Available online at www.sciencedirect.com
SCIENCE DIRECT®
The Veterinary Journal 167 (2004) 129–142
The Veterinary Journal
www.elsevier.com/locate/tvjl

Current research and theories on the pathogenesis of acute laminitis in the horse

Simon R. Bailey [a], Celia M. Marr [b,*], Jonathan Elliott [a]

[a] Department of Veterinary Basic Sciences, Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, UK
[b] Veterinary Clinical Sciences, Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, UK

Accepted 1 July 2003

Abstract

A large number of studies have been undertaken in recent years aimed at furthering our understanding of the complex mechanisms underlying the common and debilitating condition of acute laminitis in the horse. Many of these studies have either reinforced or cast doubt on previously held theories on the pathogenesis of this disease, while others have suggested new mechanisms which may play a key role in its development. This review seeks to put the current hypotheses into the context of this recent body of evidence. While a unifying theory may not yet seem to be achievable, this review demonstrates that most of the current theories are not mutually exclusive. Studies utilising in vitro and in vivo models of the disease, particularly addressing the areas of inflammation, haemodynamic disturbances and enzyme activation in the hoof, as well as the preceding events occurring in the hindgut, have helped to explain many clinical observations of the disease and may possibly lead to more effective therapies and means of prevention in the future.

Keywords: Horse; Laminitis; Pathogenesis; Ischaemia; Amines

1. Introduction

Laminitis continues to have widespread implications for equine welfare. This has been highlighted by a recent survey in the US which found that 13% of horse owners/operations reported problems with laminitis in their horses over the previous 12 months (USDA, 2000). Similarly, a survey involving 113,000 horses in the UK found a prevalence of 7.1% (Hinckley and Henderson, 1996). Although it is accurate to say that the precise causes of acute laminitis in the horse are not yet fully understood, and there are still a number of different theories as to the pathogenic mechanisms involved, this is a fast moving field of research in which a large amount of recent work has improved our understanding of this common condition.

The pathogenesis of acute laminitis, in particular its link with disturbances of the hindgut following access to lush pasture, has been a subject of fascination and frustration ever since horses have been kept on 'improved pastures'. There are many other potential factors that may contribute to the onset of this condition, nevertheless, pasture-induced laminitis appears to be the most common aetiology in the UK (Hinckley and Henderson, 1996). It has become increasingly apparent that allowing horses and ponies access to excessive amounts of certain types of carbohydrate is likely to trigger changes in the hindgut precipitating this disease. The trigger factor(s) released from this process have yet to be elucidated, however various proposed candidates are now being investigated, and more knowledge has been gathered about the biochemical and functional responses within the foot which these mediators may initiate. In addition, significant advances have been made in the understanding of the form and function of the tissues of the foot, particularly the dermal and epidermal lamellae.

* Corresponding author. Tel.: +44-7717-431-749; fax: +44-1707-652090.
E-mail address: celia.marr@rossdales.com (C.M. Marr).

1090-0233/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S1090-0233(03)00120-5

Despite this, some fundamental questions remain to be answered in terms of the mechanisms leading to the development of acute laminitis. Among these questions are: (1) With so many predisposing factors and apparent clinical manifestations, can there be a single unifying theory relating to the pathogenesis of acute laminitis? (2) Why does the pathology of this disease appear to be localised to the lamellar structures of the foot? (3) What trigger factors are released from the hindgut (or septic foci such as metritis) which initiate the pathological changes to the lamellar structure? and (4) What factors (managemental or genetic) are involved in apparently predisposing certain individuals to the condition?

This review seeks to summarise, and put into context, some of the more recent research carried out into the pathogenesis of equine laminitis, in an attempt to address the above questions. Much of this research has used experimental models of laminitis, which must be born in mind when interpreting the results. Models include inducing hindgut fermentation induced by carbohydrate overload in the form of corn starch (Garner et al., 1975), and recently also inulin (Pollitt, 2002), as well as orally administered black walnut extract, whose mode of action is not yet fully understood (Eaton et al., 1995). These models are used because the prodromal stage of the naturally occurring disease, when mediators are released and the key pathogenic events take place, is not clinically recognisable and, once in the acute stages, a very different set of secondary processes takes over (Hood et al., 1993). Inducing laminitis in this way may produce such a marked effect on the hindgut contents and mucosa that the resulting systemic changes may not precisely mirror the naturally occurring disease, although the final pathogenic pathway in the foot may be the same. It is important to remember that acute laminitis is a systemic disease which is only finally manifest as a condition of the foot (Hood, 1999a), although the changes in circulating mediators in the naturally occurring disease may be much more subtle and hard to characterise compared to the changes recognised in association with some experimental models (Moore et al., 1981).

