Phenylbutazone in the horse: a review

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SUMMARY

Phenylbutazone is an acidic, lipophilic, non-steroidal anti-inflammatory drug (NSAID). It is extensively metabolized in the horse. The metabolites so far identified, oxyphenbutazone, γ-hydroxyphenylbutazone and γ-hydroxyoxyphenbutazone, account for some 25–30% of administered dose over 24 h. The plasma half-life of phenylbutazone and termination of its pharmacological action are determined primarily by its rate of hepatic metabolism.

Phenylbutazone acts by inhibiting the cyclo-oxygenase enzyme system, which is responsible for synthesis of prostanoids such as PGE_2. It appears to act on prostaglandin-H synthase and prostacyclin synthase, after conversion by prostaglandin-H synthase to reactive intermediates. It markedly reduces prostanoid-dependent swelling, edema, erythema, and hypersensitivity to pain in inflamed tissues. Its principal use in the horse

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is for treatment of soft tissue inflammation.

Phenylbutazone is highly bound (> 98%) to plasma protein. After i.v. injection, blood levels decline with an elimination half-life of 3–10 h. The plasma kinetics of phenylbutazone may be dose dependent, with the plasma half-life increasing as the drug dosage level increases. Plasma residues of the drug at 24 h after a single i.v. dose of 2 g/450 kg average about 0.9 μg/ml, but considerable variation occurs. If dosing is repeated, the plasma residue accumulates to give mean residual blood levels of approximately 4.5 μg/ml on Day 5 after 4 days of dosing. Approximately similar blood levels are found after a combination of oral and i.v. dosing. Experiments on large numbers of horses in training have been undertaken to ascertain the population distributions of residual blood levels after such dosing schedules.

Absorption of phenylbutazone from the gastrointestinal tract is influenced by the dose administered and the relationship of dosing to feeding. Access to hay can delay the time of peak plasma concentration to 18 h or longer. Under optimal conditions, the bioavailability of oral phenylbutazone is probably in the region of 70%. Paste preparations may be more slowly absorbed than other preparations and yield higher residual plasma levels at 24 h after dosing, but further controlled studies are required.

Phenylbutazone is easily detected in the plasma and urine of horses but concentrations in saliva are low. It is quantitated for forensic purposes by HPLC. The variability of this method between laboratories is about ± 25%.

Increasing urinary pH increases the urinary concentration of phenylbutazone and its metabolites up to 200-fold. However, urinary pH has little effect on the plasma half-life of phenylbutazone, which is determined mainly by hepatic metabolism and possibly by biliary secretion.

Phenylbutazone has a narrow therapeutic index in the horse. If the administered dose is greater than recommended by the manufacturer, toxic effects may be produced, especially if high dose administration is maintained for more than a few days. Signs of toxicity include anorexia, depression, oral and GI ulcers, plasma protein losing enteropathy, and death from shock. Other side-effects include toxic neutropenia, hepatotoxicity and renal papillary necrosis; the latter may occur if access to water is restricted. If phenylbutazone is withdrawn in the early stages of toxicity, the prognosis is good. Late withdrawal is associated with delayed recovery. Death may occur up to 50 days after withdrawal of the drug. This toxicity can be antagonized by administration of prostaglandins.

INTRODUCTION

Phenylbutazone is a classical non-steroidal anti-inflammatory drug (NSAID) (Gilman *et al.*, 1980; Tobin, 1981). NSAIDs are a chemically heterogenous group of drugs which share certain pharmacological actions, therapeutic uses and side-effects. All NSAIDs produce their effects by inhibiting the synthesis of prostanoids, including prostaglandins, and thromboxanes such as TXA_2. While aspirin is still the most widely used NSAID in human medicine, phenylbutazone is the drug used most extensively in equine medicine (Tobin, 1981; Lees & Higgins, 1985).

Phenylbutazone is chemically related to aminopyrine and antipyrine. It was introduced into human medicine in 1949 for the treatment of rheumatoid arthritis and related disorders. It produces adverse reactions in about 40% of human patients and causes agranulocytosis and death in a very small percentage of patients. Its use in human medicine is now limited (Gilman *et al.*, 1980); indeed, the product license for use in man in the U.K. was revoked in 1984.

Phenylbutazone was introduced into veterinary medicine in the 1950s and soon became the NSAID of choice in equine medicine. Its effectiveness was such that it became widely used in performance horses. In time, it was approved for use in racing horses in the U.S.A (although it is not permitted in some countries, including the U.K.), and by the mid-1970s, use of phenylbutazone in racing horses had spread to most American states (Tobin, 1981).

In 1977, the use of phenylbutazone in performance horses was reviewed by The Veterinary Chemists Advisory Committee to the National Association of State Racing Com-

missions (Gabel *et al.*, 1977), and also in the Equine Veterinary Journal (Jefcott & Colles, 1977). These reviews were developed in response to the need of regulators of equine sports to have available information on the actions, fate and uses of phenylbutazone in performance horses.

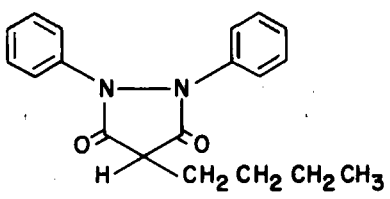
In the late 1970s, a bill in Congress entitled 'The Corrupt Horse Racing Practices Act' (H.R. 1694) was sponsored by the Humane Societies. Passage of this bill would have made medication of a racing horse with phenylbutazone a felony, under some circumstances. This event stimulated a substantial research effort on this drug, and the purpose of this review is to discuss recent studies on the actions, fate, uses, detection and contraindications for phenylbutazone in the horse.

CHEMISTRY AND METABOLISM

Phenylbutazone (4-butyl-1, 2-diphenyl-3, 5-pyrazolidinedione, $C_{19}H_2ON_2O_3$) MW 308.37 is an enolic acid with lipophilic properties (Fig. 1). The acidity is due to the presence of a dissociable proton at C(4) of the five-membered ring. The lipophilism is attributable to the benzene rings and the butyl group. It has a pKa in water of about 4.5 and a *n*-octonal/buffer partition coefficient of about 5.0. Its solubility in water is low, so that most injectable preparations are solutions of the sodium salt which are alkaline (Fig. 1) (Faigle & Dieterle, 1977). This low water solubility may be associated with the renal problems which can develop in dehydrated horses receiving this drug (Gunson, 1983).

Phenylbutazone exists in solution in three forms, a diketo, an enol, and a mesomeric anion form. In solution, it exists primarily in the diketo form and transformations between the forms are slow. These transformations probably contribute to its chemical instability and to the ability of the cyclo-oxygenase system to generate reactive intermediates and finally 4-OH-phenylbutazone (Reed *et al.*, 1985). Like other acidic drugs, phenylbutazone is highly protein-bound in plasma (Perel *et al.*, 1964), and is found in negligible quantities in equine saliva (Lambert & Kelly, 1978; Tobin, 1981). The acidic nature of phenylbutazone will tend to reduce its reabsorption from the renal tubules if the tubular luminal fluid is alkaline (Faigle & Dieterle 1977).

Because of its lipophilicity, phenylbutazone is extensively metabolized before it is excreted. In the horse, less than 2% is excreted in the urine as parent phenylbutazone over 24 h (Maylin, 1974; Lees *et al.*, 1983a). Some 25% of an intravenous dose is excreted in urine over 24 h as oxyphenbutazone (ring hydroxylation) and γ-hydroxyphenylbutazone (side-chain hydroxylation). In addition, the di-hydroxy metabolite (γ-hydroxyoxyphenbutazone) has been detected in equine urine (P. Lees and J. B. Taylor, unpublished data) in amounts that will add a few percentage points to the 24-h urinary excretion values (Fig. 2). Phenylbutazone has an elimination half-life of approximately 4.5 h, so that less



4-Butyl-1,2-diphenyl-3,5-pyrazolidinedione

Solubility

Phosphate buffer pH 7.0 (20°)	1.88 g/l
Methylene chloride	> 1.00 g/l

Partition coefficient

$$\left[\frac{C_{organic}}{C_{aqueous}}\right]$$

Methylene chloride / buffer pH 7.3	476
n-Octanol / buffer pH 7.4	5
Peanut oil / buffer pH 7.4	2.2

Acidity

pKa (water)	4.5

FIG. 1. Structure and physical characteristics of phenylbutazone.

