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Effects of Bronchodilating and Non-steroidal Anti-inflammatory Drugs on Performance Potential in the Horse

Peter Kallings

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

This study was intended to ascertain whether or not equine performance potential is affected by non-steroidal anti-inflammatory drugs (NSAIDs; phenylbutazone, meclofenamic acid, flunixin) and bronchodilators (theophylline and clenbuterol). The latter have been illicitly adminestered to normal racehorses with the intention to ease respiration and improve oxygen uptake. In some cases use of the analgesic NSAIDs is permitted in horseracing and competitive events. However, as this use of the drugs has been questioned, it was felt important to study their effects in an objective standardized way. Clinically healthy horses therefore underwent an established submaximal exercise tolerance tests on a treadmill, both with and without medication. To study the effects of NSAIDs also during more intensive exercise, a modified standard incremental treadmill test to the point of fatigue was developed. To simulate racing conditions this test was also tried out on a track. In addition, NSAID effects on equine kinematics were studied objectively on a treadmill with high-speed cinematography in horses with low-grade lameness.

The main effects of theophylline during submaximal exercise were increased heart rate and elevated blood lactate level, but no increase in oxygen uptake, thus suggesting impaired rather than enhanced performance potential. Clenbuterol had no major effects, thus indicating its inability to improve the performance of healthy horses.

NSAIDs also increased heart rate and caused changes in lactate responses to exercise, but as neither flunixin nor phenylbutazone altered the oxygen uptake, they too were considered not to improve performance. However, flunixin did appear to have a beneficial effect on locomotory pattern, possibly attributable to its analgesic effect on subclinical lameness or pain. In the studied lame horses, the effects of phenylbutazone remained even after its plasma concentration had fallen to a very low level.

It could then be considered that NSAIDs do not directly improve performance but, by alleviating pain, allow subclinically lame horses to run at their full potential. In racing and other competitive sports this type of therapeutic use could jeopardize the wellbeing of equine athletes.

Keywords: horse , NSAIDs, bronchodilators, treadmill, track, performance, doping.

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Doctoral thesis
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To my family

it was just a
matter of time....

Abstract

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Contents

Appendix

Papers I-VII

The present thesis is based on the following papers, which will be referred to by their roman numerals.

I. Kallings, P., Persson, S., Appelgren, L-E., Eneroth, P. and Wiese, B. (1987) Effects of phenylbutazone on exercising horses: Studies of cardiorespiratory and metabolic parameters, plasma concentrations and inhibition of prostaglandin synthesis. In: Proc. 6th Internat. Conf. Racing Analysts and Vet., Hong Kong, 1985, MacMillan Publ., pp 53-58.

II. Ingvast-Larsson, C., Kallings, P., Persson, S. Appelgren, L-E. and Wiese, B. (1989) Pharmacokinetics and cardio-respiratory effects of oral theophylline in exercised horses. J Vet Pharmacol Ther. **12**, 189-199.

III. Kallings, P. Ingvast-Larsson, C., Persson, S., Appelgren, L-E., Förster, H.J. and Rominger, K.L. (1991) Clenbuterol plasma concentrations after repeated oral administration and its effects on cardio-respiratory and blood lactate responses to exercise in healthy Standardbred horses. J Vet Pharmacol Ther. **14**, 243-250.

IV. Johansson, I.M., Kallings, P. and Hammarlund-Udenaes, M. (1991) Studies of meclofenamic acid and two metabolites in horses - pharmacokinetics and effects on exercise tolerance. J Vet Pharmacol Ther. **14**, 235-242.

V. Drevemo, S., Johnston, C., Kallings, P. and Roepstorff, L. (1995) Effects of phenylbutazone at low plasma concentrations on the locomotion pattern in lame horses. In: Proc. 10th Internat. Conf. Racing Analysts and Vet., Stockholm, 1994, R&W Publications, Newmarket, UK. pp 23-27.

VI. Kallings, P., Johnston, C. and Drevemo, S. (1998) Effects of flunixin on movement and performance of Standardbred trotters on the track. Submitted to Equine vet J, Suppl. / Proc. 5th ICEEP, Japan 1998 (Abstract accepted).

VII. Kallings, P., Persson, S.G.B. and Essén-Gustavsson, B. (1998) Effects of flunixin on cardiorespiratory, plasma lactate and stride length responses to treadmill exercise in Standarbred trotters. (Submitted).

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Introduction

Background

Doping, medication and equine welfare

The term "doping" is usually referred to the use of drugs with the aim to enhance performance, e.g. with stimulant "cocktails" containing narcotics and cocain . Drugs could also be given with the purpose to decrease the performance capability, e. g. "nobbling" a competitors horse with sedatives. The abuse of narcotics, which in low doses stimulate a horse´s nervous activity, can if adminestered in large amounts sedate or even kill a horse.

Doping and medication may also be used to disguise injuries, mask defects in temperament and to improve the functioning of various body systems of horses offered for sale. These are problems for organizers and veterinarians controlling equestrian events.

The non-steroidal anti-inflammatory drugs (NSAIDs) are probably the most commonly used drugs in racehorses and since they are in some cases even permitted to be used in competition, the question has often been raised whether these drugs affect the physiological responses to exercise and hence performance potential. In the late 1970s the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners in the USA stated that phenylbutazone allows a horse to run up to its potential, i.e. relief of inflammation enables the horse to race up to its maximum capacity. Currently also flunixin and other NSAIDs are permitted to use in the USA. This view is not shared by the rest of the world - racing horses on NSAIDs is illegal in most countries.

Most racing jurisdictions initially advocated the principle that horses should run on "hay, oats and water". Any horse having in its systems any substance which could affect its performance in any way was barred from competition, and severe penalties were levied. This type of rule, usually called the "foreign substance rule" is most effectively policed through random testing of entries in a field. In practice winners are usually selected and tested, ensuring that top prize money will not go to a "doped" horse. Still most horse competitions require that the horses are free of "foreign substances" which may affect performance. Apart from that the rules of competition should ensure that competitors compete on equal terms, abuse of drugs could be detrimental to the welfare of the horse.

Bronchodilating drugs have been used illegally with the intention to "open up the airways" and increase the oxygen uptake and thereby improve performance in normal racehorses.

There is a lack of information as regards effects of drugs on performance, especially at low concentrations ("therapeutic endpoints"). Studies have been undertaken, both of effects on treadmill exercise tolerance and

pharmacological activity (e.g. prostaglandin inhibition) but usually with therapeutic concentrations.

It could be concluded that there are still to many problems with a system of threshold values, mainly because of the uncertainty in estimating the therapeutic endpoints. There is an obvious need for research of effects of drugs at low concentrations in the horse.

To study if NSAIDs and bronchodilators have an influence in healthy horses on cardiorespiratory, lactate and locomotory responses to exercise, standardized tolerance tests as well as analyses of effects on lameness of drugs at low concentrations were considered.

Drugs and their effects

Bronchodilating drugs

Theophylline

The xanthine derivative theophylline has in addition to the bronchodilating effect a wide range of pharmacological effects. Apart from relaxing smooth muscle, theophylline stimulates the central nervous system, has a diuretic effect and has positive chronotropic and inotropic effects on the myocardium (Rall, 1980; Rang *et al.*, 1995). It is also known that theophylline increases the release of noradrenalin, adrenalin and free fatty acids (Andersson *et al.*, 1985). Its mode of action is still uncertain, but suggested mechanisms are inhibition of phosphodiesterase enzymes (Bergstrand, 1980; Persson, 1987) by inhibition of cyclic AMP-phosphodiesterase (Rang *et al.*, 1995), which may be involved in the relaxant effects on smooth muscle, and/or adenosine receptor antagonism. Adenosine is an important regulator in several cells and tissues, e.g. it increases the release of mediators from mast cells (Fredholm, 1980 a,b; Stiles, 1986).

The clinical relevance of theophylline in equine medicine is its use as a bronchodilator in horses suffering from chronic obstructive pulmonary disease (COPD) (Beech, 1979; Petermann, 1981; von Botz, 1982; Deegen, 1982). Theophylline has a relative narrow therapeutic window and adverse effects, such as central nervous symptoms, sweating and muscle tremor, have been reported in horses with plasma concentrations above 15 µg/ml (Errecalde *et al.*, 1985).

Beside the pharmacokinetic and pharmacodynamic effects of theophylline on COPD , it was also of interest to study whether these central stimulating and bronchodilating xanthines might affect performance in healthy horses. Theophylline was then chosen for investigation as a representative of this kind of drugs, as this drug had previously been studied at our university (Ingvast-Larsson *et al.*, 1985). As there had been some controversy about the effects of the xanthine derivatives on performance and their use as potential doping agents, it was of interest to evaluate the effects of theophylline on cardiorespiratory and metabolic responses to exercise in healthy horses.

Clenbuterol

Other bronchodilators are the ß$_2$-adrenoceptor agonists, e.g. clenbuterol, which relax the airways by stimulating ß-adrenoceptors in their smooth muscles. In contrast to the unselective ß-agonists, which have undesirable effects on heart and skeletal muscles, the ß$_2$-stimulators act more selective on smooth muscles in the airways (and uterus). The growth-promoting effect has been reviewed for several species (Westergaard and Sejerson, 1991), but there are no reports on such effects in horses. Other effects reported in horses are increased mucociliary clearance and mucus secretion (Derksen, 1987; Turgut and Sasse, 1989).

The ß$_2$-sympathomimetic agent clenbuterol is available on the Swedish market (Ventipulmin® vet.) for use in horses suffering from COPD or other respiratory distress. Its clinical use in coughing horses has frequently been described (Sasse and Hajer, 1978; Genetzky and Loparco, 1985; Teitzel, 1982), but few studies have been reported in healthy exercising horses (Lieske and Deegen, 1980; Rose *et al.*, 1983; Rose and Evans, 1987). More recently, Slocombe *et al.* (1992) have studied the effect of clenbuterol on respiratory mechanics during stepwise treadmill exercise tests.

Although there are no published reports of significant effects on respiratory or cardiovascular capacity in healthy horses, clenbuterol has been used with the intention of enhancing performance in normal racehorses. It is reported to be given illegally (as an aerosol) before racing in Australia (Slocombe *et al.*, 1992). Therefore, it was of interest to include clenbuterol in this study.

Non-steroidal anti-inflammatory drugs

NSAIDs are commonly used in equine practice, mainly for musculoskeletal inflammatory conditions. Phenylbutazone ("Bute") has been the preferred drug for treating musculoskeletal disorders since it was introduced in veterinary medicine in the 1950s. During the last two decades, a number of NSAIDs have appeared exclusively for use in veterinary medicine, e.g. flunixin meglumine (Banamine or Finadyne) and meclofenamic acid (Arquel). Flunixin is also used to treat colic and abdominal pain (Tobin 1981; Lees and Higgins, 1985; Kallings, 1993).

NSAIDs act basically by inhibiting the synthesis of prostanoids (Lees and Higgins, 1984; Soma *et al.*, 1992), i e prostaglandins (PGs) and thromboxanes (TXs), by blocking the cyclo-oxygenase enzymes (COX-1, COX-2). See Figure 1. The major effects of the NSAIDs are anti-inflammatory, analgesic and antipyretic - all related to their prostanoid-inhibiting effect and depending on the differing influence on the constitutive COX-1 and the inflammation-induced COX-2 enzymes (Rang *et al.*, 1995). NSAIDs could also give other effects since prostanoids have numerous functions in the body (e.g blood pressure control, renal function, thrombus formation and various reproductive functions).

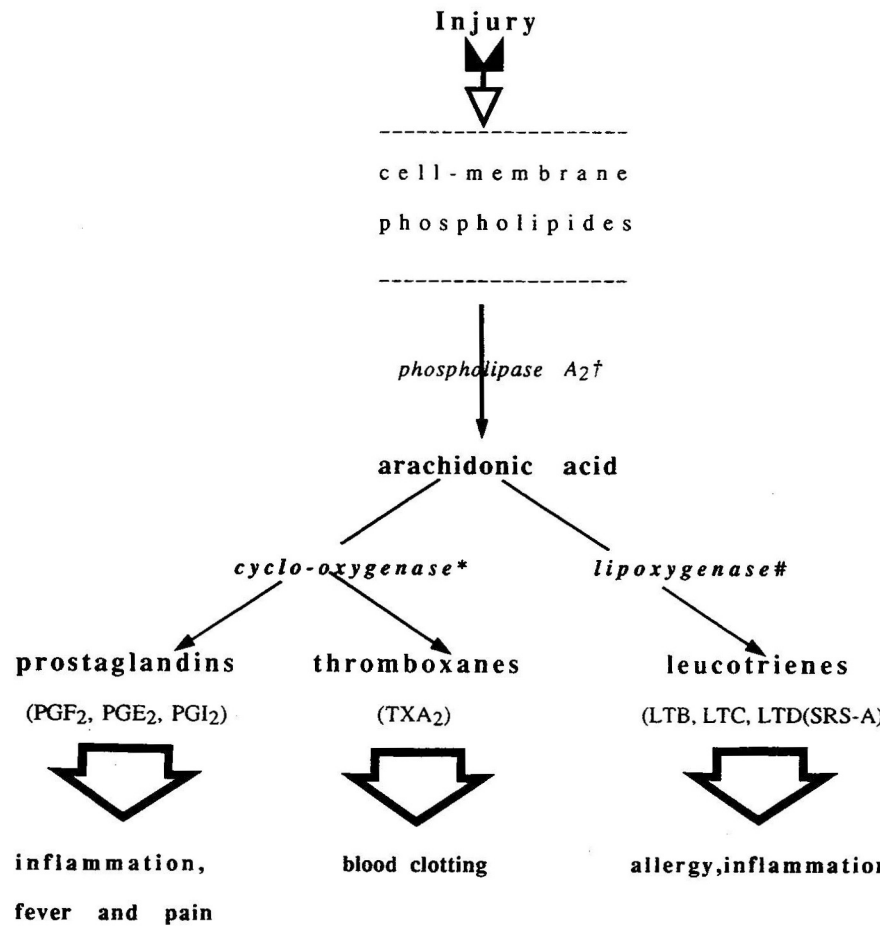

Figure 1. The formation of eicosanoids via the arachidonic acid cascade. Starting with an injury initiating the build-up of arachidonic acid from phospholipids in the cell membrane (by phospholipase A_2) the cyclo-oxygenase enzymes (COX-1, COX-2) transform the synthesis of the prostanoids, i.e. prostaglandins (PG) and thromboxanes (TX). NSAIDs (*) inhibit this synthesis by blocking the cyclo-oxygenase enzymes. The other pathway via the enzyme lipoxygenase generates the leucotrienes. (This is reported to be blocked by some novel NSAIDs, e.g. ketoprofen (#) - although not shown in horses). Corticosteroids (ƒ) inhibit the formation of all eicosanoids by indirectly blocking the enzyme phospholipase A_2 (Kallings, 1993).