2. Foot pathology

The lamellar structures, lying between the pedal bone and the hoof wall, have inevitably proved very difficult to study, in terms of visualising or measuring the pathological processes taking place in the developmental and acute stages of laminitis in the live horse. Indirect measurements of blood flow and inflammatory mediators in the live horse have been widely applied (Hood et al., 2001; Hoffmann et al., 2001a), although relating the results of these studies to the processes occurring at the level of the secondary lamellae has proved difficult.

Histological analysis of sections of lamellar tissue at different stages of the disease provides snapshots of the pathology involved (Hood et al., 1993). Few histological changes have been noted to occur in the developmental phase prior to the onset of clinical signs; among them has been the presence of platelet microthrombi (Weiss et al., 1994, 1995). Evidence of systemic coagulopathy in this disease does not, however, seem to be apparent (Weiss et al., 1996; Prasse et al., 1990), although endothelial cell dysfunction or injury within the digital vasculature could lead to localised platelet adhesion (Vanhoutte, 1991). Platelets may release various vasoactive mediators when activated, such as 5-hydroxytryptamine (5-HT; serotonin) and thromboxane A_2, therefore their activation within the digit would certainly be consistent with impaired local perfusion. Furthermore, prevention of platelet activation using fibrinogen inhibitors has been shown to prevent laminitis (Weiss et al., 1998a). These findings strongly indicate that platelets are involved in the pathogenesis of the condition, although if platelet activation does occur through potential trigger factors such as endotoxin, one would expect to see a systemic coagulopathy. Therefore, it seems likely that the microthrombus formation may occur secondary to earlier activation or damage to the digital endothelium.

The fact that there does not appear to be a persistent inflammatory infiltrate of the vessel wall in either the developmental or early acute phases of laminitis had previously led to the assertion that this was not primarily an inflammatory condition. However, new evidence suggests that inflammation may play an early role in the pathogenesis after all. In situ hybridisation has demonstrated the expression of interleukine (IL)-1β in small perivascular pockets of cells early in the prodromal phase (Fontaine et al., 2001). The exact cell type(s) involved have not been determined, but one could speculate that these were infiltrating white blood cells. Differential upregulation of cyclo-oxygenase (COX)-2 mRNA has also been described, as well as the expression of other inflammatory signalling molecules in venous vascular smooth muscle cells (Waguespack et al., 2002). Little is yet known about the factors stimulating this upregulation, although they could include reperfusion injury and toxins such as endotoxin.

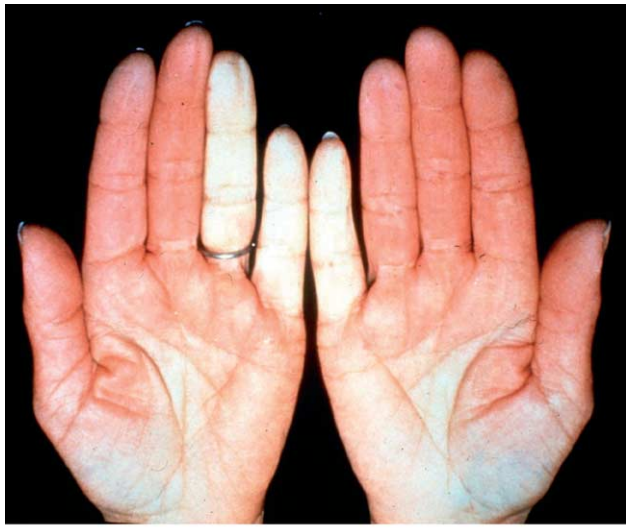
The histological changes in the laminar tissues seen following the onset of lameness are consistent with ischaemia of the tissues followed by reperfusion injury (Hood et al., 1993). Reperfusion injury may result from the sudden generation of oxygen free radicals following re-oxygenation of ischaemic tissues (Flaherty and Weisfeldt, 1988), and can lead to severe damage to phospholipid cell membranes, including disruption of the endothelial barrier protecting against neutrophil and platelet adherence to the blood vessel wall (Verrier, 1996). In the ischaemic lung, myocardium, skeletal