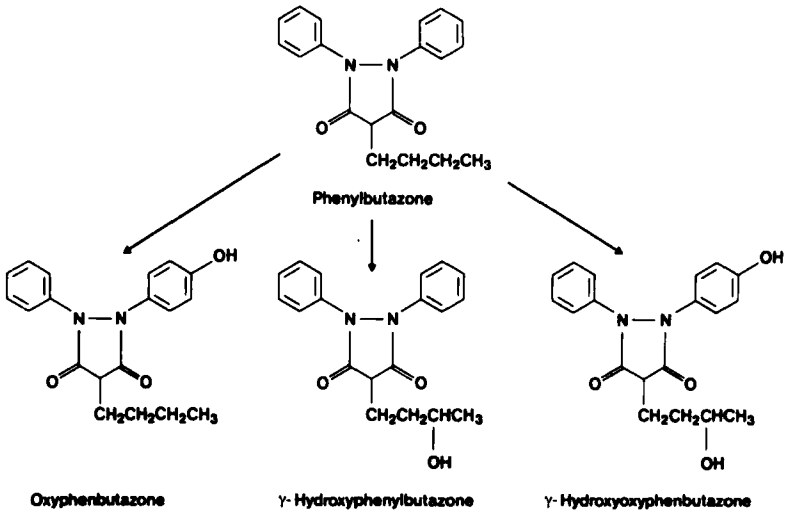


FIG. 2. Major metabolites of phenylbutazone in the horse.

than 2% of the dose should still be present in the body as unchanged drug after 24 h. There is, therefore, approximately 70% of the drug which has not been accounted for. Undoubtedly, a significant proportion of this will be excreted in urine as hydroxylated metabolites after 24 h, but another fate that has to be considered is biliary excretion of phenylbutazone and its metabolites, with or without subsequent reabsorption. Smith *et al.* (1985) have shown that 37% of an intravenous dose and 40% of an oral dose were detected in the feces. The rate of hepatic biotransformation and possible biliary excretion are probably the main factors determining the plasma half-life of phenylbutazone in the horse. No glucuronide metabolites have been reported from the horse. However, according to Reed *et al.* (1985) 4-OH-phenylbutazone is produced by the cyclo-oxygenase system, and either this metabolite or its glucuronide is therefore the potential metabolite in the horse. In this regard, it is worth noting that the 4-glucuronide of phenylbutazone has been reported in man (Dieterle *et al.*, 1976).

The plasma half-life of phenylbutazone in the horse is not affected by chloramphenicol or quinidine, but it is increased by oxyphenbutazone (Tobin *et al.*, 1977). The finding that oxyphenbutazone inhibits the metabolism of phenylbutazone in horses may explain the reports of dose-dependent kinetics for phenylbutazone in the horse (Piperno *et al.*, 1968). It is also possible that phenylbutazone itself saturates mixed function oxidase to inhibit its own metabolism.

The metabolite γ-hydroxyphenylbutazone exists in two interchangeable forms, the lactone and straight-chain forms. In the crystalline state in storage, the lactone predominates. In solution, the lactone form changes slowly to the straight-chain form* (Von Rechenberg, 1966). It appears likely that γ-hydroxyphenylbutazone found in the horse is the straight-chain form, but that change to the lactone form can occur during the drug testing process. These changes affect the R_f values of this metabolite in chromatographic systems (W. E. Woods, S. Chay and T. Tobin, unpublished data).

Phenylbutazone is chemically unstable. This creates problems in quantitative assay of this drug for forensic purposes, and with the running of relatively longer time-course experiments (Bellward *et al.*, 1972). Unfortunately, the degree of degradation of the drug

*G. Haas and K. Scheibli, Ciba-Geigy Ltd, Basel, Switzerland, personal communication (1984).

occurring under laboratory conditions has not been fully characterized (however, see Taylor *et al.*, 1981).

ANALYTICAL METHODOLOGY

As quantitative assays are used to regulate phenylbutazone in racing and eventing horses, the accuracy, reproducibility, and sensitivity of these assays are of considerable importance. The first assays for phenylbutazone employed ultra-violet spectrophotometry (Burns *et al.*, 1953). This method lacked sensitivity (> 10 μg/ml), and a further problem was that it did not distinguish between phenylbutazone and its hydroxylated metabolites.

Perego *et al.* (1971) reported a method for the determination of phenylbutazone by gas chromatography which was reproducible (standard error of less than 5%) and sensitive (lower limit of detection of 1 μg/ml from serum and urine). Although relatively specific for phenylbutazone, it was unsuitable for detection of metabolites. Other gas chromatographic methods were developed which had similar limitations.

By the early 1980s, high performance liquid chromatography (HPLC) methods were developed. They proved to be superior to previously reported methods. One of the earliest HPLC methods was that of Alvinerie (1980). Acidified plasma was extracted into *n*-hexane and analysed using a C-18 reverse-phase column with a UV detector absorbance of 240 nm. The lower limit of detection was 50–100 ng. Phenylbutazone extraction recovery was 65 ± 2%, while recovered oxyphenbutazone concentrations were lower and γ-hydroxyphenylbutazone levels were undetectable. Taylor *et al.* (1981) reported a method modified from Pound's work (Pound *et al.*, 1974) which employed a normal-phase column with a UV detector absorbance set at 240 nm. By increasing solvent polarity with tetrahydrofuran in the mobile phase and the flow rate to 100 ml/h, they were also to detect phenylbutazone, oxyphenbutazone and γ-hydroxyphenylbutazone simultaneously, with percentage recoveries of 100, 90, and 60, and limits of detection of 0.01, 0.05 μg/ml, and 0.1 μg/ml, respectively.

In the interlaboratory studies referred to in this review, the HPLC method usually used was based on that of Marunaka *et al.* (1980). With modifications in the extraction procedures, we were able to obtain recoveries of 99% (phenylbutazone), 88% (oxyphenbutazone), and 75% (γ-hydroxyphenylbutazone), with a lower limit of detection of 0.25 μg/ml (Chay *et al.*, 1984).

In these studies, there were no significant differences in the assay values between laboratories, and within-laboratory variance was less than 5%. However, the mean range between estimates from different laboratories was about 25%. Furthermore, when HPLC and electron capture data from one laboratory were compared, the HPLC data were found to be superior (Soma *et al.*, 1985). Nevertheless, despite the accuracy of the HPLC method, the reproducibility of the method is only fair. In a study of the factors contributing to analytical errors, McDonald (1982) determined that he could not conclude for forensic purposes that a plasma sample of phenylbutazone was greater than 2 μg/ml until the reading from his assay system was greater than 2.8 μg/ml.

Low resolution mass spectral analysis of phenylbutazone produces a base peak at m/e 77 and a strong molecular ion at m/e 308. The first neutral loss corresponds with loss of the butyl group, yielding a m/e 752 fragment. The next loss requires breaking of the pyrazolidinedione ring, to yield a fragment consisting of benzene rings, linked by the double nitrogen bridge. The other major peak at m/e 77 represents the phenyl rings(s).

Oxyphenbutazone shows a base peak at m/e 199 corresponding to the extra oxygen atom on the major diphenyl fragment, and a molecular ion at m/e 324. The first neutral loss is of m/e 43 corresponding with loss of a propyl group, and the next fragment of mass corresponds with the loss of a butyl group. The other major fragments at m/e 77 and m/e 93 represent the phenyl and phenoxy ions, respectively.

γ-Hydroxyphenylbutazone shows a spectrum with a base peak at m/e 183, and a small molecular ion at m/e 324. The first neutral loss of m/e 62, corresponding with the loss of a propyl alcohol group. Another small peak at m/e 252 corresponds with the loss of the butyl alcohol group. As with phenylbutazone, the benzene rings at m/e 77 are well represented.

ACTIONS AND MECHANISM OF ACTION

All classical NSAIDs probably share a common mechanism of action, and their therapeutic uses and toxic effects are also generally similar. Their actions are due to blockade of the cyclo-oxygenase enzyme system (Fig. 3). This enzyme utilizes arachidonic acid as a substrate to form, first, the cyclic endoperoxides PGG_2 and PGH_2, and then, the classical prostaglandins (such as PGE_2), prostacyclin (PGI_2) and thromboxane A_2 by the action of further specific enzymes (Moncada & Vane, 1979). Since certain prostanoids (notably PGE_2 and PGI_2) are important mediators of the vascular changes in inflammation (Ferreira *et al.*, 1974; Jones, 1977), inhibition of their formation suppresses, but does not abolish, the inflammatory response (Fig. 3). Inhibition of cyclo-oxygenase by phenylbutazone and aspirin is probably irreversible, whereas inhibition by oxyphenbutazone is thought to be reversible (Ku & Wasvary, 1973). The precise site and mode of action of NSAIDs are still unknown, but the two principal classes of agent, the carboxylic acids and enolic acids, *may* act on different enzymes, the former inhibiting cyclo-oxygenase and the latter blocking endoperoxide isomerase (Flower, 1974). On this hypothesis, one would predict that phenylbutazone would block the formation of PGE_2 but not of PGI_2 (Fig. 3). In fact, a therapeutic dose of phenylbutazone does block the synthesis of both compounds in the horse, so that final elucidation of its mode of action must await further studies (Higgins & Lees, 1984b).