Non-prostanoid dependent effects of the NSAIDs have also been described, such as inhibition of the activity of a variety of enzymes, transmembrane ion refluxes and oxidative phosphorylation (Abrahamson and Weissmann, 1989; Weissmann, 1991).

Effects on pain

Prostaglandins are believed to bind to receptors on the sensory nerve endings, thus promoting the discharge of impulses and consequently causing an increase in pain. They also sensitize afferent nerve endings to the effects of a number of physical and chemical stimuli. The overall effect of prostaglandins and other mediators is to provide an amplification of the peripheral pain mechanism by reducing the nociceptor threshold (Jenkins, 1987). Some prostaglandins act like inflammatory mediators and increase the sensivity to histamine and bradykinin, all sensitizing pain receptors. Thus, the NSAIDs have anti-inflammatory and analgesic effects, but according to Tobin *et al.* (1986) they do not exert a direct effect on normal pain perception. They rather act to reduce the hypersensitivity to pain caused by the inflammatory response. They should therefore have no effect on pain perception in non-inflamed tissue (Tobin *et al.*, 1986; Tobin, 1989). In a study of the actions of local anaesthetics and phenylbutazone on pain perception in normal tissues, phenylbutazone had no effect on pain perception for 24 hours, whereas mepivacaine had a blocking effect for up to 3 hours (Kamerling *et al.*, 1985).

However, it was reported recently that the analgesic effects of NSAIDs can be dissociated from their anti-inflammatory actions. Some NSAIDs were shown to exert a direct spinal action by blocking the excessive sensitivity to pain induced by the activation of spinal glutamate and substance P receptors. This argues for a probable spinal cyclo-oxygenase inhibition, mediated by a mechanism independent of the peripherally increased sensitivity due to inflammation (Malmberg and Yaksh, 1992). NSAIDs may also have central pain relief mechanisms unrelated to arachidonic acid metabolites. These include inhibition of a variety of enzyme systems, some of which catalyse the formation of neurotransmitters involved in the central pain pathways (Liles and Flecknell, 1992). NSAIDs also produce changes in transmembrane ion fluxes (Abramson and Weissmann, 1989). The enhanced membrane potentials resulting from treatment with salicylates could reduce the probability of generating action potentials in nerves and so reduce the effectiveness of synaptic output, indicating a direct inhibitory action of the salicylates on nerve impulse conduction (Rainsford, 1985). It is not clear if the influence of flunixin on abdominal pain in horses with colic might also be due to a centrally mediated action.

Since the pain alleviating effect is one reason to use/abuse NSAIDs with the intention to conceal lameness, it was of interest to study effects of NSAIDs on the locomotion pattern in clinically healthy horses as well as in lame horses.

Effects on the cardiovascular system

Prostacyclin (PGI_2) inhibits platelet aggregation and, like PGE_2, is a potent vasodilator (Oates *et al.*, 1988; Adams, 1995). These actions have been observed in both arteriols and post-capillary venules (Birks *et al.*, 1991). Conversely, PGF compounds have been shown to cause constriction of venules and veins (Birks *et al.*, 1991; Ronni-Sivula *et al.*, 1993). TXA_2 contributes to platelet aggregation and blood vessel constriction (Oates *et al.*. 1988; Adams, 1995).

It has been discussed whether prostanoids serve in the regulation of regional blood flow during exercise - increased blood flow to active skeletal muscles and reduced blood flow to organs not requiring it, would be beneficial - but there are varying results from different studies on prostanoids in different systems (Birks *et al.*, 1991). However, there is increasing evidence that prostanoids are involved in the haemodynamic and metabolic responses to exercise (Nowak and Wennmalm, 1978; Demers *et al.*, 1981; Mehta *et al.*, 1983, Ronni-Sivula *et al.*, 1993). In humans, release of vasodilatory PGs contributes to exercise induced arteriolar vasodilatation and hypaeremia in skeletal muscle (Wilson and Kapoor, 1993). Marathon runners have increased plasma PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) (Demers *et al.*, 1981; Ronni-Sivula *et al.*, 1993, while treadmill and cycle exercise increase plasma 6-keto-$PGF_{1\alpha}$ and PGE_2 concentrations (Mehta *et al.*, 1983).

In horses performing treadmill exercise, increases in plasma concentrations of TXB_2 (the stable metabolite of TXA_2) and 6-keto-$PGF_{1\alpha}$ have been reported (Birks *et al.*, 1991; Hinchcliff *et al.*, 1994; Mitten *et al.*, 1995).

There are a number of studies indicating that inhibition of prostanoid production by NSAIDs affects the cardiovasculary and metabolic responses to exercise in humans, horses and other species (Kallings and Persson, 1983; Cowley *et al.*, 1984; Stassen *et al.*, 1984; Cowley *et al.*, 1985; Wilson and Kapoor, 1993; Mitten *et al.*, 1996). Prostaglandin inhibition in humans is accompanied by increases in systolic and diastolic arterial pressure both at rest and during exercise (Stassen *et al.*, 1984). The NSAIDs acetylsalicylic acid and indomethacin attenuate the exercise-induced increase in blood flow to the calf and also attenuate the increasde blood flow to a non-exercising limb in humans performing cycle exercise (Cowley *et al.*, 1984; Cowley *et al.*, 1985). Indomethacin reduces forearm blood flow both at rest and during exercise (Wilson and Kapoor, 1993). The effects of prostanoid inhibition on the cardiovascular responses to submaximal exercise have been documented in humans (Cowley *et al.*, 1984; Stassen *et al.*, 1984; Cowley *et al.*, 1985; Wilson and Kapoor, 1993) and in horses performing treadmill exercise (Kallings and Persson, 1983; Olsen *et al.*, 1992; Hinchcliff *et al.*, 1994). However, there has been limited information of the effects of NSAIDs on the systemic hemodynamic responses to intense exercise in horses. It was therefore of interest to further evaluate the effects of NSAIDs on cardiovascular responses to exercise in the horse.

Aims

The aims of the present study were to determine if:

- *bronchodilating drugs* have cardiocirculatory and metabolic effects on clinically healthy horses performing submaximal standardized exercise tests on a treadmill

- *non-steroidal anti-inflammatory drugs* (NSAIDs) have cardiocirculatory and metabolic effects in clinically healthy horses performing submaximal standardized exercise tests on a treadmill

- *NSAIDs* exert cardiocirculatory and metabolic effects on clinically healthy horses performing incremental exercise to fatigue on a treadmill

- *NSAIDs* exert effects on movement and performance in clinically healthy horses trotting on a track

- *NSAIDs* at low plasma levels influence locomotory pattern in lame horses on the treadmill and to assess if this method is appropriate to reveal subclinical changes in locomotion

- standardised treadmill and track testing with high-speed filming are adequate methods for testing of drug-effects on performance in horses.

Materials and Methods

For a more detailed account of the Materials and Methods used, see the separate papers (Studies I-VII).

Horses

Standardbred trotters; 1-2 mares and 5-6 geldings, 3-16 years of age and weighing between 400 and 600 kg, were used in Studies I-IV and VI-VII. At pre-examination they were found to be clinically healthy and they were well accustomed to the treadmill before the tests. In horses used for determination of blood gases in arterial blood, a part of their right carotid artery was surgically relocated to a subcutaneous position (using a modification of the technique described by Tavernor, 1969) to facilitate arterial blood sampling during high speed exercise. These experimental horses were trained and used in different research projects, allowing sufficient wash-out periods in between. Almost every week they performed submaximal/maximal exercise work outs either on treadmill or track. They were housed in conventional stables and fed a normal diet consisting of grain and hay and had water *ad libitum*. In Study V five slightly lame horses were used. They were provided by either clients or the Stockholm Police Cavalry, plus one experimental horse from our Department.

The experiments were performed after approval of the local Ethical Committee on Animal Experiments.

Exercise tolerance tests

Submaximal exercise on treadmill (Studies I-IV)

In Studies I, II and III, all horses performed a standardized exercise tolerance test on a high-speed treadmill (Persson, 1983) on two consecutive days - one without and one with a respiration mask and in Study IV two of the horses performed this test. After one week this was repeated with drug administration, i.e. to compare pre- vs. post-drug values (each horse served as its own control). These submaximal exercise tests were performed approximately 2 h (II, II) or 4 h (I, IV) after the last drug administration.

In the standardized exercise tolerance test the horses exercised on the treadmill set at a slope of 6.25 % (approx. 3.5 °). They trotted at velocities of 6, 7, 8 and 9 m/s for 2 min at each speed. In the tests with a respiratory mask they ran at one speed step lower i.e. 5, 6, 7 and 8 m/s. Recordings and sampling procedures were carried out before exercise, during the last 15 s at each speed, immediately after cessation and then at 2 and 5 min after exercise. All heart rate, blood lactate and blood-gas parameters were determined from the test without the mask.

Incremental exercise to fatigue on treadmill (Study VII)

The horses conducted the standardized exercise tests on a high-speed treadmill. The exercise test consisted of an incremental test to fatigue on an inclined (6.25 %) treadmill. The horses were fitted with a face mask and trotted, after a warm-up at 1 minute stepwise speed increments of 6, 7, 8, 9, 10 and 11 m/s or until they could no longer keep pace with the treadmill at a trot. Time to fatigue was defined as the tpoint at which the horses could no longer maintain their position at the last speed on the treadmill , despite intense but humane encouragement.

Measurements of the exercise parameters were made before, during each speed step and after exercise. If a horse was unable to complete the 60 s at the last speed step, samples were taken and measurements made just before the treadmill was stopped.

Track testing (Study VI)

The horses trotted on two occasions with a one- or two- week interval on a 1000 m test track. After a warm-up the horses performed a standardized stepwise incremental test of three laps at 10.5 m/s, 11 m/s and 12.5 m/s (with a finish at maximum speed). HR was recorded with a telemetric system during each lap. The horses were recorded on film on the last straight of each lap. Blood samples were drawn 5 min pre- and post-exercise to determine the plasma lactate accumulation and flunixin concentration in plasma.

The same horses were used in Study VI and VII. They participated during four weeks in two trials in the same flunixin study, the treadmill test and the track test each with and without medication. The whole study had a blind three-way cross-over design (Williams balanced design). After a baseline test (without drug), in randomized order, some horses started with the track test after administration of flunixin or saline (control), and some horses with the treadmill test after treatment with flunixin (at least one-week intervals).

Measurements and recordings

Heart rates (HR) were in the treadmill tests monitored electrocardiographically and recorded before, during the last 15 s of each exercise step and 2 and 5 min after exercise. The HR response to exercise was expressed as the velocity (V) causing a HR of 200 bpm, V_{200} (m/s) calculated from the linear HR/V relationship (Persson, 1983; Persson, 1997) (Studies I, IV and VI).

In the track test (VI) HR was recorded at each lap with a telemetric system.

Oxygen uptake (VO_2, l/min) was in studies I, II, III and IV determined with a respiratory mask (Persson 1983). The respiratory minute volume (VE, l) was measured with a flow meter and the expired oxygen and carbon dioxide

concentrations were determined by mass spectrometry. Tidal volume (TV, l) was calculated from VE and respiratory rate. The respiratory exchange ratio (R) was calculated from the CO_2/O_2 ratio.

In study VII, a face mask was fitted to the horses muzzle and by collecting gases from the expired air in an open flow system,VO_2 and carbon dioxide production (VCO_2, l/min) were determined. The gases were then analysed for oxygen and carbon dioxide content using a gas analyser. Registrations were made at 15 s intervals during the test. The VO_2 -max was defined as the levelling off of VO_2 at increasing speed, a high lactate concentration and R exceeding 1.