muscle and brain, activation of matrix metalloproteinase enzymes (MMPs) occurs as a result of reperfusion injury (Etoh et al., 2001; Yano et al., 2001; Jiang et al., 2001). The fact that hoof heat and bounding digital pulses are characteristic of the acute phase of laminitis (Stashak, 1987) and that the onset of clinical signs coincides with an increase in hoof wall surface temperature (Hood et al., 2001; Pollitt and Davies, 1998) is consistent with a reactive hyperaemia of the digit following ischaemia/reperfusion injury.

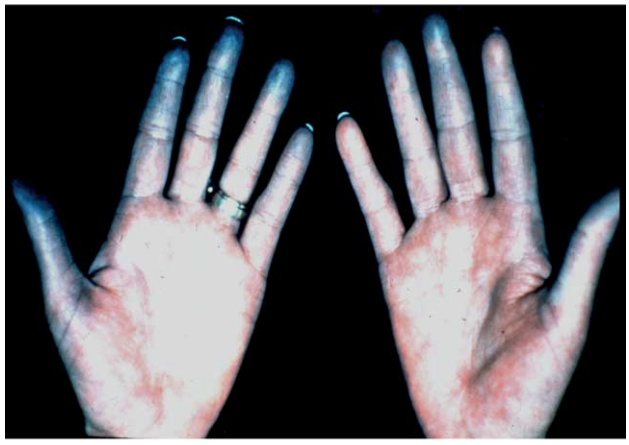
As already noted by others, acute laminitis bears many similarities with the human condition of Raynaud's phenomenon (Hood et al., 1990). This is a relatively common peripheral vascular condition, involving ischaemia/reperfusion of the digits (see Figs. 1a and b), brought on by cold as well as other factors. Vasoconstrictors, such as 5-HT and endothelin, as well as vasodilators such as calcitonin gene-related peptide (CGRP), have been implicated in its pathogenesis (Biondi et al., 1988; Bunker et al., 1996) and repeated episodes lead to changes to the vascular architecture and the connective tissues (Burch et al., 1979). As with laminitis, much about the key pathogenic mechanisms remains enigmatic, and treatment usually relies on vasodilator drugs (Wigley and Flavahan, 1996; Cerinic et al., 1997).

It has been shown that MMPs are activated in the developmental and acute phases of laminitis (Johnson et al., 1998; Pollitt et al., 1998). It appears that these zinc-containing proteases are responsible for the active destruction of the basement membrane which bonds the interlocking lamellar leaflets, leading to the loss of structural integrity of the attachment between pedal bone and hoof wall. Thus, these enzymes are the key effectors causing the clinical manifestations of pedal bone displacement, which have such serious implications for the prognosis of affected cases. MMP-9 activity in particular has been localised to the basal/parabasal epithelial cells of the epidermal lamellae (Mungall et al., 1998).

As with the induction of IL-1β and COX-2, it has yet to be confirmed whether MMP activation in acute laminitis, is specific to the digit or represents a systemic tissue response. There are a number of structural and functional features of the equine digit which may make it susceptible to selective damage in laminitis. Many unique features of the digital circulation mean that it is finely balanced between maintenance of capillary flow and excessive capillary pressure, therefore perhaps predisposing the lamellae to ischaemic injury (Moore and Allen, 1996). Its capillaries are particularly leaky to proteins, predisposing to increases in interstitial tissue pressure (Allen et al., 1988). MMP enzymes are important in normal tissue remodelling in many parts of the body other than the digits. However, the basal epithelial cells of the epidermal lamellae, which produce these enzymes, may be particularly sensitive in terms of glucose requirement, which might account for their selective activation (Pass et al., 1998).



(a)



(b)

Fig. 1. Appearance of human digits during the prodromal phase of Raynaud's phenomenon. Initially one or more fingers become blanched due to vasoconstriction, then they turn blue as they become progressively ischaemic. The return of blood flow to the fingers is associated with pain, heat and bounding pulse. (Picture courtesy of Professor C. Black, Royal Free Hospital, London.)