Recent work on the inactivation of prostaglandin-H synthase and prostacyclin synthase by phenylbutazone has shown that phenylbutazone undergoes peroxide-dependent co-oxygenation (Fig. 4) catalysed by prostaglandin-H synthase. This work suggests

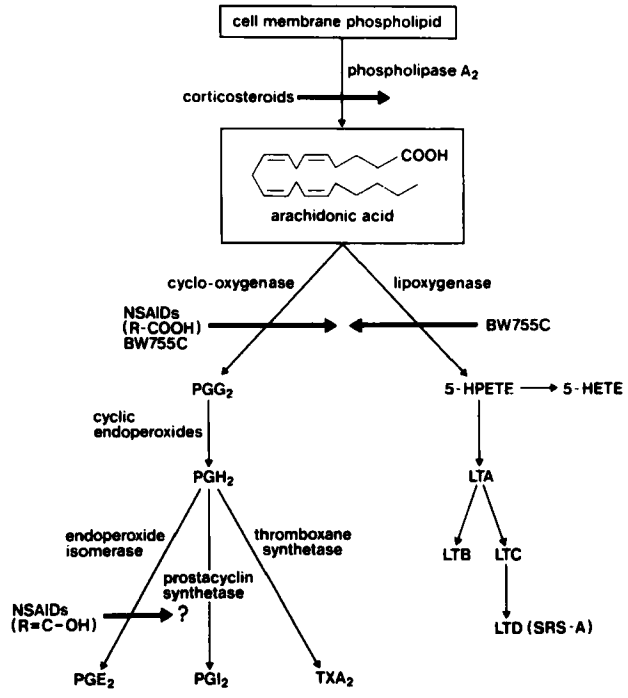


FIG. 3. Inflammatory mediators formed from arachidonic acid. Code: PG, prostaglandin; TX, thromboxane; LT, leukotriene; SRS-A, slow-reacting substance of anaphylaxis; HPETE, hydroperoxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid.

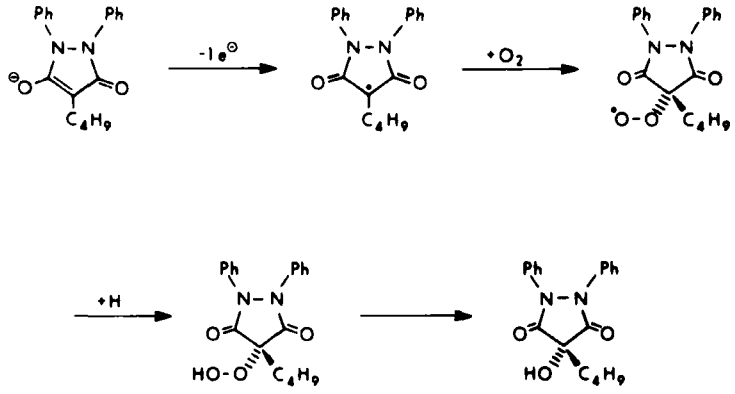


FIG. 4. Prostaglandin-H synthase conversion of phenylbutazone to reactive intermediates. Phenylbutazone donates a single electron to the peroxidase during hydroperoxide reduction. The resulting phenylbutazone radical traps molecular oxygen to yield a peroxyl radical, a phenylbutazone hydroperoxide, and eventually forms the stable end product, 4-OH-phenylbutazone (reproduced with permission from Reed *et al.*, *Molecular Pharmacology*, **27**, 109, 1985).

that it is an oxygenated derivative of phenylbutazone rather than the parent compound which leads to the inactivation of prostaglandin formation, by inactivating prostaglandin-H synthase and prostacyclin synthase. It appears that inactivation of prostacyclin synthase is probably the more clinically important inhibition (Reed *et al.*, 1985).

Newer compounds, such as BW755C, which inhibit both the cyclo-oxygenase pathways and lipoxygenase pathways of arachidonic acid metabolism, are now being developed (Fig. 3). It is likely that such dual inhibitors will be more effective anti-inflammatory drugs than single enzyme inhibitors, since at least one leukotriene (LTB_4) has been detected in inflammatory exudate in nanogram quantities (Simmons *et al.*, 1983; Higgins & Lees, 1984a). LTB_4 is an extremely potent chemotactic agent, and it may be an important mediator in chronic inflammatory conditions (Davidson *et al.*, 1983). NSAIDs inhibit the synthesis of prostaglandins and prostacyclin in many tissues and in many species, including the horse (Higgins & Lees, 1983). The potency of several NSAID analogs as anti-inflammatory agents *in vivo* correlates with their ability to inhibit cyclo-oxygenase *in vitro*. Moreover, stereoisomers of NSAIDs with widely different anti-enzyme activities vary similarly in their anti-inflammatory activities (Moncada & Vane, 1979).

Inhibition of prostanoid synthesis produces a spectrum of clinical and toxic effects. Prostaglandins of the E series produce erythema, while prostacyclin and PGE_2 also induce paw edema in rats. Prostaglandins and prostacyclin also sensitize blood vessels to the permeability effects of other inflammatory mediators, such as histamine and bradykinin, and the action of NSAIDs on edema is associated with blockade of this potentiation. In addition, prostaglandins possess the ability to produce hyperalgesia at the low concentrations in which they occur in inflammatory exudate. Furthermore, this hyperalgesic action of the prostaglandins can be cumulative, since it is related to both the duration and concentration to which tissues are exposed. As these actions of the prostaglandins are exerted locally, their inhibition by anti-prostaglandin drugs is also local. Therefore, NSAIDs do not produce analgesia in non-inflamed tissues (Moncada & Vane, 1979; Gilman *et al.*, 1980).

Prostaglandin E_1 is a powerful pyretic agent, and although it does not occur in animals, there is a general increase in the concentration of PGE-like substances such as PGE_2 in the CNS during fever. The NSAIDs inhibit both the generation of prostaglandins in the CNS and the fever caused by pyrogens or 5-hydroxytryptamine injected into the cerebral ventricles. They are all, therefore, anti-pyretic (Moncada & Vane, 1979).

When platelets aggregate, they release

thromboxane A2, a potent platelet aggregator. Formation of thromboxane A2 is blocked by NSAIDs reducing thrombosis and hemostasis (Meyers *et al.*, 1979). Some NSAIDs such as aspirin and phenylbutazone irreversibly inhibit platelet cyclo-oxygenase, and hence no new cyclo-oxygenase is formed during the life of that platelet. The effects of these drugs on platelet aggregation and hemostasis are therefore especially potent and long-lasting (Moncada & Vane, 1979). This action may, in part, explain the therapeutic benefit provided by phenylbutazone in navicular disease.

The side-effects of NSAIDs can also be explained by inhibition of prostanoid formation. Prominent among these effects is gastrointestinal irritation. Reduction of prostacyclin formation in the stomach is thought to lead to increased acid secretion and local vasoconstriction leading to tissue hypoxia, both of which may cause mucosal damage. However, the principal site of ulcers in phenylbutazone-treated horses seems to be not the stomach but the caecum and colon (*vide infra*)

The NSAIDs also cause varying degrees of nephrotoxicity, such as renal papillary necrosis. Sodium and water retention may also occur transiently. Prostaglandins are known to be natriuretic, and a result of interference with this action may be a retention of sodium and water. Analgesic nephropathy is not an uncommon toxic reaction to extensive NSAID use in the human (Moncada & Vane, 1979).

In summary, from their basic mechanism of action and effects in other species we may expect the NSAIDs to reduce erythema, edema, tissue swelling and the hypersensitivity to pain associated with inflammation in the horse. It is also predictable that these agents will affect platelet function and prolong bleeding times at high dose levels. The toxic effects are likely to involve the gastrointestinal tract and the kidney. In general, the clinical actions, side-effects, and toxicities of these agents follow these patterns in the horse (Tobin, 1979).

CLINICAL USES

The several clinical uses of phenylbutazone in veterinary medicine are encompassed by the phrase 'reduction of soft tissue inflammation'. Phenylbutazone can be administered to animals before surgery (Mansmann *et al.*, 1982) to animals with tissue tears of lacerations, or other tissue trauma (Oehme, 1962; Ebert, 1962), or to animals with soft tissue injuries associated with racing or other performance events. In all these instances, the inflammatory response is non-infective, so that phenylbutazone alone is effective. The drug may be useful in septic soft tissue inflammation as well, although it should be used in association with anti-infective (antibiotic) medication. Much of the early work with phenylbutazone clearly demonstrates this suppression of soft tissue inflammatory responses (Davidson & Franks, 1966; Tobin, 1981; Snow, 1983; Higgins & Lees, 1983).