To determine *lactate concentration*, venous blood samples were collected from an indwelling catheter in a jugular vein before, duringthe last 15 s at each speed and 5 min after exercise.

Blood lactate (BLA, mmol/l) was in the earlier studies (I-IV) determined in whole blood and analysed enzymatically. The lactate threshold, V_{LA4}, was estimated from the exponential function of blood lactate on treadmill velocity (Persson, 1983).

In Studies VI and VII *plasma lactate* concentration (PLA, mmol/l) was determined with a lactate analyser.

Calculations for blood lactate (BLA) were made by using the regression between the plasma and blood lactate concentrations;

$$BLA = PLA \times 0.55 + 0.74.$$

This was done to enable calculation of the V_{LA4} and for comparison with previous results when lactate was determined in whole blood (Persson, 1997).

Stride frequency (SF) was calculated by timing 50 paces at each speed and stride length (SL, m) was calculated from SF and speed.

Locomotion analysis

Treadmill (Study V)

In Study V film recordings on treadmill and blood sampling were carried out before treatment and at 6, 24 and 48 h after the last administration of phenylbutazone.

The kinematics of the horses at the trot on a treadmill (4 m/s) were recorded under standardized conditions by means of high-speed cinematography (250 frames/s). The kinematic analysis was based on coordinate determination of reference markers fixed to the skin over well defined anatomical structures at the end of the bone segments. The tracking of the reference points and data processing were carried out in a TrackEye® film/video analysis system and comprised basic temporal and joint and segment variables (Drevemo *et al.*, 1993).

Track (Study VI)

The horses were filmed on the last straight of each lap on the track. The kinematics for five consecutive strides were filmed left laterally using a panning high-speed camera at 250 frames/s along a straight side of the track (Drevemo and Johnston, 1994). The developed films were converted to video images and digitized in the TrackEye® system (Drevemo *et al.*, 1993). The events of first contact (FC) of the hoof with the track surface and the toe-off (TO) when the hoof leaves the ground, were determined from the video images. Swing time is the time between TO and FC and strance time is the time between FC and TO.

Drug analysis and pharmacokinetics

Bioanalysis of drugs

The concentrations of phenylbutazone (I, VI), theophylline (II), meclofenamic acid (IV) and flunixin (VI,VII) in plasma were determined by high performance liquid chromatogarphy (HPLC).

Analysis of *clenbuterol* concentrations were carried out using a gas-chromatographic mass-spectrometric (GC-MS) method (III).

Pharmacokinetic analysis

Non-linear regression analyses with a computer program/IBM system were used in Studies II and III. In Study IV the non-compartmental approach based on statistical moments was used to calculate the basic parameters in individual horses (Gibaldi, 1984). Log-linear regression analyses or other standard pharmacokinetic methods were used to estimate terminal half-lives.

Statistical analysis

The results are expressed as means and standard deviation (± SD). Statistical calculations in Studies II and III were carried out by standard methods using the SAS program on a VAX 8530 computer with Digital's operating system VMS. The GLM procedure in SAS was used for unbalanced variance analyses to compare the values before and after drug administration. Wilcoxon's signed rank test was used (StatView SE program for Macintosh) to compare differences at each observation, with vs. without drug treatment, in Studies VI and VII and Student's t-test in Studies I and IV. The level of significance was set at $p < 0.05$.

Results

Submaximal exercise on treadmill

Study I: Phenylbutazone significantly decreased the V_{200} value by 2 % compared with the baseline (without drug) situation. The blood lactate and respiratory responses to exercise did not change after treatment with phenylbutazone. When the effect on prostanoid synthesis was studied, significantly lower levels of thromboxane B_2 for 12 h were seen in serum after administration of phenylbutazone. See Figure 2. The mean plasma concentration curve and the mean half-life of elimination are shown in Figure 3 and Table 1, respectively.

Table 1. Dose, plasma concentration at the time for the tests (mean value or range) and the terminal half lives of elimination ($T_{1/2\beta}$) for the studied drugs

Drug	Dose	Plasma concentration (mean/range)	$T_{1/2\beta}$	Study
Phenylbutazone	2.5 mg/kg BID (P.O.)	14.4 µg/ml	6.5 h	I
Theophylline	5 mg/kg BID (P.O.)	13.3 µg/ml	17.0 h	II
Clenbuterol	0.8 µg/kg BID (P.O.)	0.45-0.75 ng/ml	10.4 h	III
Meclofenamic acid	2.2 mg/kg BID (P.O.)	1.11-2.18 µg/ml	3,0 h	IV
Flunixin	1.1 mg/kg BID (P.O.)	1.6-2.3 µg/ml	1.6 h #	VI
		1.2-2.4 µg/ml		VII

BID = Bis In Die (twice daily); # Chay *et al.*, 1982

Study II: Theophylline caused significantly increased heart rate and blood lactate levels during and after exercise (Fig. 4 a, b). Arterial oxygen tension after exercise and arterial carbon dioxide values before and after exercise were significantly lower than before medication.

The mean half-life of elimination is shown in Table 1 and the plasma concentration curve in Figure 5.

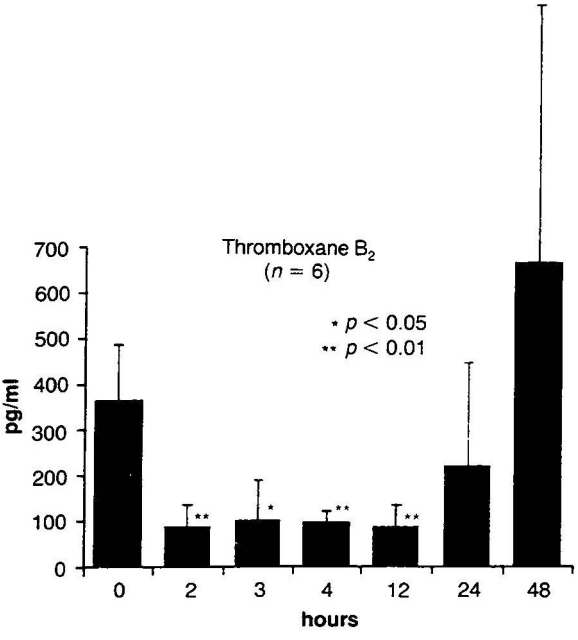

Figure 2. Concentration of thromboxane B_2 (the stable metabolite of TXA_2) in serum before (0) and after phenylbutazone administration. Mean values, SD and significant difference (p) after inhibition compared to 0-values.

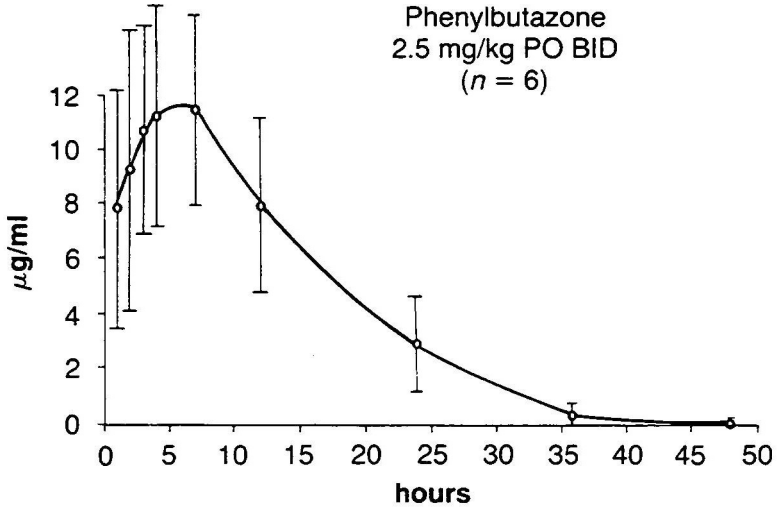

Figure 3. Phenylbutazone concentration in plasma after repeated oral dministration. Mean values (n=6) ± SD.

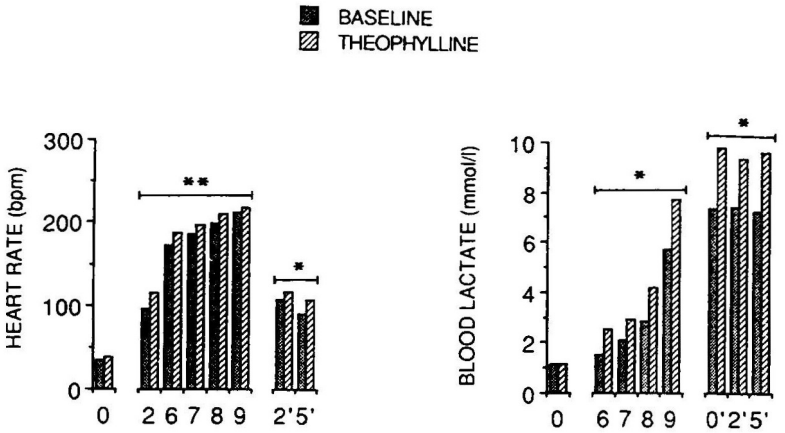

Figure 4. Mean baseline values (■) and mean values after theophylline administration (▨) (n=6) of heart rate and blood lactate concentrations at different treadmill velocities and post exercise. 0 = rest, before exercise; 2, 6, 7, 8 and 9 = treadmill velocities (m/s); 0' = immediately at cessation of exercise; 2' and 5' = post exercise (min).
Level of significance: * =$p < 0.05$, ** = $p < 0.01$.

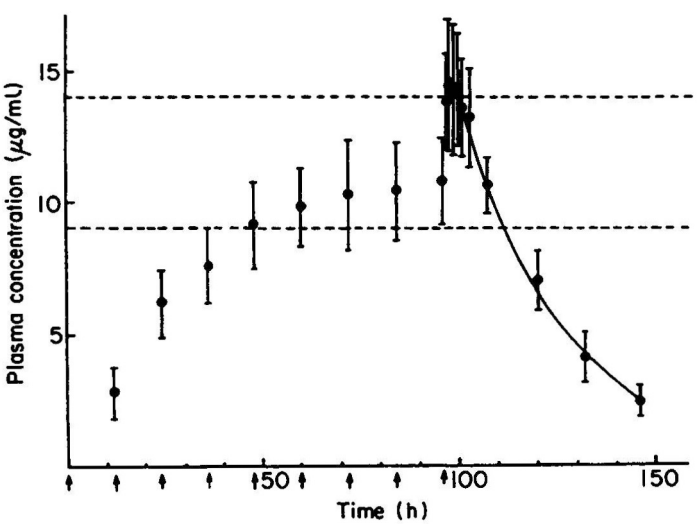

Figure 5. Plasma concentration of theophylline after repeated oral administration (5 mg/kg). Each arrow indicates a drug administration and each point represents the mean ± SD (n=6). Before the last administration up to 96 h the points indicate the mean minimum plasma concentration during a dosing interval. The solid line represents the curve after the last administration (96 h). The dotted lines refer to the predicted maximum and minimum plasma concentration levels at staedy state.

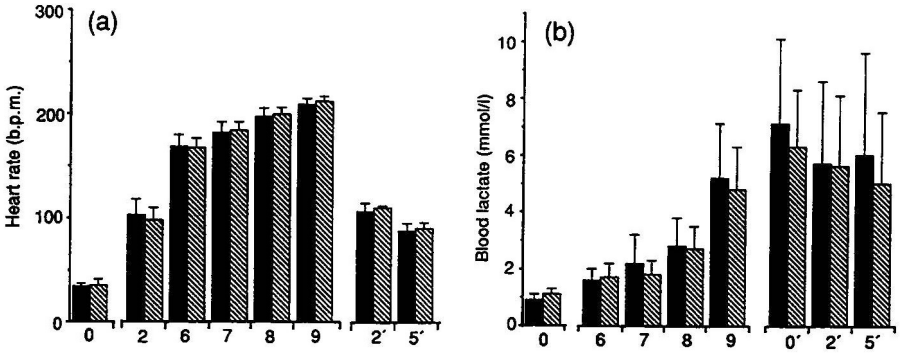

Figure 6. Mean baseline values (■) and mean values after clenbuterol administration (▨) (n=6) of (a) heart rate and (b) blood lactate concentration at different treadmill velocities and post exercise. 0 = rest, before exercise; 6, 7, 8 and 9 = treadmill velocities (m/s); 0' = immediately at cessation of exercise; 2' and 5' = post exercise (min). Bars represent standard deviation from the mean.