These findings, taken as a whole, suggest that vasoconstriction, possibly accompanied by an inflammatory stimulus, lead to local platelet aggregation and disturbances in nutrient blood flow, which cause injury to the lamellar epithelium, possibly due to its normally high metabolic rate, resulting in MMP activation (Fig. 2). Further studies integrating the evaluation of these different processes at different times during the developmental phase is required to adequately address the issue of cause and effect.

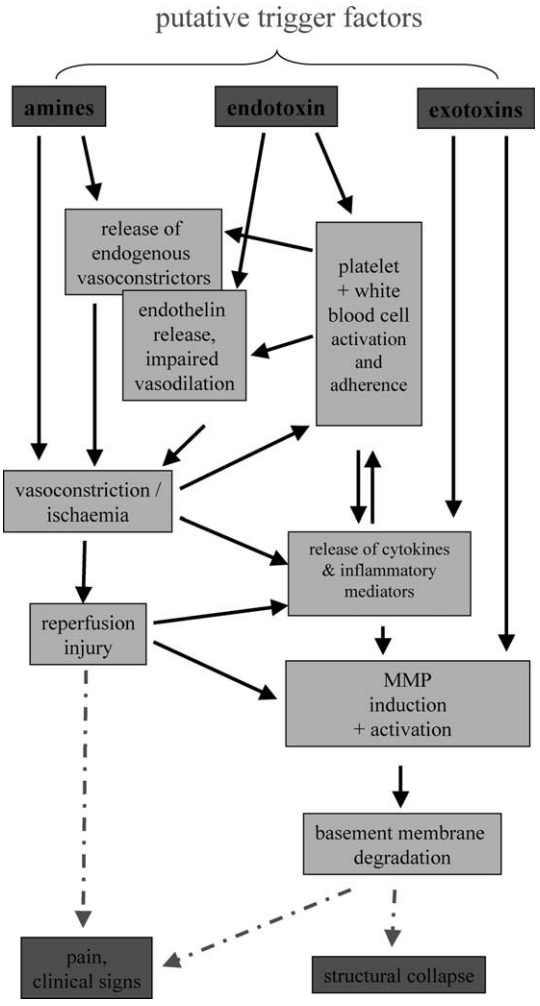


Fig. 2. Interaction between possible pathogenic pathways involved in the developmental phase of acute laminitis associated with different trigger factors. There are many areas of cross talk possible between these pathways at every level, demonstrating that they are not mutually exclusive.

3. Changes in digital haemodynamics

The results of studies using different techniques in an attempt to quantitate blood flow in the lamellar tissues of the live horse, following the experimental induction of laminitis, have proved equivocal although many well conducted studies have shown a period of hypoperfusion during the developmental phase. The apparently incompatible results may be largely due to the complexity of the digital vascular anatomy. The vascular supply is very different to other parts of the body; operating at very high hydrostatic pressures (Hunt, 1991), tending to be maintained under a degree of tonic vasoconstriction rather than vasodilation (Bailey and Elliott, 1998a; Elliott, 1997), and with capillaries relatively permeable to large molecular weight compounds (Allen et al., 1988). Intimately associated with the axially arranged arterioles and venules of the lamellar circulation are a large number of arteriovenous anastomoses (AVAs; Molyneux et al., 1994). These play a role in thermoregulation, periodically opening to increase the blood flow and warm the lamellar tissues (Mogg and Pollitt, 1992). Therefore, overall blood flow may not reflect the nutrient supply to the lamellar capillaries, if blood is passing through the AVAs at the expense of capillary flow.

In order to distinguish effective capillary perfusion from AVA shunt flow, it is necessary to use tools such as radiolabelled microspheres, which pass through AVAs but get trapped in capillaries. Studies have shown considerable amounts of AV shunt flow using technetium-99m tagged macroaggregated albumin, but in terms of the changes seen in the early stages of laminitis, these studies have produced equivocal findings (Trout et al., 1990; Hood et al., 1978). Aggregated albumin particles tend to be variable in size, some clogging in AVAs (Robinson, 1990), and as it is not possible to repeat measurements frequently, transient changes may be missed.