Phenylbutazone and other NSAIDs have no direct effect on pain perception, but they reduce hypersensitivity to pain by reducing the inflammatory response. They have no effect on pain perception in non-inflamed tissues (Moncada & Vane, 1979). NSAIDs such as phenylbutazone are therefore effective against the dull throbbing pain of inflammation, but not against sharp stabbing pains, which are produced by the direct stimulation of sensory nerves (Gilman *et al.*, 1980).

These actions of phenylbutazone on pain perception have been experimentally demonstrated in the horse. Phenylbutazone had no effect on pain perception in normal cutaneous tissues (Fig. 5), a finding that is consistent with the concept that it acts to reduce the formation of prostaglandins only in inflamed tissues (Kamerling *et al.*, 1983).

The actions of phenylbutazone on soft tissue inflammation are of particular value in performance horses. In inflamed joints, more than 99% of the resistance to movement is due to soft tissue inflammation (Simon, 1981). Reduction of soft tissue inflammation largely eliminates this resistance, and thus largely restores normal joint function.

The basis for the action of phenylbutazone on degenerative joint disease and osteoarthritic joint disease is less clear. Phenylbutazone is unlikely to affect long-standing degenerative articular or bony change in or around joints that reportedly characterize most cases of 'racetrack' lameness. Further,

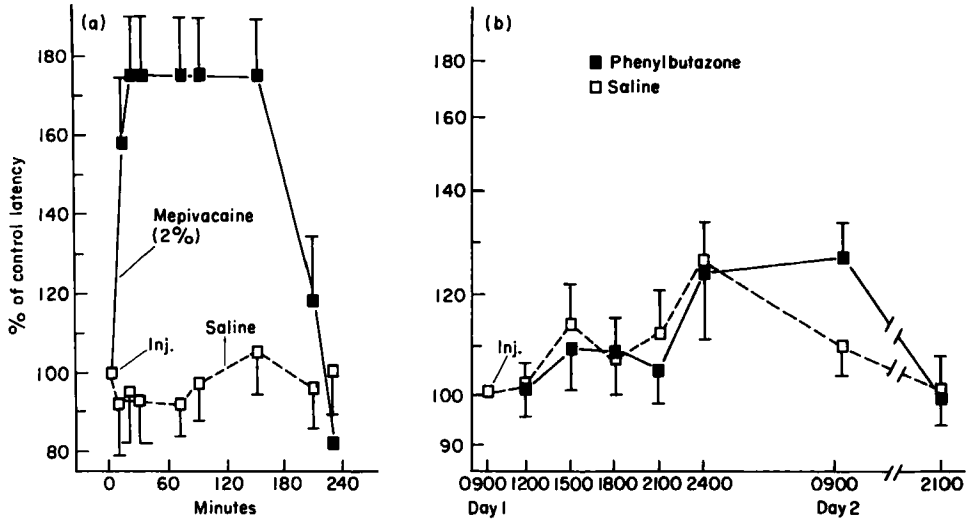


FIG. 5. Comparison of the effects of mepivacaine and phenylbutazone on hoof withdrawal reflex. Radiant light is focused on the coronary bands of horses and the time to hoof withdrawal measured. This time is called the latency, and is a measure of pain perception by the horse. (a) Effect of 5 liters of 2% mepivacaine injected as a palmar nerve block. The effects on pain perception are maximal within minutes and maintained for 3 h. The ceiling on the stimulus is an experimental constraint to prevent tissue injury. (b) Effect of 3.0 g phenylbutazone i.v., on the time of hoof withdrawal reflex in horses. (□–□) Effect of saline treatment. (■–■) Data for phenylbutazone. No significant difference was observed between the effects of phenylbutazone and saline in contrast with the marked effects of mepivacaine (Kamerling *et al.*, 1985).

there are no pain receptors in articular cartilage. Nevertheless, phenylbutazone is apparently useful in both osteoarthritic joints and degenerative joint disease. While these effects may be due to actions of phenylbutazone on the relatively permanent structural changes seen in these conditions, it seems much more likely that the therapeutic responses are attributable to effects of phenylbutazone on soft tissue changes associated with these conditions.

In man, phenylbutazone is particularly effective in the enthesopathies, in which tendon insertions become swollen and inflamed. Ankylosing spondylitis is the best known of these conditions. To our knowledge, enthesopathies are not recognized as a clinical entity in the horse.

In racetrack practice in the U.S.A., the principal use of phenylbutazone is as an adjunct in the training of 'sore' horses (Cannon, 1973). Horses with chronic arthritic and ligamentous problems also derive benefit. In some countries, the use of phenylbutazone to keep a horse in training and to enable it to race longer than in the absence of the drug is regarded as permissible, but others contend that it can never be acceptable to use phenylbutazone to allow an unsound horse to run. Some equine practitioners believe that horses race consistently on phenylbutazone, and that they race for longer (Cannon, 1973; Gabel *et al.*, 1977; Tobin, 1981). However, these views are controversial, and there is concern over possible hazards to the horse arising from this kind of prophylactic use.

Clinical conditions responding to phenylbutazone therapy include sore feet (pedal osteitis), cunean tendon bursitis (jacks), spavins, minor sprains and muscle soreness, splints, navicular disease, osselets and ringbones. Further, it is common practice for many horsemen to administer phenylbutazone after a race, as this is said to prevent the animals from 'cooling out sore'. Under these circumstances, when used with great care, phenylbutazone seems to be beneficial, and it may allow a horse to run up to his best form (Tobin, 1981).

A major concern regarding the use of phenylbutazone in performance horses relates to possible long-term effects on joints.

The joint damage that occurs in rheumatic disease is related to the intensity and duration of the inflammatory process, and this may be exacerbated by the stresses of racing and eventing. In principle, anything that reduces the inflammatory response should retard the rate at which joint deterioration occurs, and should prolong the period of useful joint function. In practice, however, there are no reports in human rheumatology that support this hypothesis, and this is due, at least in part, to the unpredictable course of most joint disease. There is a general belief, however, that while NSAIDs relieve the symptoms of chronic inflammatory joint diseases, in many they do not retard the rate of development of the organic disease process. Indeed, it is even possible that the progress of the disease may sometimes be hastened by treatment with NSAIDs; this might be due to 'substrate diversion', i.e. increased availability of arachidonic acid to lipoxygenase when cyclooxygenase is inhibited and a resultant increase in the production of compounds such as LTB_4 (Higgins & Lees, 1984a).

Hamm (1978) administered the NSAID naproxen to about 50 quarterhorse yearling colts during their training and racing careers. Treated horses lost only 3% of their training time, compared with a 13% loss for non-treated animals. Similarly, when the treated groups reached the racetrack, they raced more often than the control group, and their injuries were reduced dramatically, by four-fold during training and by 30-fold during racing. These results suggest that there may be a case for using anti-inflammatory medication in training and racing horses, but others consider the conclusion controversial.

Although limited, the available experimental evidence may also support the horseman's contention that phenylbutazone allows a horse to 'run up to its potential'. Studying the action of drugs on performance, Sanford administered phenylbutazone to four horses and tested them 24 h later (cited in Tobin, 1981). Sanford was surprised to find that performance was improved, and he could explain the improvement only by assuming that the horses, which he had considered sound, were actually subclinically lame. Similarly, J.-M. Jouany (personal communication, 1982) in France has proposed that small doses of phenylbutazone improve the performance of horses, supporting the data of Sanford. One possible interpretation of these findings is that there is no such thing as a completely 'sound' horse, and that the closest approximation to this ideal is a 'clinically sound' horse treated with phenylbutazone.

Phenylbutazone has been given to breeding mares for long periods with little apparent effect on their ability to conceive or to carry foals to term (Ellsworth *et al.*, 1983). It has also been used at high dose rates in the treatment of endotoxin shock, when it relieves the acute symptoms of the condition, but has little effect on the final mortality associated with this syndrome (Burrows, 1981).

Because of the widespread use of phenylbutazone in performance horses, its ability to influence performance, and the uncertainties surrounding the risks and benefits for the horse, the use of phenylbutazone in performance horses is a vigorously debated area. Differing views on the ethics of such usage have been presented by Scott-Dunn (1972), Hopes (1972), Moss (1972), Gerber (1984), Cannon (1974), O'Dea (1976) and Tobin (1981). In general, the use of phenylbutazone in performance horses is a matter for the regulators of individual sports, rather than a question in clinical medicine, although the possible long-term effects on the well-being of the horse is the concern of clinical veterinarians.

