," Am J Vet Res 44:57-63, 1983.

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