Techniques such as the pump-perfused digit preparation have enabled the measurement of capillary and interstitial fluid pressures in the digits of horses in the prodromal phase of experimentally induced laminitis (Allen et al., 1990), although the necessity for general anaesthesia and severing of nerves to the limb will have some major effects on baseline values, possibly causing the dilation of arteriovenous shunts. The very high capillary pressures (over 50 mm Hg) were suggestive of high vascular resistance from the venous side of the circulation, which may predispose to fluid accumulation in the interstitium and capillary collapse (Eaton et al., 1995).

Non-invasive or minimally invasive methods have enabled blood flow to be measured in the standing horse during the course of the disease. Laser Doppler flowmetry has been used to measure lamellar tissue microvascular flow following black walnut-induced acute laminitis (Adair et al., 2000) and hoof wall surface temperature has also been validated as an accurate indicator of subcapsular blood flow and used to follow perfusion in the experimental induction of laminitis by carbohydrate overload (Hood et al., 2001). In both of these separate studies using different techniques, a period of digital hypoperfusion was documented during the prodromal phase, between 12 and 8 h prior to the onset of clinical signs. A similar study by Pollitt and Davies (1998), however, did not document a significant decrease in hoof wall temperature during this phase but did note a hyperaemia at the onset of clinical signs.

Although it is difficult to interpret the discrepancies in the results of these various studies, the majority of experiments specifically measuring flow to the area of the laminae or measuring resistance vessel function within the foot, point to a period of vasoconstriction at some

time during in the developmental phase. However, these changes may be masked by increased shunt flow or the different times that the measurements were made relative to the onset of clinical signs.

At present, it seems likely that the most accurate methods for measuring subcapsular blood flow are likely to be the most invasive and that by their nature they are likely to disturb blood flow themselves. Technological advances in microvascular blood flow measurement and imaging, both invasive and non-invasive, may be able to answer these questions in time. For example, near infrared spectroscopy is another potentially useful tool which has been used for the measurement of tissue perfusion in the foot, since it provides an index of oxygenated haemoglobin or reduction–oxidation status. The technique has been validated in a small number of animals (Hinckley et al., 1995), but has not yet been applied to time-related changes in tissue oxygenation during the prodromal phase of natural or experimentally induced laminitis.

4. Changes in the hindgut leading to acute laminitis

The link between the ongoing processes in the equine hindgut and the pathological mechanisms in the digit are probably crucial to the understanding of the pathogenesis of this disease. Knowledge of the changes taking place in the hindgut contents and its mucosa will enable the development of preventive treatments and will provide the ability to give more effective information to clients regarding preventive measures.

Carbohydrate overload, in the form of corn starch administered orally, has been the most commonly used experimental model of this disease to study the changes during this critical period (Garner et al., 1975). Allowing the hindgut bacteria access to fermentable carbohydrate results in overgrowth of Gram-positive bacteria, with resulting production of lactic acid plus other factors which are yet to be fully characterised (Garner et al., 1977; Garner et al., 1978; Bailey et al., 2002a). However, it has not been shown whether the same changes in hindgut flora are found during naturally occurring, pasture induced laminitis, and so this may not necessarily represent a fair comparison with the sort of carbohydrate ingestion causing laminitis in the field. Furthermore, the marked clinical signs and changes in the hindgut flora induced using this model may actually complicate investigations and obscure some of the key pathogenic pathways of laminitis. For example, it was hypothesised that endotoxin was the single factor triggering acute laminitis, since laminitis is a common sequel to certain forms of colic (Hunt et al., 1986) and endotoxin could be detected in the plasma of horses given carbohydrate overload (Sprouse et al., 1987). Subsequently, studies administering endotoxin to horses have failed to induce laminitis (Clark and Moore, 1990) and in other models of experimental laminitis, no endotoxin was detected (Eaton et al., 1995). Therefore, the role of endotoxin in laminitis has been brought into question (Hood, 1995), although it could nevertheless play a facilitatory role. Indeed, the acute intravenous administration of endotoxin does cause a profound decrease in digital blood flow (Ingle-Fehr and Baxter, 1998), although the resulting pattern of vascular resistance was not the same as that observed in anaesthetised horses with laminitis (Allen et al., 1990), suggesting perhaps that endotoxin, if involved, is not acting alone.