PHARMACOKINETICS

Bioavailability

Studies on the bioavailability of phenylbutazone are complicated by the fact that this agent may show dose-dependent kinetics. Classical bioavailability studies, comparing the area under the curve (AUC) after oral and i.v. administration, can only provide approximate values for bioavailability.

When phenylbutazone was given intramuscularly, the bioavailability was much lower and the rate of absorption slower than after oral administration (Sullivan & Snow, 1982). This was probably due to precipitation of the drug in the neutral pH of the muscle. Sullivan & Snow (1982) studied the factors affecting

absorption of phenylbutazone from the gastrointestinal tract. In general, access to feed reduced both the peak plasma concentration and the time to reach this concentration, both in thoroughbreds and in foals. Rose *et al* (1982) studied phenylbutazone following administration with a small bran mash or after a full feed. Prolongation of the time to peak concentration and reduction of AUC under full feed conditions were recorded. It should be noted that in both investigations plasma concentrations were monitored for 12 h only. In similar studies, Lees *et al.* (1983b) recorded a profound delay in the time of peak phenylbutazone concentrations in plasma in ponies allowed free access to hay. The peak level occurred after a mean time of 13.2 h. However, neither AUC nor peak concentration was significantly affected, and the findings of Rose *et al.* (1982) and Sullivan & Snow (1982) might therefore simply reflect the shorter sampling period adopted. Lees *et al.* (1983b) proposed that delayed absorption might be due to adsorption on to roughage in the feed, with subsequent release in the large intestine as a result of fermentative digestive processes. It was further suggested that such adsorption could have several consequences: it could explain why large doses of phenylbutazone generally produce ulcers in the caecum and colon rather than the stomach; it would lead to delay in the onset of the therapeutic action, and it would carry major implications for horses competing under FEI regulations, in view of the 4 μg/ml concentration in plasma upper limit set by this authority. Subsequent *in vitro* studies have shown that phenylbutazone adsorption on to hay does occur (J. B. Taylor, P. Lees and A. J. Higgins, unpublished data).

Comparing the bioavailability of paste and powder preparations, Snow & Douglas (1983) concluded that the bioavailability was greater for the paste, and that a paste preparation would, therefore, more readily produce toxicity. In similar studies, Rose *et al.* (1982) using higher doses of phenylbutazone reported on the bioavailability of paste and powder preparations. Absorption of the drug by the oral route was very variable, and readily affected by feeding. One horse in Rose's group of four showed consistently less than 25% bioavailability for the drug, no matter which dosage form was used. No explanation for this phenomenon was offered.

Little is known about the bioavailability of phenylbutazone preparations marketed in North America. Soma *et al.* (1983) reported about 90% bioavailability of phenylbutazone in their kinetic studies using Jen-Sal phenylbutazone. Chay *et al.* (1984) reported lower plasma levels of phenylbutazone after dosing with one commercial preparation as compared with the Jen-Sal product, which might be due to bioavailability differences. According to Maitho *et al.* (1986), approximate bioavailability values for orally administered phenylbutazone were 69% and 77%, in fed and fasted ponies, respectively. These authors used a powder formulation. Breed differences in phenylbutazone absorption may also occur. Sullivan & Snow (1982) recorded higher AUC values in thoroughbreds than in ponies, but unpublished studies (P. Lees and J. B. Taylor) in our laboratory have shown the reverse effect.

Plasma kinetics

The first pharmacokinetic study on phenylbutazone in the horse was that of Piperno *et al.* (1968) (Tables I and II). Using a relatively non-specific analytical method, they reported on the disposition of phenylbutazone and oxyphenbutazone in a mixed group of thoroughbreds, standardbreds and quarterhorses. The plasma half-life of phenylbutazone was reported to be 3.5 h at the clinically recommended dose of 2 g/1000 lb i.v., increasing to about 6 h when the dose of phenylbutazone was 8 g/1000 lb i.v. Piperno also noted that the plasma levels of phenylbutazone did not increase in proportion to the dose administered; thus, doubling of the dose from 4.4 to 8.8 mg/kg increased the 1-h plasma level by 33%, but quadrupling the dose to 17.6 mg/kg raised the plasma concentration by 300%. Piperno *et al.* (1968) also compared the plasma half-life of phenylbutazone in horses with acidic (pH 5.2) and alkaline (pH 8.3) urines; plasma half-life was not affected by urinary pH. This finding is not unexpected, since binding to plasma protein (> 99%) greatly limits the amount of

TABLE I. Pharmacokinetic parameters for phenylbutazone following single dose administration

	Dose (mg/kg)	Route of administration	$t_{1/2}$ (h)	Elimination rate constant (h)	Volume of distribution (V_d) (l/kg)	Cl_B total (ml/kg/h)	$C_{p\ max}$ (μg/ml)	t_{max} (h)
Piperno *et al.* (1968)	4.4	i.v.	3.5					
	13.2	i.v.	6.0					
Jenny *et al.* (1979)	6.0	i.v.	6.2	0.112	0.24	27.6		
Rose *et al.* (1982)	8.9	i.v.	4.3	0.1568	0.25	41.95		
Gandal *et al.* (1969)	6.5	i.v.	7.0					
Tobin *et al.* (1977)	6.6	i.v.	5.46					
Gerring *et al.* (1981)	1.1	Oral					1.2	4
	2.2	Oral					2.8	12
	4.4	Oral					4.5	4
	6.6	Oral					8.6	12
	8.8	Oral					17.5	2
	13.2	Oral					20.6	10
Maitho *et al.* (1986)	4.4	i.v.	4.71	0.325	0.164	26.1		
	4.4	Oral*					9.5	3.8
	4.4	Oral†					11.9	13.2
	4.4	Oral‡					11.8	5.9

* Partial access to food (fasted before, access after dosing).
† Full access to food (access before and after dosing).
‡ No access to food (fasted before and after dosing).

TABLE II. Pharmacokinetic parameters for phenylbutazone following multiple dose administration

	Dose	Route of administration	$t_{1/2}$ (h)	Bioavailability (%)	$C_{p\ max}$ (μg/ml)	t_{max} (h)	Volume of distribution (V_d) (l/kg)	Cl_B (ml/kg/h)
Norheim *et al.* (1978)	2.5 g 2 g × 3	i.v.	10.9					
	6 g 4 g × 3	i.v.	7.56					
	6 g 4 g × 4	i.v.	8.32					
Maylin (1974)	4.4 mg/kg × 4	i.v.	5.1 1st dose 5.3 2nd dose 5.5 3rd dose 6.1 4th dose					
Soma *et al.* (1983)	8.8 mg/kg × 5	Oral × 4 i.v. × 1	6.2	91.8	61.1	24 h post i.v. injection	0.152	16.8

phenylbutazone passing into glomerular ultrafiltrate and hence into urine, irrespective of urinary pH.

The plasma and urinary concentrations of phenylbutazone and its metabolites after repeated intravenous dosing for 4 days with 4.2 mg/kg were reported by Maylin (1974). Peak plasma levels increased from approximately 18 μg/ml on Day 1 to about 31 μg/ml on Day 4. Maylin also reported small increases in the plasma half-life of the drug with repeated doses, although the increases he observed were not as large as those observed by Piperno with increasing dose rates.

More recently, Soma *et al.* (1983) has reported on the kinetics of phenylbutazone in four standardbred and two thoroughbred mares. The dose rate was 8.8 mg/kg, and this was administered orally for 4 days and intravenously on the fifth day. The apparent plasma half-life, which was approximately 6.2 h did not change over the 4-day period of the experiment. However, the mean residual plasma level of phenylbutazone at 24 h after dosing increased from about 1.7 μg/ml on Day 1, to 5.3 μg/ml on Day 4. This confirms the earlier report of Maylin (1974). No changes in the urinary phenylbutazone concentrations occurred in Soma's study, but the concentrations of oxyphenbutazone and the γ-hydroxy metabolite increased. Soma concluded that the kinetics of phenylbutazone were not dose dependent 'in the therapeutic range'. Lees *et al.* (1985) administered phenylbutazone (4.4 mg/kg) intravenously to six Welsh Mountain ponies. In three 3-year-old ponies, plasma clearance was almost twice as rapid as in three ponies aged 8–10 years. These authors reached the tentative conclusion that age might significantly influence phenylbutazone clearance, although they recognized that more detailed studies were required. If aged horses do eliminate phenylbutazone more slowly than younger animals, they could also be much more susceptible to the drug's toxic effects, and it might be appropriate to recommend lower dose rates for older animals.

Another factor that may influence the plasma clearance of phenylbutazone is breeding (Chay *et al.*, 1984). Experiments on phenylbutazone kinetics performed in thoroughbred and standardbred horses versus half-bred ponies showed only one-third the residual plasma levels of phenylbutazone at 24 h after dosing in ponies, as compared with thoroughbred and standardbred horses. This finding may indicate that ponies are able to metabolize phenylbutazone more quickly than thoroughbred or standardbred horses.