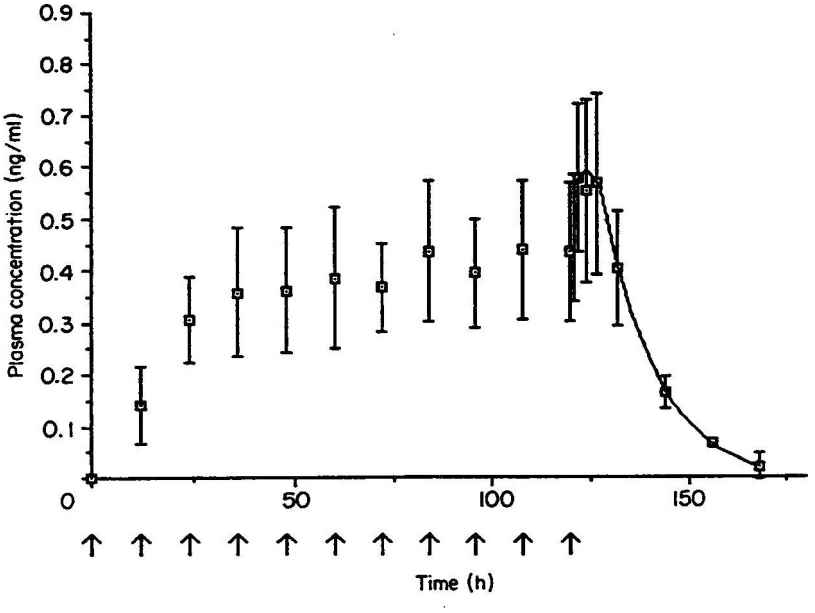

Figure 7. Plasma concentration of clenbuterol after repeated oral administration (0.8μg/kg). Each arrow indicates a drug administration and each point represents the mean ± SD (n=6). The point at 156 h is the mean of two observations only. Before the last administration up to 120 h the points indicate the mean minimum plasma concentration during a dosing interval.

Study III: Clenbuterol administration did not affect heart rate, blood lactate, (Fig. 6 a, b) arterial oxygen tension, oxygen uptake or respiratory minute volume. The only significant effect of clenbuterol was an increase in arterial pH during work. The mean half-life of elimination in plasma was 10.4 h (Table 1). The plasma concentrations are shown in Figure 7.

Study IV: Meclofenamic acid did not affec heart rate response during exercise (V_{200}) compared with the baseline test. The blood lactate accumulation was significantly decreased and the lactate threshold, V_{LA4}, was increased after administration of meclofenamic acid (Table 2). No effects on the respiratory response to exercise were seen in the 2 horses performing the mask test. The plasma concentration curve is shown in Figure 8 and the mean half-life of elimination in Table 1.

Table 2. Effects of the studied NSAIDs on the heart rate (HR) response, expressed as the treadmill velocity performed at HR 200, V_{200} (m/s), and on the lactate threshold, i.e. the velocity at the blood lactate concentration of 4 mmol/l, V_{LA4}. Mean values (±SD)

Test:	**Submaximal**				**Incremental to fatigue**	
Study:	**I**		**IV**		**VII**	
	Baseline	**Phenyl-butazone**	**Baseline**	**Meclo-fen. acid**	**Baseline**	**Flunixin**
V_{200} (m/s)	8.10 (±0.42)	7.92* (±0.49)	7.72 (±0.48)	7.82 (±0.73)	8.52 (±0.52)	7.95* (±0.57)
V_{LA4} (m/s)	8.12 (±0.93)	8.00 (±0.68)	7.86 (±0.80)	8.51* (±0.96)	8.67 (±0.57)	8.26* (±0.42)

* $p<0.05$ (difference between baseline and drug test)

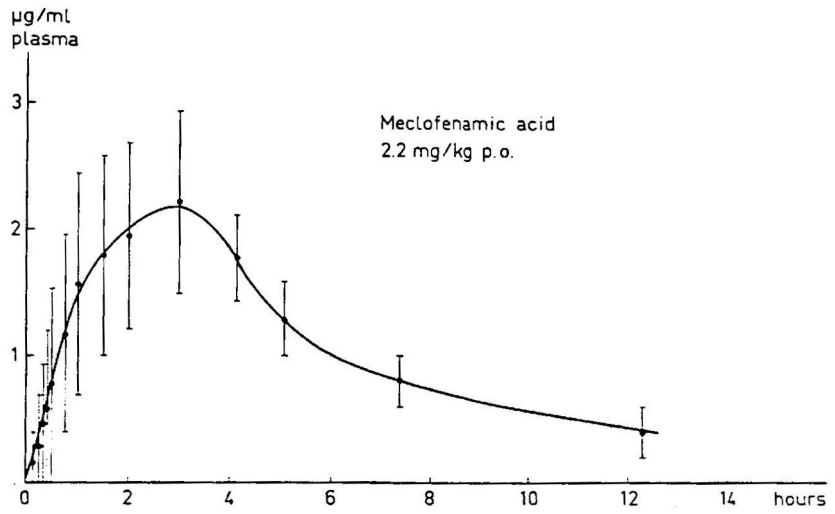

Figure 8. Plasma concentrations of meclofenamic acid. Mean values (n=5) ±SD.

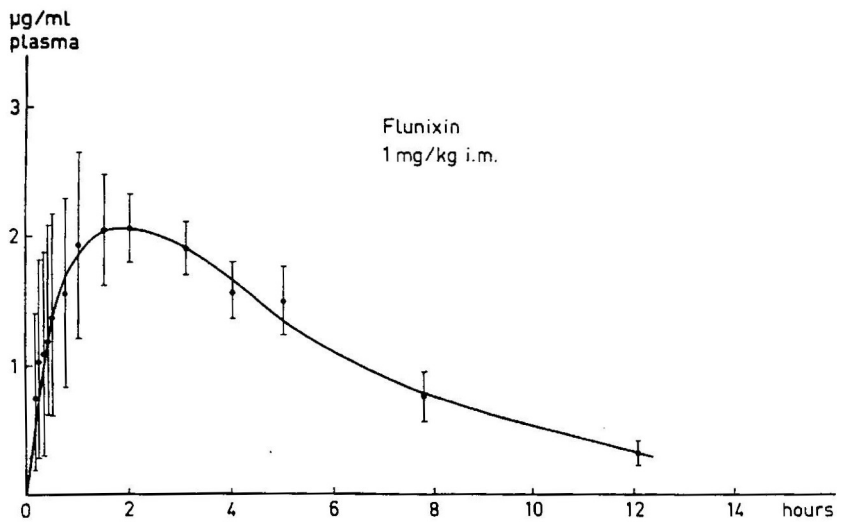

Figure 9. Plasma concentrations of flunixin. Mean values (n=5) ±SD.

Incremental exercise to fatigue on treadmill

Study VII: Flunixin increased HR significantly during submaximal exercise and consequently, V_{200} was decreased. Maximal HR was unchanged. See Figure 10 a. Plasma lactate concentrations were higher at all velocities (Fig. 10 b) and the VLA_4 was then decreased, compared with baseline values (see Table 2). SL was increased during submaximal work but not at higher velocities (Fig. 10 c) and oxygen uptake was virtually unaffected (Fig. 10 d) after flunixin treatment. A plasma concentration curve from a preliminary studie is presented in Figure 9 (Kallings and Persson 1983).

Incremental exercise to fatigue on track

Study VI: Mean HR at 10.5 m/s and plasma lactatate accumulation 5 min post-race did not differ between flunixin and saline treatment. See Table 3.

Table 3. Heart rate (HR) and stride frequency (SF) at maximal exercise and plasma lactate concentration 5 min after exercise (PLA-5') for 5 horses performing both treadmill and track exercise to fatigue, with and without administration of flunixin. Mean values (± SD)

Test:	**Track (flat, dirt)**		**Treadmill (inclined 6.25%)**	
Study:	**VI**		**VII**	
	Saline	**Flunixin**	**Baseline**	**Flunixin**
HR (bpm)	217 (±9)	220 (±13)	221 (±11)	220 (±11)
SF (st/min)	125 (±4)	124 (±6)	127 (±6)	126 (±8)
PLA-5' (mmol/l)	19.2 (±5.6)	20.3 (±1.0)	20.3 (±5.7)	25.6 (±6.0)

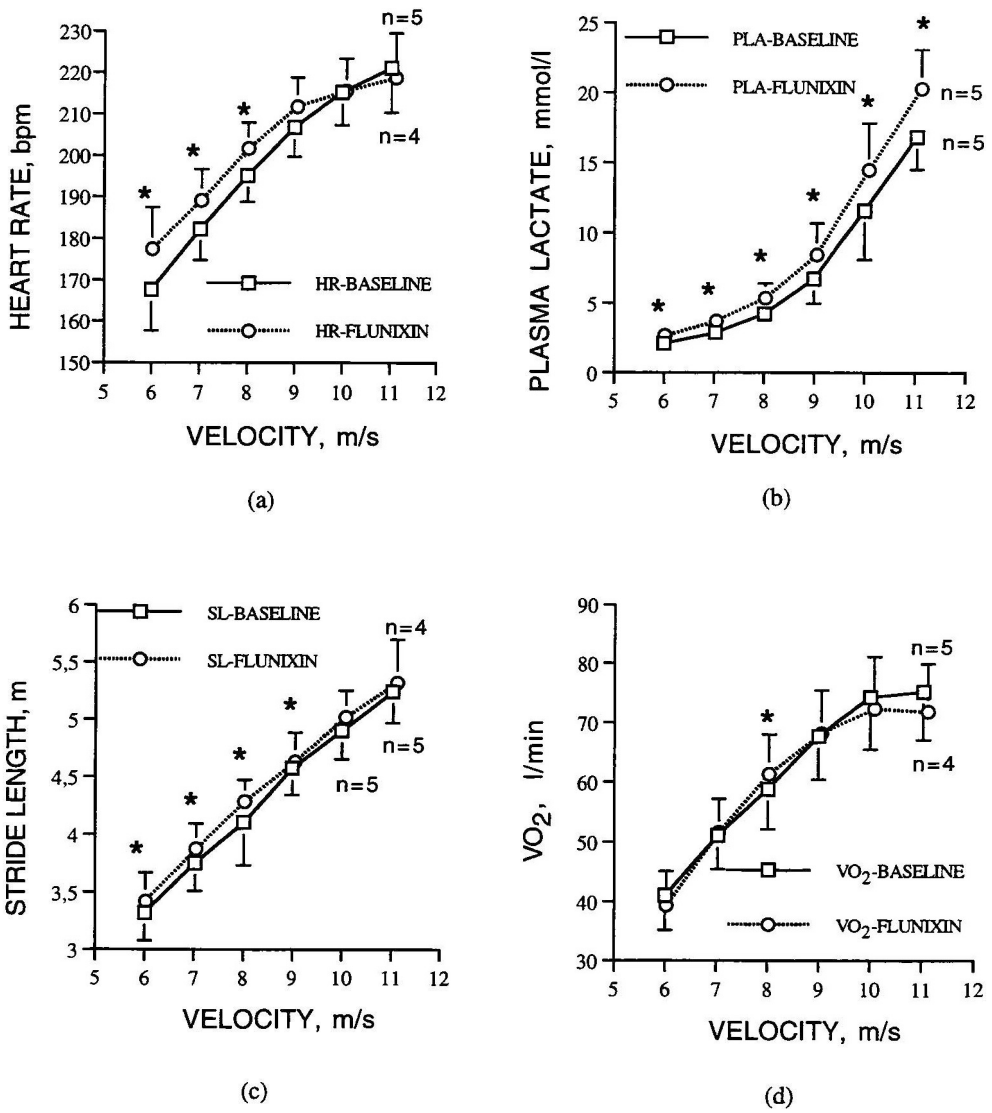

Figure 10. Mean baseline values (□) and mean values after flunixin administration (○) of (a) heart rate, (b) plasma lactate, (c) stride length and (d) oxygen uptake at different treadmill velocities. Bars represents standard deviation from the mean (n=6 if not otherwise is noted) * $p<0.05$

Locomotion pattern

Track (normal horses)

Study VI: Flunixin did not affect the mean velocities for the finishing maximum speed (12.1 m/s). In the forelimb, swing time values increased while those for stance time decreased significantly after medication (Fig. 11). In the hind limb, very few changes were observed.

Treadmill (lame horses)

Study V: Phenylbutazone did not affect stride, swing or stance time in the 5 lame horses. Significant differences in locomotion pattern were observed in all horses, mainly in the fetlock joint angle, which on the lame limb decreased after treatment, whereas the corresponding minimal angle in the contralateral limb was greater after medication. Locomotion was influenced by medication in all horses and the effect lasted for at least 48 h and the plasma levels of phenylbutazone and the active metabolite oxyphenbutazone were below 2 µg/ml. See Figure 12 and 13.

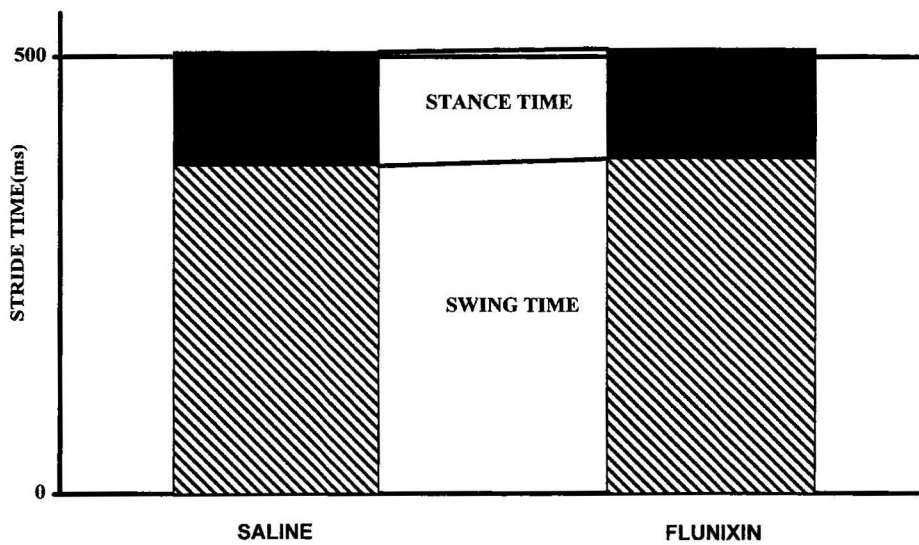

Figure 11. Relationship of stride time, swing time and stance time in the left forelimb for the group of horses before and after treatment with flunixin (at 10.5 m/s).