Fructans, a group of fructo-oligosaccharides of varying molecular size and branching structure, are produced as a storage carbohydrate in grasses, and levels increase under climatic conditions favouring photosynthesis over growth (Longland and Cairns, 1998; Longland et al., 1999). These conditions (bright sunshine during the day, with cool nights) are similar to those associated with increased incidence of pasture laminitis (Katz et al., 2000). The carbohydrates do not appear to be digested/absorbed by the small intestine and, therefore, are present as a fermentable substrate for hindgut bacteria (Potter et al., 1992). Recently, fructans have been shown experimentally to induce laminitis (Pollitt, 2002). Although studies have not yet been published correlating the threshold amount required to cause laminitis with the likely intake expected of normal animals on lush pasture, it is quite conceivable that intake of large amounts of lush grass at certain times would cause a significant change in hindgut pH in some individuals, as a result of carbohydrate fermentation.

The administration of an aqueous extract of black walnut (*Juglans nigra*) heartwood also consistently causes laminitis under an experimental conditions (Eaton et al., 1995), with no endotoxin being detected in the plasma (although the methods used to measure endotoxin may not have been sensitive enough to pick up low concentrations). It is still unclear what component of this extract causes laminitis and by what mechanism. Blood flow to the foot appears to decrease (Galey et al., 1990a) and, although the extract does not have a direct effect on digital blood vessel contractility, it may impair endothelial function and augment the response to other mediators (Galey et al., 1990b; Holm et al., 2002a). Recent evidence also suggests that black walnut extract causes an inflammatory response in the colonic mucosal epithelium, accompanied by oedema and haemorrhage, an effect which may increase intestinal permeability (McConnico et al., 2002).

Several studies have documented damage to the hindgut mucosa in experimentally induced laminitis (Kreuger et al., 1986; Masty and Stradley, 1991). Intestinal permeability increased shortly after the administration of carbohydrate overload in vivo (Weiss et al., 1998b) and in vitro when colonic mucosa was exposed to

caecal contents incubated with carbohydrate (Weiss et al., 2000). These effects have been attributed to the low pH and/or lactic acid produced from carbohydrate fermentation, or changes in osmolality of the caecal contents. Thus, factors that increase intestinal permeability, such as acidic conditions generated by carbohydrate fermentation, irritants such as black walnut extract or mucosal damage following ischaemia, by allowing mediators from the hindgut contents to leach into the systemic circulation, may be the critical first step in causing laminitis. Indeed, it may not be the intraluminal production of trigger factors per se which is the initiating event in acute laminitis, rather, their rate of access to the systemic circulation, which determines the outcome of the gastrointestinal insult.

It is attractive to speculate, therefore, that one factor which may provide a common mechanism linking naturally occurring cases of laminitis (grass-induced; those occurring secondary to gastrointestinal diseases leading to stasis and/or ischaemia in the bowel) and the well characterised experimental models of laminitis (carbohydrate overload; ingestion of black walnut) is a change in intestinal mucosal permeability. If significant changes in hindgut pH, bacterial flora, and by-products of bacterial metabolism occur in naturally occurring causes of laminitis, as they do in the experimental models, subsequent changes in hindgut permeability, if not gross mucosal damage, might result, allowing access of preformed trigger factors to the systemic circulation.

A number of mediators have been recently proposed as trigger factors for laminitis, being produced in the equine hindgut contents and causing the events described in the digit. The absence of a systemic inflammatory response and pathological changes in other tissues in some forms of laminitis suggests that whatever factors are released from the hindgut may have a direct or selective effect on the digit; therefore they must gain access to the peripheral circulation, overwhelming uptake processes in the liver and other clearance mechanisms. What their mode of action might be remains unclear, but it is presumed, from what is known about the pathology, that their effects could lead directly or indirectly to the impairment of blood flow through the lamellar tissues and/or the local induction and activation of MMP enzymes. It should be stressed that these different mechanisms may be closely inter-related, and are not mutually exclusive (Fig. 2).