In summary, the plasma half-life reported for phenylbutazone in the horse has ranged from 3.5 to 10.9 h, increasing with the administered dose. The half-life is probably determined by biliary secretion and hepatic metabolism, and only small amounts are excreted unchanged in the urine. The kinetics of phenylbutazone may be age dependent, but further studies are required to clarify this possibility.

Plasma protein binding

Phenylbutazone is usually reported to be highly bound to plasma protein in mammalian blood (at least 98%). Gandal *et al.* (1969) reported that binding was about 96% at a total plasma concentration of 69 μg/ml, while Gerring *et al.* (1981) found that therapeutic doses of phenylbutazone produced at least 98% binding. Similarly, Maitho *et al.* (1986) were unable to detect any free drug in plasma when the concentration was less than 27 μg/ml. Only in the 60-min period following intravenous dosing (4.4 mg/kg) did the unbound concentration exceed 0.5% of the total. Lambert & Kelly (1978) reported similar binding in horse serum albumin. They considered that there were four high affinity binding sites and 1.5 low affinity binding sites for phenylbutazone on the serum albumin molecule, but they did not report the percentage binding. Most plasma protein binding studies with phenylbutazone have been undertaken with methods of marginal accuracy (Aarbakke, 1978; Bellward *et al.*, 1972), so that accurate comparison of the degree of binding between species is difficult. Nevertheless, it is clear that phenylbutazone binding in horses is essentially similar to that in other species.

Plasma residues of phenylbutazone

With the increasing awareness of phenyl-

butazone's narrow safety margin in the horse, and worldwide concern about the administration of drugs to performance horses, a number of studies to determine residues of phenylbutazone in the plasma and urine of performance horses have been carried out. The first of these was carried out by the Veterinary Chemists Advisory Committee to the National Association of State Racing Commissioners, in 1980. Forty-nine horses received a dose rate of 8.8 mg/kg orally each day for 3 days, followed by 4.4 mg/kg i.v. on the fourth day (Tobin, 1981). The mean plasma level at 24 h in these horses was 4.1 μg/ml, and the distribution was log-normal, so that one horse in 1000 would be expected to have a 24-h plasma level of 24 μg/ml. The wide intersubject variation in plasma values obtained in this experiment was at that time unexpected.

In another study performed about the same time, Gerring *et al.* (1981) dosed thoroughbreds, hunters, and polo ponies with 8.8 mg/kg of phenylbutazone daily in two doses for 4 days, followed by 4.4 mg/kg in divided doses for the next 4 days, and then 2.2 mg/kg for the subsequent 3 days. As in the NASRC study, considerable animal-to-animal variation in plasma levels occurred, and the time to reach peak plasma levels after oral dosing also varied considerably. While the mean residual plasma level at 24 h after the first day of dosing was 4.4 μg/ml, by the end of the high dose period the peak blood levels had risen to 20 μg/ml and the 24-h level was 10 μg/ml. When the dose was reduced to the 4.4 mg/kg level for 4 days the mean plasma level on the fifth day was approximately 4 μg/ml, and when the dose was reduced to 2.2 mg/kg the mean residual (24-h) plasma level fell to less than 1 μg/ml. From this data, it was predicted that one horse in 20 would exceed the 4 μg/ml limit set by Federation Equestre Internationale (FEI) for phenylbutazone.

In a recent study in the U.S.A., 53 horses were dosed with 2 g/1000 lb phenylbutazone 24 h before racing, no phenylbutazone having been administered 48 h previously, and doses of phenylbutazone which approximated 2 mg/1000 lb were administered 72 h before post time. The residual 24-h plasma levels in these horses were about 0.9 μg/ml, and the population distribution was normal. A statistical projection suggested that the plasma level residue for one horse in 1000 dosed with this schedule would exceed 2.2 μg/ml at 24 h (Table III) (Soma *et al.*, 1985; Houston *et al.*, 1985).

In a second study in the U.S.A., 34 horses were dosed with 2 g phenylbutazone at 48 h, and approximately 2.3 g 24 h prior to racing. The mean serum level in these horses was 4.1 μg/ml immediately before racing, and the distribution was log-normal. A statistical projection from this data suggested that one horse in 1000 might be expected to have a serum level of about 28.2 μg/ml of phenylbutazone (Houston *et al.*, 1985; see also Soma *et al.*, 1985).

In a third (Keystone Racetrack) study in the U.S.A., 43 horses received 2 g/1000 lb i.v. each day for 4 days. The mean plasma level 24 h after the final dose was 4.75 μg/ml, with a maximum value of 9.9 μg/ml. The data were log-normally distributed, and it was predicted that one horse in 1000 would have a plasma level of 16.2 μg/ml (Soma *et al.*, 1985; Houston *et al.*, 1985).

In a fourth study undertaken on 62 horses in training, a dose rate of 8.8 mg/kg phenylbutazone was administered orally for 3 days, and this was followed by 4.4 mg/kg i.v. on the fourth day. The mean plasma level 24 h after the final dose was 5.32 μg/ml. The population distribution was again log-normal, and a statistical projection indicated that one horse in 1000 would have a plasma level in excess of 23.5 μg/ml (Table II) (Chay *et al.*, 1984). A survey of plasma and urinary phenylbutazone plus metabolites was undertaken in 200 horses racing in Kentucky (U.S.A.) in 1983, there being no restriction on the use of phenylbutazone at the time of the study (Houston *et al.*, 1985). The levels were broadly similar to those obtained after dosing with phenylbutazone following a no-race-day medication rule. In particular, the mean level of phenylbutazone (4.5 μg/ml) was less than that reported from the Keystone study.

All of these plasma residue studies must be interpreted in the light of reports that phenylbutazone absorption is considerably delayed in horses permitted free access to hay. Lees *et al.* (1983b) and Maitho *et al.* (1986) found that t_{max} values were 5.9, 3.8 and 13.2 h, respectively, in six ponies with no access, partial

TABLE III. Comparison and statistical projection from data on horses running in Kentucky and California and 'no-race-day medication rule' studies

Phenylbutazone*	Range (μg/ml)	Median (μg/ml)	Mode (μg/ml)	Mean (μg/ml)	5% (μg/ml)	1% (μg/ml)	0.1% (μg/ml)
Kentucky (post-race)	0.20–15.00	2.52	1–2	3.49	11.07	15.8	35.8
Keystone (i.v.)	1.5–9.88	4.00	3–4	4.75	8.8	11.8	16.2
Keenland (oral-i.v.)	1–14	5.16	2–3	5.32	10.3	18.6	23.5
California (serum)	0.44–9.97	3.65	3–4	4.09	10.45	16.78	23.18
Florida (serum)	0.27–1.80	0.76	1–2	0.94	1.62	1.90	2.21

* All data reported here refer to drug or drug metabolite concentrations from these studies as assayed by the Kentucky Equine Drug Testing and Research Programs. Unless otherwise noted, the data refer to plasma levels of drugs.

access and full access to hay, and the mean 24-h plasma levels in these horses, each of which received a single dose of 4.4 mg/kg orally, were 0.9, 1.8 and 2.8 μg/ml. The profound delay in absorption in hay-fed ponies was attributed to phenylbutazone adsorption on to the feed with ultimate release in the large intestine. *In vitro* studies using both chopped hay and equine gut contents have shown that a high level of adsorption does in fact occur (P. Lees, A. J. Higgins and J. B. Taylor, unpublished data).

Urinary residues and effect of pH

Piperno *et al.* (1968) were the first to demonstrate that urinary pH does not affect the plasma half-life of phenylbutazone. Subsequently, Moss & Haywood (1973) showed that the ^{14}C from [^{14}C]phenylbutazone appeared initially at high concentrations in basic urines, but persisted for longer in acidic urines. More recently, Houston *et al.* (1983) have shown that urine pH greatly affects the concentrations of phenylbutazone and its metabolites in post-race urines from horses racing in Kentucky. As the pH of urine samples increased from 4 to 8.5, the concentrations of phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone increased 200-, 65- and 30-fold, respectively. There were no corresponding changes in the plasma concentrations. These data are most readily interpreted in terms of the classic ion trapping theory, which assumes that acidic compounds will remain in basic urines because the ionized form of the drug is less lipid-soluble and hence is not reabsorbed.

Since only 1–2% of an administered dose of phenylbutazone is excreted unchanged in the urine over 24 h, these effects have little influence on the plasma half-life of phenylbutazone in the horse, as suggested by Piperno *et al.* (1968), Maylin (1974), Gerring *et al.* (1981) and Lees *et al.* (1985).