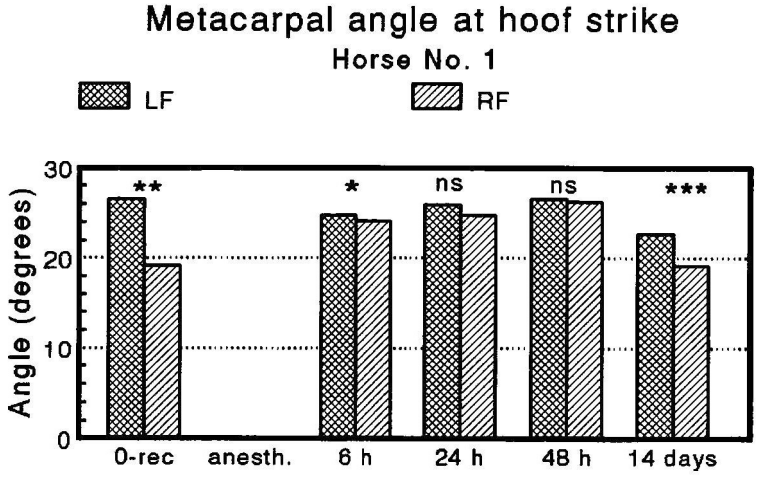

Figure 12. Mean metacarpal angle at hoof strike during the stance in 1 horse prior to medication and 6, 24, 48 h and 14 days after administration of phenylbutazone (2.5 mg/kg p.o. BID). Mean values for 4 strides and level of significant difference between the lame left foreleg (LF) and right foreleg (RF); * = $p< 0.05$, ** = $p< 0.01$, *** = $p< 0.001$.

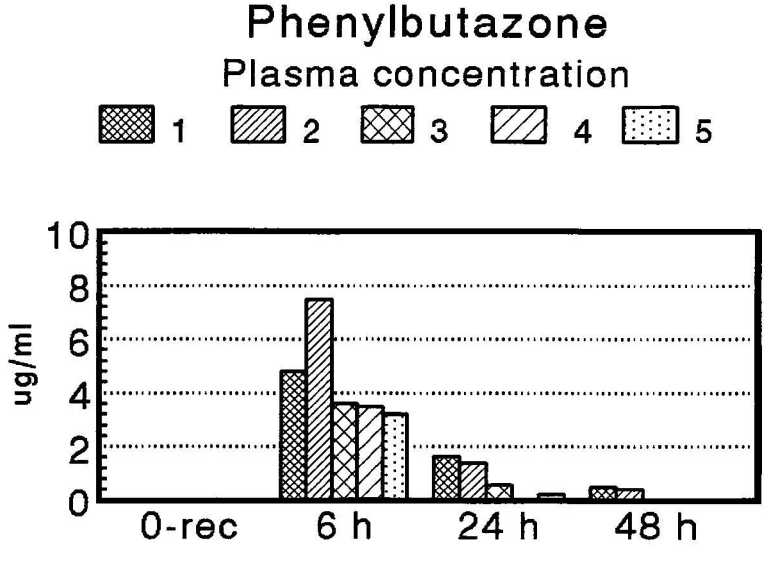

Figure 13. Concentration of phenylbutazone in plasma before and 6, 24 and 48 h after administration (2.5 mg/kg p.o. BID) in 5 horses.

General Discussion

Bronchodilating drugs

Theophylline

The predominant effect of theophylline on response to exercise (Study II) was the increased heart rate, probably a reflection of the direct chronotropic effect on the myocardium or indirect by an increased release of catecholamines from the adrenal medulla (Robertsson *et al.*, 1981; Stubbs *et al.*, 1984). This ougth to result in a hyperkinetic circulation, i.e. a high cardiac output in relation to the oxygen uptake and a concurrent reduction of the arteriovenous oxygen difference. Beside stimulating the heart, xanthines cause vasodilation in most blood vessels (Rang *et al.*, 1995).

The blood lactate accumulation in Study II was significantly increased by theophylline. It was suggested that this might indicate an increased dependence on the glycolytic pathway, caused by a stimulation of glycolytic enzymes or an insufficient oxygen supply due to limiting drug effects on the cardiovascular system. The increased R also indicated an increased metabolism of carbohydrates. The increased blood lactate concentration could at least in part be due to an increase in the levels of catecholamines in plasma (Snow, 1979). The slight decrease in pH probably reflects the higher lactate production.

The methyl-xanthines have been used in doping of racehorses (Tobin, 1981). Caffeine has been reported to enhance performance in Thoroughbreds running on a track (Fuijii *et al.*, 1972) and to increase speed in gallop and lunge tests (Sanford, 1983), although the validity of these results is difficult to evaluate. In man caffeine has been reported to improve exercise performance (Costill *et al.*, 1978; Ivy *et al.*, 1979). Increased VO_2-max and elevated HR values during maximal exercise, but not in submaximal exercise have been reported (Toner *et al.*, 1982). However, Gaesser and Rich (1985) observed no effects of caffeine on VO_2 and HR during maximal exercise in humans, but they did observe increased blood lactate production and a decrease in HR during submaximal work. No effect of caffeine on the rate of blood lactate accumulation during maximal exercise was found in a study by Powers and co-workers (1983).

It was considered in Study II that theophylline *per se* probably did not affect respiratory ventilation or oxygen uptake during exercise, and that the increased HR and lactate accumulation suggested that theophylline impaired rather than enhanced performance in healthy horses.

Clenbuterol

In contrast to theophylline, clenbuterol did not cause any major effects on cardio-respiratory and blood lactate parameters studied in healthy Standardbred horses performing submaximal exercise (Study III). Only arterial pH was significantly changed following clenbuterol medication. Nor did Rose and Evans (1987) observe any major effects on cardiorespiratory function in healthy Thoroughbreds after injecting intravenously the same dose as we administered orally (0.8 µg/kg b.wt). The only significant effect they found was a slight decrease in tidal volume at the highest work rate. In our study tidal volume tended to decrease, although not significantly ($p=0.05$). The clinical relevance of these minor effects remains to be clearified.

When Slocombe *et al.* (1992) studied respiratory mechanics during exercise, they found that clenbuterol treatment did not affect any of these parameters in response to exercise.

Non-steroidal anti-inflammatory drugs

Cardiorespiratory responses

It was found in Study VII that flunixin treatment influenced physiological responses during treadmill exercise to fatigue. The higher heart rates and the decrease seen in V_{200} were concistent with previous preliminary findings during submaximal treadmill exercise (Kallings and Persson, 1983). In Study I phenylbutazone was studied in a similiar way during submaximal exercise in treadmill trials. The V_{200} was significantly decreased (by 2 %) compared with that in a test without drug. The oxygen uptake, ventilation and other exercise tolerance related variables did not change significantly.

During sustained submaximal exertion (1 h on an inclined treadmill), however, no effects of phenylbutazone on HR, cardiac output, right atrial pressure or other haemodynamic variables were found (Hinchcliff *et al.* 1994). Also Short and colleagues have studied responses to submaximal racetrack exercise in Standardbreds with and without administration of phenylbutazone and other drugs. The hypothesis was that heart rate response to exercise would be the predominant physiological parameter that changes with medication. Phenylbutazone (2 g daily for 3 days, orally) did not affect heart rate, but there were elevated blood pressure values compared with control (non-medicated) values (Short *et al.*, 1990).

However, increased heart rate and right atrial pressure have been reported recently in Standardbred horses during exertion in treadmill tests after administration of phenylbutazone (8.8 mg/kg bwt p.o. for 2 days, then 4.4 mg/kg i.v. 1 h pre-exercise). It was also shown that phenylbutazone abolished the exertion-induced increases in plasma 6-keto-$PGF_{1\alpha}$ and TXB_2 and it was

concluded that prostanoids probably mediate or modulate some of the systemic haemodynamic responses to exertion in horses (Mitten *et al.,* 1996).

The accentuation of the exercise-induced increase in heart rate by flunixin in Study VII and phenylbutazone in previous study (I) as well as in the study by Mitten *et al.* (1996), may have been due to inhibition of prostanoid production and an increased sensitivity to sympathetic stimulation of the SA node in the heart, as suggested by Mitten *et al.* (1996).

In contrast, phenylbutazone (4.4 mg/kg b.wt, i.v., for 2 days with the final dose 4 or 24 h before tests) showed no effect on the heart rate or on right atrial and pulmonary vascular pressures in Thoroughbreds undergoing strenuous treadmill exercise in a study by Manohar *et al.* (1996). They suggested that these conflicting findings with the previous study by Mitten et al. (1996) might be due to differences in breeds and/or fitness level of the horses in the two studies. However, this was studied during strenuous exercise, and submaximal exercise responses were not reported in that study. The fact that phenylbutazone showed no effect on HR in the study by Manohar *et al.* (1996) at maximal exertion is in agreement with the findings in both studies VI and VII. In Study VI, on the track, flunixin had no significant effect on heart rate response during a simulated race and in study VII no effect was seen at the higher intensities on the treadmill.

The apparently conflicting results of HR responses of NSAIDs reported in the literature are probably related to different doses, dosage intervals and route of administration as well as to breed differences, fitness level and exercise performedby the studied horses.

Lactate response

In Study VII the lactate concentrations during exercise were increased following flunixin medication, and consequently, V_{LA4} was decreased. At the end of exercise all horses hadhigh lactate levels in plasma and their respiratory exchange ratio was > 1, implying maximal exertion.

Five out of 6 horses showed higher plasma lactate accumulation 5 min post-exercise after flunixin treatment; why the sixth horse had lower lactate values after work may be because this horse performed less intense exercise as it became fatigued after only 10 s in the last speed step. In the track study (VI) flunixin had no effects on plasma lactate accumulation measured 5 min post-race. The lactate concentrations reached similar high levels at fatigue, both in the treadmill test and on the track (see Table 3).

Previous preliminary studies have shown that the V_{LA4} was not significantly affected by flunixin treatment and the lactate accumulation after exercise was lower than or similiar to the corresponding baseline value (Kallings and Persson, 1983). Similarly, the blood lactate accumulation did not change significantly during submaximal exercise (with 2-min steps) after administration of phenylbutazone as shown in Study I and by Mitten *et al.* (1996).

Meclofenamic acid, on the other hand showed in the preliminary studie significantly higher V_{LA4} and lower lactate accumulation (Kallings and Persson, 1983), which was confirmed in Study IV.

These contradictory results could be explained by the fact that in the previous exercise test model the horses were running for a longer duration at each speed step (2 min at each step) (Kallings and Persson, 1983) than in the present study (VII), allowing the blood lactate to be distributed to other tissues and organs.

In Study VII with more intense exercise, the speed increase was faster, with 1 min at each speed step up to 11 m/s. This indicates an earlier recruitment of fast-twitch glycolytic muscle fibres (type IIA and B). It has been shown that fibre recruitment pattern occur from type I (slow twitch) to type IIA and type IIB (fast twitch) fibres with incremental speed (Lindholm *et al.* 1974; Valberg 1986). There will thus be a quicker increase in the accumulation of lactate, which was relatively higher after flunixin treatment. Lactate concentration in blood is dependent not only on the release of lactate from muscle, but also on its rate of removal from blood. The increased level of plasma lactate after flunixin administration in the current study could thus be due to factors like a reduced uptake in surrounding tissues (e.g. muscle fibres) or organs (heart, liver, kidneys, etc). It may also have been a result of an increased production (glycolysis), an increased efflux from muscle to blood, or reduced transport into the erythrocytes.

In StudiesVI and VII, lactate was measured in plasma, and in the earlier studies (I-IV) in whole blood, which could suggest that the uptake of lactate by red blood cells is reduced by flunixin treatment. It is reported that NSAIDs may exert non-prostanoid effects such as inhibition of transmembrane anion transport in erythrocytes (Abramson and Weissmann, 1989).

Inhibition of prostanoids by the cyclo oxygenase pathway as the sole mechanism of action of NSAIDs has recently been discussed. Non-prostanoid effects have also been described (Boothe, 1995). At high concentrations, NSAIDs seem to uncouple protein-protein interactions within the plasma membrane and thus interfere with a variety of cell membrane processes, like oxidative phosphorylation in mitochondria and cellular adhesion (Abramson and Weissmann, 1989, Weissman, 1991, Rang *et al.*, 1995).

The increased plasma lactate levels seen in Study VII could thus have been a result of interference by flunixin with the blood flow, and/or the oxidative phosphorylation, in skeletal muscle. It could also reflect a change in muscle fibre recruitment, with an increased contribution from the glycolytic type II fibres.