Relationship between plasma and urinary concentrations of phenylbutazone and its metabolites

During the 1970s, some racing authorities regulated the use of phenylbutazone by quantitating the concentrations of 'phenylbutazone and its metabolites' in post-race urines. Most commonly, the maximum permitted level was set at 165 μg/ml of phenylbutazone plus its metabolites. In addition, there were also reports that estimates of the time of administration of the last dose of phenylbutazone could be made from the ratios of the different metabolites to the parent drug in the urine samples (Gabel *et al.*, 1977). More recently, however, Soma *et al.* (1985) showed that there was no correlation between plasma and urinary levels of phenylbutazone and its metabolites, further supporting the findings of Houston (1985) and Tobin (1979, 1981). In spite of this, some authorities continue to regulate the use of phenylbutazone by means of quantitation in urine.

'Detection times' for phenylbutazone

It can be readily calculated that about 77 elimination half-lives are required to eliminate completely a dose of phenylbutazone from 99% of horses. This complete 'clearance time' is approximately 26 days, depending on the actual half-life of phenylbutazone in the individual horse (Tobin *et al.*, 1982). In performance horses, a number of factors affect the time for which phenylbutazone can be detected ('detection time'). One factor is the sensitivity of the analytical method used. When using a sensitive method, the longest reported 'detection time' is 7 days for both plasma and urine (Norheim *et al.*, 1978). Using less sensitive methods, other workers reported detection times which ranged from 24 to 96 h (Tobin, 1979). Urinary pH can also exert a marked effect on the period for which phenylbutazone and its metabolites can be detected in urine. As shown by Moss & Haywood (1973), urine pH can extend the period of detection from 24 to 72 h. Because of this effect, the Veterinary Chemists Advisory Committee of the National Association of State Racing Commissions (U.S.A.) has suggested that plasma is the fluid of choice for regulating the use of phenylbutazone.

Plasma and exudate levels of phenylbutazone and therapeutic effects

Estimates of the therapeutic plasma levels

of phenylbutazone in man have ranged from 50 to 150 μg/ml. However, some workers have suggested that there is no relationship between the plasma levels and toxic effects of this drug in man (Aarbakke, 1978).

It is clear that the therapeutic levels of phenylbutazone in equine plasma are much less than this, probably being of the order 5–20 μg/ml (Gerring *et al.*, 1981; Jenny *et al.*, 1979; Gabel *et al.*, 1977). The reason why much lower plasma levels are effective in the horse is unclear, although this might be due to species differences in the structure of cyclo-oxygenase, the enzyme in equine tissues possibly having a low inhibitory constant (Lees & Higgins, 1985). Estimation of the concentration of phenylbutazone in plasma at one point in time may only have limited value in predicting the level of therapeutic effect according to Lees & Higgins (1985). These authors pointed out that the concentration of both phenylbutazone and its active metabolite, oxyphenbutazone, was initially greater in plasma than in inflammatory exudate after intravenous administration of phenylbutazone. By 12 h, however, the concentration ratio (plasma : exudate) was less than unity, and after 24 h it was even smaller. The slower decline in exudate concentration relative to plasma concentration has implications for therapeutic efficacy; it may partially explain why efficacy persists when plasma concentrations have fallen to very low levels. According to Lees & Higgins (1985) the varying plasma : exudate ratio (initially greater than and subsequently less than one) also makes the interpretation of plasma concentration data much more difficult in regard to competitive equine sports. From the plasma concentration at a single time-point, it is not possible to say whether concentration at the site of action will be greater or less than the plasma level. This observation is consistent with that of Piperno *et al.* (1968) which implied saturation of a receptor site.

Since some authorities consider that horses may legitimately train on phenylbutazone, but may not, at least in some jurisdictions, race on pharmacologically effective levels of phenylbutazone, the concentrations required for therapeutic effects of phenylbutazone are of considerable practical importance. It is likely that this field will be investigated in the near future, and researchers will have to bear in mind that phenylbutazone is converted to an active metabolite, oxyphenbutazone, and that an inconsistant relationship exists between plasma and inflammatory exudate concentrations for both the metabolite and the parent compound (Lees & Higgins, 1985).

Interference or masking by phenylbutazone

In the U.K. and some other countries, no level of phenylbutazone in racehorses is permitted in any fluid. In North America, a major concern regarding the approval of phenylbutazone for use in racing horses is the possibility that phenylbutazone or its metabolites can interfere with or 'mask' the detection of other drugs in post-race urine samples. Moreover, it has been suggested that horsemen may deliberately administer 'high' levels of phenylbutazone to mask or interfere with the detection of other drugs (Houston *et al.*, 1983).

While the concept of masking is well established in the racing community and has considerable forensic currency, there is virtually no published scientific literature on this subject. More recently, however, studies in this area have been initiated.

In a survey of the effectiveness of illegal drug detection in America, Woods *et al.* (1985b) compared the number of 'positive call rates'. The medication rules of these jurisdictions were divided into those which did not allow any level of phenylbutazone, those permitting approximately 2 μg/ml, and those allowing even higher plasma concentrations. In order to ensure accurate comparison of the 'positive call rates' from jurisdictions with differing definitions of illegal medication, only those drug classes (stimulants, depressants, tranquilizers, narcotic analgesics and local anesthetics) illegal in all jurisdictions were included. The results indicated that jurisdictions that did not permit any level of phenylbutazone had a mean positive call rate of 1.3 for each 1000 samples tested. On the other hand, jurisdictions that allowed the use of phenylbutazone also have a positive call rate for drugs of 1.3/1000 samples tested.

In another approach to the 'masking' problem, Woods *et al.* (1985a) used thin layer

chromatography to investigate the ability of phenylbutazone and its metabolites to interfere with the detection of the 55 drugs classified as illegal in North American racing since 1980. In these experiments, oxyphenbutazone did not extract in detectable concentrations, and γ-hydroxyphenylbutazone either co-migrated with phenylbutazone or was also undetectable. Hence, only phenylbutazone had any potential to interfere with illegal drug detection. It was found that the only drug that could have been obscured was phenothiazine. Since phenothiazine is an anthelmintic with no behavioral effects, its abuse potential is negligible. Moreover, phenothiazine is readily distinguishable from phenylbutazone by its reaction with visualization reagents. It was concluded that phenylbutazone and its metabolites had virtually no ability to interfere with the detection of illegal drugs under the conditions of testing in Kentucky (Woods *et al.*, 1985a).

ADVERSE REACTIONS

Until 1979, phenylbutazone was considered to be virtually non-toxic to horses. During 20 years of clinical use, there had been only occasional reports suggesting that its use might be linked to low PCV and hemoglobin concentrations, intestinal ulceration, fatal hemorrhage, depression, and shock (Gabriel & Martin, 1962; Gabel *et al.*, 1977; Jefcott & Colles, 1977; Roberts, 1981; Chandler, 1979).

In 1979, Snow *et al.*, (1981), however, reported that ponies receiving doses of 8–14 mg/kg phenylbutazone daily for 7–14 days showed clear signs of phenylbutazone toxicosis, and three of eight ponies died. Similar but less detailed findings were reported by Wanner *et al.* (1980) at about the same time. Subsequently, several groups have undertaken similar studies, and a well-defined syndrome of phenylbutazone toxicity in the horse has now been established.

Currently available evidence suggests that toxicity arises when manufacturers' recommended doses are exceeded. In general, the recommended dose of phenylbutazone in North America is not more than 4 g/450 kg orally/day* for not more than 5 days. Some manufacturers recommend a maximum dose of 2 g/horse/day by the intravenous route, and this is now the recommended dose of the American Association of Equine Practitioners. A similar dosage schedule was recommended by one British manufacturer (8.8 mg/kg daily for 4 days followed by a reducing dosage) (Lees & Gerring, 1983). However, toxicity occurred with this dose rate, and this group therefore recommended that a loading dose of 8.8 mg/kg on Day 1 should be followed by a dose level of 4.4 mg/kg for 4 days and 2.2 mg/kg thereafter (Taylor *et al.*, 1983). Ponies may be more susceptible to the toxic effect of phenylbutazone than thoroughbred horses (Sullivan & Snow, 1982), but further studies are required to clarify this suggestion.

The initial signs of phenylbutazone toxicity are inappetence and depression, and decrease in body-weight. As death approaches, the extremities are cold, heart rate increases, and rectal temperature is reduced. These are the classical signs of hypovolaemic shock. Signs of toxicity can persist for weeks after cessation of treatment. Death can occur weeks after treatment in some animals (Snow *et al.*, 1981).