Locomotion responses and drug concentrations in plasma

Phenylbutazone

Studies to evaluate effects of NSAIDs in horses are sparse. They often rely on subjective evaluation with absence of control and placebo treatment. When

e.g. a comparison was made between naproxen and phenylbutazone in induced myositis, both subjective and quantifiable parameters were measured. Stride length was determined by walking the horse over freshly raked sand. The distance between the imprints of the toe were measured. The results of this and the other studied variables led to the conclusion that naproxen was superior to phenylbutazone for more rapid relief of inflammatory swelling and associated lameness (Jones and Hamm, 1978).

Another way to determine more quantifiable data on onset, duration of activity and effectiveness of NSAIDs on limb lameness is the use of a "force-plate", which allows small variations in the load on a horse's leg to be measured. It was shown that 2 g of phenylbutazone orally had an onset of action after several hours and lasted of about 24 hours (Pratt, 1977).

Similar studies conducted at our laboratory are described in Study V. Horses with mild but significant lameness were studied with a high-speed camera and a force-measuring shoe during exercise on the treadmill before and after treatment with phenylbutazone. In a pilot study the computerized film analyzing system, TrackEye®, was utilized to study a horse with lameness caused by osteo-arthritis in the fetlock of a foreleg and to assess the effect of a recommended oral dose (2.5 mg/kg BID) of phenylbutazone on the fetlock and carpal joint angle movements. The minimum fetlock angle and carpal joint angle were significantly greater in the lame limb before treatment than those at 6, 37 and 49 h after the last administration of phenylbutazone (Drevemo *et al.*, 1993). During the experiment, blood samples were drawn to determine the concentration of phenylbutazone in plasma. At 49 h, when an effect was apparently still noted, the phenylbutazone concentrations in the horse was as low as 0.4 µg/ml plasma.

According to Tobin (1988) most of the NSAIDs begin to act within 3-6 h of administration, are effective for about 24 h after a single dose, and after repeated doses for somewhat longer time. Further, Tobin states that if phenylbutazone was used to conceal lameness, a blood sample would show a concentration of phenylbutazone > 2 µg/ml pharmacoldynamic effective. After about 24 h when lameness might reappear, a blood sample would show only traces (< 1 µg/ml or so), and in the urine would show low levels of metabolites (Tobin, 1988).

Pharmacological effects in relation to plasma and inflammatory exudate concentrations have been intensively studied by Higgins and Lees and their co-workers. Their observation that therapeutic effects following intravenous dosing with phenylbutazone may last longer than 24 h has been substantiated by the finding that the drug inhibits the formation of prostaglandins (PGE_2 and PGI_2) in inflammatory exudate for at least 24 hours (Higgins *et al.*, 1984; Higgins and Lees, 1983). At that time the mean concentration of phenylbutazone in exudate was 2.9 µg/ml, while the plasma concentration had fallen to 0.7 µg/ml. The slower clearance from exudate was indicated by approximate half-lives of elimination of 4.8 h for plasma and 24 h for exudate (Lees *et al.*, 1986). This, together with the observations that

phenylbutazone binds irreversibly to cyclo-oxygenase and that the pharmacologically active metabolite oxyphenbutazone is also cleared more slowly from exudate, may explain the prolonged effect in spite of the relatively short half-life (approximately 5 h) and low concentration in plasma (even below 1 µg/ml) of phenylbutazone (Lees *et al.*, 1986).

These factors may help to explain the clinical observation that the response seems to last for more than 24 h after a single dose and for several days after the last administration in a course of treatment (Scott, 1972).

The "therapeutic window" of phenylbutazone in horses has earlier been estimated to be in the order of 5-15 µg/ml plasma (Gerring *et al.*, 1981; Jenny *et al.*, 1979; Lees and Higgins, 1985). Obviously, this should be modified according to more up-to-date knowledge. It seems that pharmacological and therapeutic effects could be demonstrated even when plasma concentrations have fallen to values well under 5 µg/ml.

When the effects of drugs on performance of horses were studied it was found that phenylbutazone improved the performance in time trials. Phenylbutazone was administered (6.6 mg/kg b.wt, intramuscularly) to 4 horses 23 h before the trial. The rather surprising improvement could be explained by assuming that the horses, regarded as sound, were apparently not so (Sanford, 1983). This conclusion that phenylbutazone relieved subclinical lameness rather than actually stimulating and improving performance was supported by a study on the effects of drugs on locomotory response in laboratory experiments. Phenylbutazone neither stimulated nor depressed the horses at clinically used doses (Tobin, 1981).

When studying training effects on heart rate responses in submaximal standardised exercise tests on the racetrack, Foreman *et al.* (1990) found that heart rates in lame horses were significantly higher than in sound horses. It was therefore suggested that heart rate monitoring could be used to indicate the presence of undetected lameness. Phenylbutazone in doses of 4.4-8.8 mg/kg (orally) was given to horses which became lame during the experiment. However, heart rates of untreated and phenylbutazone-treated lame horses were remained alike. It was concluded that since workouts in untreated horses were compared with workouts in horses to which phenylbutazone had been administered the preceding evening, drug levels may have fallen below the therapeutic concentration (Foreman *et al.*, 1990).

Flunixin

In Study VII the stride length increased at submaximal velocities after treatment with flunixin compared with the baseline situation. Since the treadmill velocities were the same on both occasions, the mean stride frequencies subsequently were decreased, although not significantly so (except at 7 m/s).

When the same horses were raced in the track trial (VI), no significant changes in maximum speed, stride length (SL) or stride duration could be proven after administration of flunixin, although some effects on the

locomotion pattern were observed. In the foreleg, stance time was decreased and swing time increased after flunixin was administered. The change in stance time relative to stride time was decreased,which is in agreement with a study of induced lameness (Buchner *et al.*, 1995). When we studied the effects of phenylbutazone on lame horses we obtained t similar results (V). A possible explanation is that these changes might be due to allevating of pain in the musculoskeletal system and with a suspicion of subclinical lameness.

In Study VII the increased SL after flunixin administration might, if not caused by changes in the muscle fibre recruitment, equally well have been due to subclinical lameness and a consequently altered locomotion pattern in these horses.

SL is considered to be sensitive to the action of NSAIDs and for quantitating the effect on lameness (Tobin, 1981). In a pharmaco-kinetic/pharmacodynamic model with induced carpal arthritis it was reported that NSAIDs had a potential ability to increase SL by 10 %. Flunixin increased the stride length of horses with induced joint inflammation by 6-16% compared with control (saline), with a peak effect at 4-6 h and an EC_{50} of 0.9 µg/ml plasma. (Toutain *et al.*, 1994).

To show that the levels of flunixin during the present trials were within the therapeutic range, the plasma concentrations of the drug were determined and found to be between 1.2 and 2.8 µg/ml (4-4.5 h after administration). The drug concentration at the recommended dose in horses is reported to peak at 1.6 µg/ml and the onset of action to occur within 2 h, with peak effects between 2 and 16 h (Boothe, 1995). The median maximum concentration (Cmax) after intramuscular administration has been shown to be 2.3 µg/ml plasma (range 1.8-3.3 µg/ml), the median time to reach Cmax (Tmax) to be 76.6 min (31.7-97 min) and the median elimination half-life ($T_{1/2\beta}$) to be 189 min (Dyke *et al.*, 1997). Previous, $T_{1/2\beta}$ is reported to be about 1.6 h (Chay *et al.*, 1982). The mean plasma flunixin concentration of 2 µg/ml in our study was thus within the suggested range of efficacy (Toutain *et al.*, 1994, Dyke *et al.*, 1997).

The greatest effect of flunixin seems to be obtained between 2 and 16 h and persists for up to 24-36 h (Houdeshell and Hennessey, 1977; Lees and Higgins, 1985). The suppression of thromboxane (TXB_2) release produced by flunixin is reported to vary in the literature: from 12 h (Semrad *et al.*, 1985) and 24 h (Hardee and Moore, 1986) in horses (less than 24 h in Thoroughbreds and Quarterhorses) (Kopp *et al.*, 1985) - up to 48 h in ponies (Lees *et al.*, 1987). Soma *et al.* (1985) showed that the serum concentration of TXB_2 in Thoroughbreds returned to baseline values 12-16 h after flunixin administration (1.1 mg/kg bwt). It was also suggested that the detection of low concentrations of flunixin in urine 24 h post-administration may not represent pharmacologically effective concentrations of flunixin in plasma (Soma *et al.*, 1985). On the other hand, the rapid metabolism of flunixin will reduce the detectability in plasma, through the prostanoid synthesis inhibition (Lees and Higgins, 1984) and the masking of lameness (Houdeshell and Hennessey,

1977) will persist. This indicates a clinical effect despite our inability to detect flunixin in plasma (Higgins *et al.*, 1986; Lees and Higgins, 1985) which could have implications for racing and medication control.

Exercise and effects on pharmacokinetics

Apart from differences in metabolism due to age, breed and drug interactions, the question has been raised whether exercise and training status could have any effect on the pharmacokinetics of these drugs.

Exercise-induced changes in urinary pH might affect drug excretion. The urinary concentrations of phenylbutazone and metabolites increase with higher urinary pH, but this should not significantly affect the plasma half-life of the drug (Tobin *et al.*, 1986) since renal clearance of phenylbutazone is only a small fraction of total body clearance.

A decrease in blood flow to the liver might alter the pharmacokinetics of drugs with hepatic metabolism. Studies in man have, according to Tobin (1981), shown that exercise can lead to reduced hepatic blood flow. However, as Tobin (1981) states, there is no evidence that the very brief period of exercise that most racing horses are involved in is likely to significantly affect drug metabolism (at least not to affect pre- and postrace drug testing). As phenylbutazone has a hepatic biotransformation which partly determines the plasma half-life in the horse (Tobin *et al.*, 1986), is it plausible that heavy exercise might increase the plasma half-life, at least when exercise is prolonged (e.g. eventing and endurance). The plasma half-life of elimination of phenylbutazone found in study I described above (Kallings and Persson, 1983) in a 10 minute exercise-test was 6.5 h (with a dose of 2.5 mg/kg BID). This is somewhat longer than reported in other studies (Lees and Higgins, 1985; Piperno *et al.*, 1968). Unfortunately, this half life was not compared within the same individual horses at rest.

In a study of effects of age and training on the pharmacokinetics of flunixin in Thoroughbreds, it was concluded that age (but not training status) influenced the disposition of the drug (Jensen *et al.*, 1990). In agreement with this, Soma and co-workers (1985) studied flunixin and its effects on thromboxane (TXB_2) production. The observed variation in depression of serum TXB_2 seemed to be related to age rather than to training status (Soma *et al.*, 1985).

Ethical and regulatory considerations

Bronchodilators

The methyl-xanthines, theophylline, caffeine and theobromine, have also been involved in doping of racehorses (Tobin, 1981). In horseracing and the equestrian sports there is an internationally established threshold for

theobromine in horse urine of 2 µg/ml and in human sports, caffeine has a threshold of 12 µg/ml urine. A doping test is considered as a positive if drug concentrations are above these values and below as food contamination.

Clenbuterol has been used illegally with the intention of improving performance potential in normal racehorses. Also, misuse by treating racehorses with mild, subchronic bronchitis with the aim of making them perform better under exertion than they would normally have been capable to doing without medication, has been critisized and is now forbidden in Sweden.

Slocombe *et al.* (1992) rather surprisingly suggested that clenbuterol may have a role similar to phenylbutazone, in that it may serve to return tan ailing horse to normal functioning, as no performance enhancing effects were observed. This kind of therapeutic use has been quite vigorously debated in Sweden, although a withdrawal time (96 h) was in force according to the racing regulations at the time. This was then considered to be too short since a horse suffering from respiratory distress or mild COPD must be healthy when competing. Because of ethical and medical reasons the withdrawal time therefore was increased (14 days) to ensure the wellbeing of racing horses.

NSAIDs

The controversy overthe use of NSAIDs in competing horses has been a "hot" issue for a long time. In particular, the use of phenylbutazone has been discussed. It has been questioned whether it is fair, with respect to both competition and animal welfare, to permit a horse to compete under the influence of NSAIDs (Snow, 1981). According to Tobin (1989) phenylbutazone in racetrack practice in the USA is regarded as an adjunct in the training of "sore" horses and some equine practitioners believe that if horses race on "Bute", they will race longer. Others say that either there is an increased incidence in breakdowns, or there is no increase in the number of breakdowns but there are more severe injuries when horses are raced on phenylbutazone. However, this remains to be proven and medication status has been suggested to be included in the reporting of racing breakdowns (proposed at the 9th Internat. Conf. of Racing Analysts and Vet., New Orleans, 1992).

Many racing jurisdictions in North and South America as well as the American Horse Shows Association permit the use of NSAIDs in competition, but phenylbutazone and flunixin may be restricted up to certain levels in blood (or in urine). The International Racing Conference and the European and Australian Jockey Clubs and Harness Racing Associations do not allow NSAIDs at racing. The International Equestrian Federation (FEI) permitted until 1994 concentrations of phenylbutazone and oxyphenbutazone up to 2 µg/ml plasma, respectively (Kallings 1990). The earlier total level was set at 5 µg/ml, but was reduced to the lower level after much debate preceding the 1st World Equestrian Games in Sweden and when the FEI introduced its revised Veterinary Regulations in 1990. Since the pharmacologicaleffects of the

drugs have been proven even when plasma concentration is diminishing, the relevance of permitted levels in plasma and urine is questioned and should be reconsidered (Kallings, 1990). The Veterinary Committee of the FEI proposed that there should not be a permissible level of phenylbutazone/oxyphenbutazone and in the Veterinary Regulations, valid from January 1st, 1994, this was finally decided. Several of the European countries already operated this ban at national level, e.g. Switzerland and Sweden (national law).

As pain is a signal to spare injured tissues, competing during treatment with analgesic drugs like the NSAIDs may allowfurther damage to occur. One reason for not permitting medication in competing horses is that the use of NSAIDs could lead to abnormal demands on the horse's ability, i.e. the concealment of pain and trauma, resulting from too frequent competition and the construction of too difficult courses (Snow, 1981).

The pharmacodynamic effect of NSAIDs seems to last longer than their plasma concentrations indicate. This has implications for current regulations for competing horses and the relevance of permitted levels has been questioned.

NSAIDs do not appear to enhance performance, but rather to allow the horse to run up to its potential by alleviating pain and lameness. There is concern over the possible hazards to the horse by allowing this kind of therapeutic use.

Summary of findings

Findings in relation to the aims adressed:

¤ -*theophylline* increased heart rate and blood lactate in clinically healthy horses performing submaximal standardized exercise tests on treadmill.

-*clenbuterol* had no effect on cardiorespiratory or metabolic parameters.

¤ -*phenylbutazone* increased heart rate and had no effect on lactate response in clinically healthy horses performing submaximal standardised exercise tests on a treadmill.

- *meclofenamic acid* gave no effect on HR but a decreased blood lactate response.

¤ -*flunixin* exerted increasing effects on submaximal heart rates and plasma lactate concentrations in clinically healthy horses performing incremental exercise to fatigue on a treadmill. Stride length was increased at submaximal velocities. HR and SL were not changed at higher velocities.

¤ -*flunixin* exerted effects on forelimb movement, but not on heart rate or plasma lactate accumulation post exercise in clinically healthy horses trotting on a track.

¤ -*phenylbutazone* at low levels influenced for at least 48 h locomotion pattern in lame horses on treadmill (less lame), and it was found that the method used (TrackEye®) is appropriate for reveal ing subclinical changes in an objective way.

¤ - it was concluded that standardized treadmill and track testing and high-speed filming were adequate methods for performance testing of drug effects in horses, although all methods had their advantages and limitations.

Methodological considerations and future research

Reproducibilities

The standardized submaximal treadmill exercise tolerance test was considered to be an adequate and a reliabel method to assess effects on performance potential in horses. The reproducibilities of the studied parameters are reported to be high for pulse/work relationship, e.g. the velocity at HR 200, V_{200} (r = 0.98) and oxygen uptake, VO_2 (r = 0.99). The blood lactate variables, e.g. V_{LA4}, were less repeatable (r = 0.63) (Persson, 1983). This circumstance and the relatively low number of studied horses must be taken in consideration when evaluating the results of these studies. In a previous study, 5 horses were treated with 4 different drugs and a wash-out period of one week in-between during a couple of months. Two reference tests (without medication) were done, one before and one in the middle of the tests period, (with a couple of weeks in between). There were no significant differences in the studied exercise variables betweeen the two occasions, the mean values (of e.g. V_{200} and V_{LA4}) were almost identical, i.e. no training effect over time could be shown.

Future research

The reported results in the thesis concerning lactate accumulation in plasma vs. whole blood generates a need for further investigations. In vitro studies on diffusion, efflux and influx of lactate in erythrocytes is one approach. Another approach, already preliminary tested in a pilot study, is to use microdialysis to measure lactate concentration in the working muscle and simultaneously in the venous blood, with the aim to clarify the lactate accumulation.

An interesting challenge should be to further develop a computerized telemetric system, to evaluate the present findings by recording more data on the track. These concepts will be analysed more in depth in the future.

Conclusions

Bronchodilating drugs

The predominant effects of theophylline on exercise were the increases in HR and blood lactate levels, which may indicate a decrease rather than an increase in performance potential in healthy horses.

Clenbuterol, in contrast to theophylline, did not cause any major effects on cardio-respiratory and metabolic responses to exercise. No change in oxygen uptake or improved respiration could be seen. It could thus be concluded that clenbuterol did not seem to enhance performance in healthy horses.

Non-steroidal anti-inflammatory drugs

Since the studies presented showed no increase in oxygen uptake and the heart rates were elevated after administration of flunixin and phenylbutazone, these drugs do not appear to have a positive effect on the physiological response to exercise in these horses. This probably reflects the inhibitory actions of flunixin on both prostanoid and non-prostanoid dependent mechanisms. On the other hand, the elongated stride length after flunixin medication rather implies a positive effect on the locomotion pattern. However, the possibility that this could be related to the analgesic/antiphlogistic effect of the drug on subclinical lameness or pain, can not be ruled out.

Final remarks

It may therefore be considered that the studied drugs do not appear to enhance performance, but rather allow the horse to run up to its potential by alleviating pain, lameness or respiratory distress. In racing and competition this kind of therapeutic use could jeopardize the welfare of the equine athlete. It is never acceptable to use drugs to enable an unsound horse to run. That horse should not take part in a competition until the lesion is healed. This is a concern for the veterinary profession.

References

Abramson S. and Weissmann G. (1989) The mechanism of action of non-steroidal anti-inflammatory drugs. *Clin Exp Rheum*, Suppl. 3, **7**, 163-170.

Adams R.H. (1995) Prostaglandins, related factors and cytokines. In: *Veterinary Pharmacology and therapeutics*, 7th edn. Ed: R.H. Adams. Iowa state university press/Ames. pp 419-431.

Andersson K.-E., Johannesson N. and Persson C.G.A. (1985) Some extrapulmonary actions of alkylxanthines. In: *Antiasthma Xanthines and Adenosine*. Ed: Andersson K.-E. and Persson C.G.A. Excerpta Medica, Amsterdam. pp 362-380.

Beech J. (1979) Principles of therapy. *Veterinary Clinics of North America/Large Animal Practise*, **1**, 73-88,

Bergstrand H. (1980) Phosphodiesterase inhibition and theophylline. *Eur. J. Respir. Dis.*, Suppl. **109**, 37-44.

Birks E.K., Gory S.N., Li C. and Jones J.H. (1991) Effect of exercise on plasma concentrations of prostaglandins and thromboxane B_2. In: *Equine Exercise Physiology* 3. Eds: S.G.B. Persson, A. Lindholm and L.B. Jeffcott, ICEEP Publications, Davis, CA. , pp 374-379.

Boothe D.M. (1995) The analgesic-antipyretic-antiinflammatory drugs. In: *Veterinary Pharmacology and Therapeutics*, 7th edn. Ed: R.H. Adams. Iowa State University Press/Ames, pp 432-449.

Buchner H. H. F., Savelberg, H. H. C. M., Schamhardt H. C. and Barneveld A. (1995) Temporal stride patterns in horses with experimentally induced fore or hind limb lameness, *Equine vet J.* Suppl., **18,** 161-165.

Chay S., Woods W.E., Nugent T., Blake J.W. and Tobin T. (1982) The Pharmacology of nonsteriodal anti-infalammatory drugs in the horse: Flunixin Meglumine (Banamine®). *Equine Practise*. **4** (10): 16-23.

Costill D.L, Dalsky G.P. and Fink W.J. (1978) Effects of caffeine ingestion on metabolism and exercise performance. *Medicine and Science in Sports*, **10**, 155-158.

Cowley A.J., Stainer K., Rowely J.M. and Hanley S.P. (1984) The effect of aspirin on peripheral haemodynamic changes following submaximal exercise in normal volounteers. *Cardiovasc Res.* **18**, 511-513.

Cowley A.J., Stainer K., Rowely J.M. and Wilcox R.G. (1985) Effect of aspirin and indomethacin on exercise-induced changes in blood pressure and limb blood flow in normal volunteers. *Cardiovasc Res.* **19**, 177-180.

Deegen E. (1982) Neuere Methoden der Behandlung chronisch hustender Pferde. *Der Praktische Tierartz*, **63**, 55-57.

Demers L.M., Harrison T.S., Halbret D.H. and Santen R.J. (1981) Effect of prolonged exercise on plasma prostaglandin levels. *Prostaglandins Med.* **6**, 413-418.

Derksen F.J. (1987) Chronic obstructive pulmonary disease. In: *Current Therapy in Equine Medicine 2*. Ed: Pedersen D. W.B. Saunders Co., Philadelphia, USA. 596-602.

Drevemo S. and Johnston C. (1994) The use of a panning camera technique in equine kinematic analysis. *Equine vet J.* **Suppl. 17**, 39-43.

Drevemo S., Roepstorff L., Kallings P. and Johnston C. (1993) Application of TrackEye® in equine locomotion analysis. *Acta Anat.*, **146**, 137-140.

Dyke T.M., Sams R.A. and Cosgrove S.B. (1997) Disposition of flunixin after intramuscular administration of flunixin meglumine to horses. *J Vet Pharmacol Therap.* **20**, 330-332.

Errecalde J.O., Button C. and Mülders M.S.G. (1985) Some dynamic and toxic effects of thephylline in horses. *Joirnal of Veterinary Pharmacology and Therapeutics*, **8**, 320-327.

Foreman J.H, Bayly W.M, Grant B.D et al. (1990) Standardized exercise test and daily heart rate responses of Thoroughbreds undergoing conventional race training and detraining. *Am J Vet Res* **51**, 914-920,

Fredholm B.B. (1980a) Theophylline actions on adenosine receptors. *European Journal of Respiratory Diseases*, **61** (Suppl. 109), 29-36.

Fredholm B.B. (1980b) Are methylxanthine effects due to antagonism of endogenous adenosine? *Trends in Pharmacological Sciences*, **1**, 129-132.

Fujii S., Inada S., Yoshida S., Kusanagi C., Mima K. and Natsuno Y. (1972) Pharmacological studies on doping drugs for race horses, II. Caffeine. *Jap. J. Vet. Sci.* **34**, 135-141.

Gaesser G.A. and Rich R.G. (1985) Influence of caffeine on blood lactate resonse during incremental exercise. *Internationel Journal of Sports Medicene.*, **6**, 270-211.

Genetzky R.M. and Loparco F.V. (1985) Clinical efficacy of clenbuterol with COPD in horses. *Equine Veterinary Sciences*, **5**, 320-323.

Gerring E.L., Lees P. and Taylor J.B. (1981) Pharmacokinetics of phenylbutazone and its metabolites in the horse. *Equine vet J* **13**,152-157.

Gibaldi M. (1984) *Biopharmaceutics and Clinical Pharmacokinetics*, 3rd edn. pp 17-26. Lea & Febiger, Philadelphia, PA.

Hardee M.M. and Moore J.N. (1986) Effects of flunixin meglumine, phenylbutazone and a selective thromboxane synthetase inhibitor (UK 38.485) on thromboxane and prostacyclin production in healthy horses. *Res Vet Sci* **40**, 152-156

Higgins A.J. and Lees P. (1983) Phenylbutazone inhibitition of prostaglandin E_2 production in equine acute inflammatory exudate. *Vet Rec* **113**, 622-623.

Higgins A.J., Lees P. and Taylor J.B. (1984) Influence of phenylbutazone on eicosanoid levels in equine acute inflammatory exudate. *Cornell vet* **74**, 198-207.

Higgins A.J., Lees P., Taylor J.B.O et al. (1986) Flunixin meglumine: Quantitative determination in and effects on composition of equine inflammatory exudate. *Br vet J* **142**, 163-169.

Hinchcliff K.W., MCKeever K.H. and Muir W.M. (1994) Effects of phenylbutazone on the haemodynamic, acid-base and eicosanoid responses of horses to sustained submaximal exertion. *Res Vet Sci.* **56**, 352-362.

Houdeshell J.W. and Hennessey P.W. (1977) A new nonsteroidal anti-inflammatory analgesic for horses. *J Equine Med Surg.* **1**, 57-63.

Ingvast-Larsson C., Paalzow G., Paalzow L., Ottosson T., Lindholm A. and Appelgren L.-E. (1985) Pharmacokinetic studies of theophylline in horses. *Journal of Veterinary Pharmacology and Therapeutics*, **8**, 76-81.

Ivy J.L., Costill D.L., Fink W.J. and Lower R.W. (1979) Influence of caffeine and carbohydrate feedings on endurance performance. *Medicine and Science in Sports*, **11**, 6-11.

Jenkins W.L (1987) Pharmacologic aspects of analgesic drugs in animals: An overview. *J Am Vet Med Assoc* **191**, 1231-1240.

Jenny E., Steinijans V.W. and Seifert P. (1979) Pharmacokinetic interaction of isopropyl-aminophenazone and phenylbutazone in the horse. *J Vet Pharmacol Therap* **2**, 101-108.

Jensen R.C., Fischer J.H., Cwik B.A. (1990) Effect of age and training status on pharmacokinetics of flunixin meglumine in Thoroughbreds. *Am J Vet Res* **51**,591-594.

Jones E.W. and Hamm D. (1978) Comparative Efficacy of Phenylbutazone and Naproxen in induced Equine Myositis. *J Equine Med Surg* **2**, 341-347.