The most sensitive biochemical indicator of toxicity is a decrease in total plasma or serum protein, caused by plasma protein-losing enteropathy. Snow *et al.* (1981) demonstrated that animals receiving high doses of phenylbutazone lost ^{51}Cr-labelled plasma protein into the gut at a rate three to four times higher than control horses. Other changes in clinical chemistry include decreases in serum urea nitrogen and plasma calcium, and increases in plasma creatinine and phosphate.

Necropsy findings are dominated by the intestinal lesions. In Snow's ponies, these ranged from shallow erosions to massive intestinal ulceration in the colon and the caecum. Microscopically, the surface and glandular mucosa of the erosions were necrotic, and the lamina propria was exposed. Large numbers of bacteria were present in the necrotic tissue, and there was perivascular hemorrhage, infiltration with polymorphonuclear leukocytes and thrombosis of submucosal venules. Lesions in the stomach were only occasionally seen. These findings can

*Unless otherwise noted, a horse is assumed to weigh 450 kg.

possibly be explained by phenylbutazone binding onto feed, providing protection for the stomach and small intestine, and release of the drug by fermentative digestion in the large intestine (Lees & Higgins, 1985). Snow's experiments with ^{51}Cr-labelled albumin suggested that loss of protein could occur into the gastrointestinal tract without grossly apparent damage.

In experimental animals, bile duct ligation considerably reduces the intensity of intestinal lesions, suggesting that it is the presence of phenylbutazone directly in the gastrointestinal tract that causes the toxic effects (Duggan *et al.*, 1975). Similarly, it is thought that the oral ulcers observed in the horse are associated with oral administration of the drug and its direct actions on the oral mucosa (Snow *et al.*, 1981). However, high doses of phenylbutazone given intravenously can also cause gut lesions in the horse (Meschter *et al.*, 1984).

McKay *et al.* (1983), MacAllister (1983) and Read (1983) have confirmed Snow's findings and additionally reported renal papillary necrosis. The severity of the latter lesion, which appeared as brownish-green, wedge-shaped segments of renal papillary necrosis under the renal crest of papillae in four of the six horses receiving high dose rates of phenylbutazone, was dose related (McKay *et al.*, 1983). Micropathology showed these to be areas of coagulation necrosis, with loss of cellular definition. Similarly, the medullary-connecting tubules showed swelling, necrosis, and sloughing of tubular epithelial cells. Tubular casts and lymphocytic cortical infiltrations were inconsistently present. Some degree of clinical renal failure was associated with these lesions in all horses.

Renotoxicity of phenylbutazone has been reported in other species. For example, renal damage is a consistent finding in phenylbutazone-treated rats, and similar lesions have been demonstrated in the dog and the cat (Kincaid-Smith & Fiariley, 1971). A comparable clinical and pathological syndrome in man which often follows abuse of NSAIDs is analgesic nephropathy.

In a retrospective study of renal papillary necrosis in 16 horses, Gunson (1983) and Gunson & Soma (1983) reported that the changes were associated with the administration of phenylbutazone and concomitant dehydration in the affected horses. Phenylbutazone treatment alone, or water deprivation alone, did not give rise to renal papillary necrosis. This presumably accounts for signs of renal papillary necrosis not being seen in other studies (Snow *et al.*, 1981).

While the renal lesions occurring in horses are not severe and would not normally be life threatening, they may lead to the loss of renal tissue and impairment of the horse's ability to concentrate urine. It is also possible that they might cause renal colic, as occurs with renal lesions in man.

McKay *et al.* (1983), Gerber (1984) and Snow & Douglas (1983) also reported neutropenia and a toxic left shift in horses given large doses of phenylbutazone (10–30 mg/kg). McKay *et al.* (1983) further showed in bone marrow aspirates that there was a severe depletion of marrow and circulating neutrophils. Because of the rapid onset of this effect and the lack of substantial change in the bone marrow cell morphology, McKay *et al.* (1983) suggested that the effect involved more than simple bone marrow suppression of neutrophilic production. A possible contributory factor could be absorption of endotoxin from the damaged gut. Unlike the idiosyncratic bone marrow suppression seen with phenylbutazone in man, this effect appeared to be dose related and reversible.

In a study on the toxicity of phenylbutazone in foals, Traub *et al.* (1983) administered 10 mg/kg phenylbutazone orally to foals aged between 3 months and 10 months. They described a significant increase in ulcers in the glandular portion of the stomach and the oral cavity. However, the principal site of damage seemed to be the colon.

In a study on the effects of phenylbutazone on electrolyte balance in ponies, Alexander (1982) reported that sodium and chloride retention occurred. Such side-effects have been described in other species. In addition, plasma pH, bicarbonate, and total carbon dioxide decreased. While Alexander felt that these findings were sufficient to render the use of this drug in performance horses unwise, others have questioned this conclusion on the ground that the changes were small and toxicologically insignificant (Lees & Gerring, 1983).

Necrotizing phlebitis has been reported in phenylbutazone-treated horses. This occurs in the portal veins when the drug is given orally, and jugular thrombophlebitis and pulmonary arterial thrombosis are seen in horses treated with i.v. phenylbutazone (McKay *et al.*, 1983; Gabriel & Martin, 1962). Meschter *et al.* (1984) described degeneration of the wall of small veins in thoroughbreds receiving high doses of i.v. phenylbutazone (13.6 mg/kg daily for 3 days). The phlebopathy, which involved venular dilatation, hyalin degeneration and phlebothrombosis, was considered to be a primary change, and other toxic effects were judged to be secondary to the vein lesions. Lees *et al.* (1983a), on the basis of serum enzyme increases (aspartate aminotransferase, sorbitol dehydrogenase, glutamate dehydrogenase) in a single horse, speculated that phenylbutazone can produce dose-dependent hepatotoxicity in the horse. Enzyme levels were raised during 4 days of treatment with 8.8 mg/kg, but decreased towards normal levels when the daily dose was reduced.

The fact that these toxic lesions are related to the action of phenylbutazone as a cyclo-oxygenase inhibitor is supported by the observation that administration of prostaglandin E_2 prevented the appearance of most of the signs of toxicity seen in ponies (Collins & Tyler, 1984a).

In summary, it is now clear that early workers underestimated the toxicity potential of phenylbutazone. When given at high dose levels, even for short periods, cumulation, and hence toxic effects, can rapidly and readily occur. Toxicity appears as inappetence, melena, depression, mouth ulcers, diarrhea and possibly abdominal edema (Gerring *et al.*, 1981; Collins & Tyler, 1984b). If the drug is being administered in food, the condition tends to be self-limiting, since the animal will refuse to eat after a few days. If dosing is maintained, however, more serious toxicity and death may occur. The sequence of events usually involves gastrointestinal ulceration and a resulting loss of plasma leading to hypovolemic shock. If the animal's access to water is restricted, renal lesions may also occur. Alternatively, the cause of death may be related to colic, and the intestinal changes may lead to septic (endotoxic) shock. If the drug is withdrawn at the first signs of toxicity, the chances of complete recovery are good. However, signs of toxicity can persist for weeks after the drug has been withdrawn (Snow *et al.*, 1981).

A major difficulty in evaluating reports of toxicity induced experimentally by phenylbutazone is that plasma levels of phenylbutazone and oxyphenbutazone producing these effects are unknown. Because of uncertainties about the ability of phenylbutazone to accumulate under the conditions of these experiments, the plasma levels producing these toxic responses and their relationship to the levels likely to be found in horses on therapeutic regimens of the drug are not known. Careful evaluation of the pharmacokinetics of phenylbutazone under conditions where cumulation and toxicity occurs is needed. Also, the contribution that the dose-dependent pharmacokinetics of phenylbutazone makes to toxicity should be evaluated. Even now, however, it is possible to speculate that the dependence of phenylbutazone half-life on hepatic metabolism, the posssiblity that therapeutic doses saturate the enzyme system involved in metabolism, and the further possibility that phenylbutazone is hepatotoxic could lead to a vicious circle of cumulation and toxicity.

While phenylbutazone is a drug with a narrow therapeutic index, it should be noted that none of the toxicity studies has led to suggestions that the doses of phenylbutazone recommended for use in North America need to be changed. In the U.K., however, the loading doses of this drug recommended by one manufacturer were reduced from 4.4 mg/kg twice daily to once daily for 4 days (Taylor *et al.*, 1983). Similarly, the American Association of Equine Practitioners has recommended that the dose for racing horses be not greater than 2.2 mg/kg i.v. each day, with the last dose occurring not more than 24 h before post time (Harvey, 1983). Under these conditions, and with due precautions to ensure proper hydration, phenylbutazone should continue to be a safe and effective medication in the horse. Clinical experience over many years certainly suggests that moderate doses can be given over prolonged periods without inducing clinically detectable side-effects.

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