Kallings P. (1990) Doping and Medication Control. In Kallings P (ed): Proceedings of International Conference on Equine Sports Medicine, Stockholm, pp 77-81

Kallings P. (1993) Nonsteroidal anti-inflammatory drugs. In: *Drug use in performance horses*, Veterinary Clinics of North America: Equine Practice, Ed: R. Sams and K. Hinchcliff. **9**, 523-541.

Kallings P. and Persson S. (1983) Effects of theophylline and nonsteroidal anti-inflammatory drugs on pulse and blood lactate responses to exercise in the horse: a preliminary report. In: *Equine Exercise Physiology,* Eds: D.H. Snow, S.G.B. Persson and R.J. Rose. Granta Editions, Cambridge, England. pp 538-542.

Kamerling S.G., De Quick D.J., Weckman T.J. et al (1985) Differential effects of phenylbutazone and local anaesthetics on nociception in the equine. *Europ J Pharmacol* **107**, 35-41.

Kopp K.J., Moore J.N., Byars T.D. et al (1985) Template bleeding time and thromboxane generation in the horse: Effects of three non-steroidal anti-inflammatory drugs. *Equine vet J* **17**, 322-324.

Lees P. and Higgins A.J. (1984) Flunixin inhibits prostaglandin production in equine inflammation. *Res Vet Sci* **37**, 347-349.

Lees P. and Higgins A.J. (1985) Clinical pharmacology and therapeutic uses of non-steroidal anti-inflammatory drugs in the horse. *Equine vet J.* **17**, 83-96.

Lees P., Maihto T.E. and Taylor J.B. (1985) Pharmacokinetics of phenylbutazone in two age groups of ponies: a preliminary study. *Vet rec.* **116**, 229-232.

Lees P., Taylor J.B.O., Higgins A.J. et al (1986) Phenylbutazone and oxyphenbutazone distribution into tissue fluids in the horse. *J Vet Pharmacol Therap* **9**, 204-212.

Lees P., Ewins C.P., Taylor J.B.O. et al: (1987) Serum thromboxane in the horse and its inhibition by aspirin, phenylbutazone and flunixin. *Br Vet J* **143**, 462-476.

Lieske R. and Deegen E. (1980) The influence of clenbuterol on the blood pressure in the pulmonary artery and on the arterial blood gases of horses. In: *Proceedings of the fifth meeting of the Academic Sosiety Large Animal Veterinary Medicine*, University of Glasgow Veterinary School, September 8-9, 103-114.

Liles J.H. and Flecknell P.A. (1992) The use of nonsteroidal antiinflammatory drugs for the relief of pain in laboratory rodents and rabbits. *Lab Anim* **26**, 241-255.

Lindholm A, Bjerneld H. and Saltin B. (1974) Glycogen depletion pattern in muscle fibres of trotting horses. *Acta Physiol. Scand.* **90**, 475-484.

Malmberg A.B. and Yaksh T.L. (1992) Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. *Science* **257**, 1276-1279.

Manohar M., Goetz T.E., Griffin R. and Sullivan E. (1996) Pulmonary vascular pressures of strenuously exercising Thoroughbreds after administration of phenylbutazone. *Am. J. Vet. Res.* **57**, 1354-1358.

Mehta J., Mehta P. and Horalek C. (1983) The significance of platelet-vessel wall prostaglandin equilibrium during exercise-induced stress. *Am Heart J.* **105**, 895-900.

Mitten L.A., Hinchcliff K.W., Pate J.L., Kohn C.W. and McKeever K.H. (1995) Effect of exercise intensity on plasma prostaglandin concentrations in horses. *Am J Vet Res.* **56**, 122-125.

Mitten L.A., Hinchcliff K.W. and Pate J.L. (1996) Phenylbutazone increases right artrial pressure and heart rate of running horses. *J Appl. Physiol.* **81**, 312-317.

Nowak J. and Wennmalm A. (1978) Effect of exercise on human arterial and regional venous plasma concentrations of prostaglandin E. *Prostaglandins Leukotrienes Med*, **1**, 489-497.

Oates J.A., Fitzgerald G.A., Branch R.A, Jackson E.K., Knapp H.R. and Roberts L.J. (1988) Clinical implications of prostaglandin and thromboxane A_2 formation. *N Engl J Med.* **319**, 689-698.

Olsen S.C., Coyne C.P., Lowe B.S., Pelletier N., Raub E.M. and Erickson H.H. (1992) Influence of cyclo-oxygenase inhibitors on furosemide-induced hemodynamic effects during exercise in horses. *Am J Vet Res.*, **53**, 1562-1567.

Persson C.G.A. (1987) The pharmacology of anti-asthmatic xanthines and the role of adenosine. *Astham Rev.***1**, 61-94.

Persson S.G.B (1983) Analysis of fitness and state of training: Evaluation of exercise tolerance and fitness in the performance horse. In: *Equine Exercise Physiology* , Eds: S.H. Snow, S.G.B. Persson and R.J. Rose. Granta Editions, Cambridge, pp 441-457.

Persson S.G.B (1997) Heart rate and blood lactate responses to submaximal treadmill exercise in the normally performing Standardbred trotter - age and sex variations and predictability from the red blood cell volume. *J Vet Med.* A **44**, 125-132.

Petermann U. (1981) Untersuchungen zur Wirksamkeit von Theophyllin bei Pferden mit chronisch obstructiver Bronchitis. Hannover. *Tierärztliche Hochschule*, Diss.

Piperno E., Ellis D.J., Getty S.M. and Brody T.M. (1968) Plasma and urine levels of phenylbutazone in the horse *J Am vet med Ass.* **153**, 195-198.

Powers S.K., Byrd R.J., Tulley R. and Callender T. (1983) Effects of caffeine ingestion on metabolism and performance during graded exercise. *European Journal of Applied Physiology and Occupational Physiology*, **50**, 301-307.

Pratt G. (1977) Breakthrough on understanding lameness. *The Blood-Horse* **103**, 3758-3760.

Rainsford K.D. (1985) In *Aspirin and the salicylates*, London, Butterworths, pp 123-129

Rall T.W. (1980) Central nervous stimulants, the xanthines. In: *The Pharmacological Basis of Therapeutics*. Eds Gilman A.G., Goodman L.S. and Gilman A. 592-607. Macmillan Publishing Co., Inc., New york.

Rang H.P., Dale M.M. and Ritter J.M. (1995) Anti-inflammatory and immunosuppressant drugs. In: *Pharmacology,* 3rd edn. Ed: H.P. Rang, M.M. Dale and Ritter J.M. Churchill Livingstone, pp 246-255.

Robertsson D., Wade D., Workman R., Woosley R.L. and Oates J.A. (1981) Tolerance to the humoral and hemodynamic effects of caffeine in man. *Journal of clinical Investigation*, **67**, 1111-1117.

Ronni-Sivula H., Malm H., Yilkorkala O. and Viinikka L. (1993) Marathon run stimulates more prostacyclin than thromboxane synthesis and differently in men and women. *Prostaglandins*. **46**, 75-90.

Rose R.J. and Evans D.L. (1987) cardiorespiratory effects of Clenbuterol in fit Thoroughbred horses during a maximal exercise test. In: *Equine Exercise Physiology 2.* Eds. Gillespie J.R. and Robinson N.E. 117-131. *Proceedings of the 2nd International Conference on Equine Exercise Physiology, San Diego 1986.* ICEEP Publications, Davis, CA.

Rose R.J., Allen J.R. and Brock K.A. (1983) Effects of Clenbuterol hydrochloride on certain respiratory and cardiovascular parameters in horses performing treadmill exercise. *Research in Veterinary Science*, **35**, 301-305.

Sanford J. (1983) Effects of drugs on performance of the horse. In: *Pharmacological Basis of Large Animal Medicine*. Eds: J. A. Bogan, P. Lees and A. T. Yoxall. Blackwell Scientific Publ., Oxford, pp 495-510.

Sasse H.L. and Hajer R. (1978) NAB 365, a beta2-receptor sympathomimetic agent: clinical experience in horses with lung disease. *Journal of Veterinary Pharmacology and Therapeutics,* **4**, 241-244.

Scott Dunn P. (1972) A Clinician's views on the use and misuse of phenylbutazone. *Equine vet J* **4,** 63-65.

Semrad S.D., Hardee G.E., Hardee M.M. et al. (1985): Flunixin meglumine given in small doses: Pharmacokinetics and prostaglandin inhibition in healthy horses. *Am J Vet Res* **46.** 2474-2479.

Short C.E., Maylin G.A. and Collier M.A. (1990) Responses to submaximal exercise in Standardbred horses with and without medication. *Equine Pract* **12**, 23-28.

Slocombe R.F., Covelli G. and Bayly W.M. (1992) Respiratory mechanics of horses during stepwise treadmill exercise tests, and the effect of clenbuterol pretreatment on them. *Aust Vet J.* **69**(9), 221-225.

Snow D.H. (1979) Metabolic and physiological effects of adrenoceptor agonists and antagonists in the horse. *Research in Veterinary Science*. **27**, 372-378.

Snow D. (1981) Non-steroidal anti-inflammatory agents in the horse. *Vet Rec*, Suppl: In Practice **3**, 24-31.

Soma L.R., Sams R. and Duer W. (1985) Plasma and serum concentrations of phenylbutazone and oxyphenbutazone in racing Thoroughbreds 24 hours after various dosage regimens. *Am J Vet Res* **46**, 932-938.

Soma L.R., Uboh C.E. and Rudy J. (1992) Plasma concentrations of flunixin in the horse: its relationship to thromboxane B_2 production. *J Vet Pharmacol Therap* . **15**, 292-300.

Stassen, J., Cattaert, A., Fagard, R., Lijnen, P., Moerman, E., DeSchaepdryver, A. and Amery, A. (1984) Haemodynamic and humoral effects of prostaglandin inhibition in exercising humans. *J Appl Physiol.* **56**, 39-45.

Stiles G.L. (1986) Adenosine receptors: structure, function and regulation. *Trends in Pharmacological Sciences,* **7**, 486-490.

Stubbs A.D., Cushley M.J. and Tattersfield A.E. (1984) Attenuation by propranolol of the airway and cardiovascular responses to oral theophylline in normal subjects. *Clinical Science*, **66**, 57-68.

Tavernor W.D. (1969) Technique for the subcutanous relocation of the common carotid artery in the horse. *American Journal of Veterinary Research*, **30**, 1881-1883.

Teitzel R. (1982) The use of Ventipulmine in the management of the 'Coughing Horse' syndrome. *Australian Veterinary Practitioner*, **12**, 56-60.

Tobin T. (1979) Pharmacology Review: The nonsteroidal anti-inflammatory drugs II: Equiproxen, meclofenamic acid, flunixin and others. *J Equine Med Surg* **6**, 298-302.

Tobin T. (1981) Phenylbutazone and its brothers: The Non-Steroidal Anti-inflammatory Drugs. In: *Drugs and the Performance Horse*, CC Thomas, Springfield, Ill., pp 85-110.

Tobin T. (1988) Horse purchase and medication testing: an overview. *J Equine Vet Sci* **8**, 272-275.

Tobin T. (1989) Non-Steroidal Anti-Inflammatory Drugs: Phenylbutazone and others. In *Proceedings of the 11th Bain-Fallon Memorial Lecture* (Equine Pharmacology and Therapy), Coffs Harbour, Sydney, pp 13-21

Tobin T., Chay S., Kamerling S., Woods W.E., Weckman T.J., Black J.W. and Lees P. (1986) Phenylbutazone in the horse: A Review. *J Vet Pharmacol Therap.* **9**, 1-25.

Toner M.M., Kirkendall D.T., Delio D.J., Chase J.M., Cleary P.A. and Fox E.L. (1982) Metabolic and cardiovascular responses to exercise with caffeine. *Ergonomics*, **25**, 1175-1183.

Toutain P.L., Autefage A., LeGrand C. and Alvinerie M. (1994) Plasma concentrations and therapeutic efficacy of phenylbutazone and flunixin meglumine in the horse: pharmacokinetic/pharmacodynamic modelling. *J Vet Pharmacol Therap.* **17**, 459-469.

Turgut K.and Sasse H.H.L. (1989) Influence of clenbuterol on mucociliary transport in healthy horses and horses with chronic obstructive pulmonary disease. *Vet. Rec.* **125**, 526-530.

Valberg S. (1986) Glygogen depletion patterns in the muscle of Standardbred trotters after exercise of varying intensities and durations. *Equine Vet. J.*, **18**, 479-484.

von Botz F. (1982) Die Wirkprinzipien der medikamentösen Therapie der chronischen Bronchitis des Pferdes. *Der Praktishe Tierartz*, **63**, 52-55.

Weissmann G. (1991) The actions of NSAIDs. *Hos Pract.* **15**, 60-76.

Westergaard M. and Sejerson K. (1991) Endocrine manipulation of animal growth. *Acta Vet. Scand.*, **Suppl 87**, 75-86.

Wilson J.R. and Kapoor S.C. (1993) Contribution of prostaglandins to exercise-induced vasodilation in humans. *Am J Physiol.* **265**, H171-H175.

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*"It is better to find out than to dope out *....."*

(* Norstedts Comprehensive English-Swedish Dictionary, 1997, **2,** p. 310.)

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