Monoamines, formed from the decarboxylation of various amino acids by bacteria, have been proposed as possible trigger factors (Bailey et al., 2000). By mimicking the effects of endogenous amine mediators such as serotonin and the catecholamines (epinephrine, norepinephrine and dopamine) and stimulating their receptors, they may cause vasoconstriction (Rang and Dale, 1995). These compounds have previously been implicated in vascular conditions in man, where their presence in fermented foodstuffs, such as cheese and wine, can lead to migraine or hypertension in some individuals (Shaw et al., 1978; Gonsalves and Johnson, 1977; Blackwell and Marley, 1966). A large number of different monoamines, such as tyramine, tryptamine and phenylethylamine, have been identified in the caecal contents of horses (Bailey et al., 2003a). The production of some of these compounds was increased when caecal contents were incubated with carbohydrate sources in vitro (Bailey et al., 2002a), and the intravenous infusion of individual amines into normal horses decreased blood flow to the digits (Bailey et al., 2002b) at dose rates which were without effect on systemic arterial blood pressure. Thus, although direct evidence showing that monoamines formed in the caecum can induce laminitis when infused into the systemic circulation is lacking, several pieces of experimental data provide strong support for these compounds playing an important role in the pathophysiology of laminitis.

Exotoxins are factors secreted by bacteria, as opposed to endotoxins, which are integral components of their cell walls. They include protease enzymes, which can cause direct destruction of mammalian tissues at septic foci. Moreover, some proteases have been shown to activate endogenous MMP enzymes, which in turn can break down collagen (Okamoto et al., 1997). A comprehensive analysis of the exotoxins produced in equine large intestinal contents has yet to be carried out, although broth cultures of streptococci and other bacteria show MMP activating capabilities when directly incubated with laminar tissues (Mungall et al., 2001). Although this hypothesis is an attractive one, a compelling explanation as to why these exotoxins should target the basement membranes of laminar tissue has yet to be put forward. Furthermore, activation of MMPs could equally well be explained by ischaemia of the laminae followed by reperfusion.

5. Actions of putative trigger factors and other mediators in the digit

Often the interpretation of findings from in vivo studies, particularly those involving experimentally induced laminitis or endotoxaemia, is made difficult by the complex interactions between multiple pathways and inflammatory cascades occurring in the whole animal. Differentiation of mediators playing a key central role from ones of minor or peripheral importance is challenging with such a complex system. Thus, the usual principles of deductive reasoning have proved to be hard to follow. However, the use of simpler models, such as isolated tissue preparations, has revealed a great deal about the basic physiology of laminar tissues. Such an approach has allowed inductive reasoning,

building hypotheses based on these basic physiological mechanisms.

Isolated rings of digital arteries and veins, maintained in oxygenated physiological salt solutions and connected to isometric force transducers, have been used to characterise the receptors mediating changes in blood flow within the digital circulation. In this way, α-adrenoreceptors (Elliott, 1997), 5-HT receptors (Bailey and Elliott, 1998b) and endothelin receptors (Katz et al., 2003a) have been characterised. The catecholamines, 5-HT and endothelin represent some of the most potent vasoconstrictors of the equine digital circulation and, therefore, may be expected to play a role in the vasospasm and ischaemia thought to initiate laminitis. The potencies of these endogenous vasoconstrictors are shown in Fig. 3.

The smooth muscle cells of equine digital arteries and veins possess a large number of different receptor subtypes for these mediators. For example they have both α_1 and post-junctional α_2 adrenoreceptors mediating vasoconstriction (Elliott, 1997), as well as both 5-HT_2 and 5-HT_1 receptors (Bailey and Elliott, 1998b). Thus, the digital blood vessels are extremely sensitive to vasoconstriction mediated by these receptors; indeed, digital arteries seem to be in the region of 30–40-fold more sensitive to the vasoconstrictor actions of 5-HT that other peripheral blood vessels (Bailey and Elliott, 1998a). In addition, the monoamines, which are produced by bacteria in the equine hindgut, cause constriction of digital arteries and veins in vitro by stimulating 5-HT and α-adrenoceptors (Berhane et al., 2002). Their effects seem to be more potent in veins than arteries, a finding which may be consistent with the selective venoconstriction described in haemodynamic studies of laminitis (Allen et al., 1990). Thromboxane and 5-HT, two mediators released by activated platelets, work synergistically to produce vasoconstriction which is partially selective for the venous side (Bailey, 1998), a finding which could be consistent with platelet involvement in laminitis.

Endothelin (ET), a small peptide mediator released from the vascular endothelium onto the smooth muscle, is another potent vasoconstrictor in this vascular bed (Katz et al., 2003a), and its increased expression has been observed in laminitis (Katwa et al., 1999). When administered into the lateral digital artery, it causes a decrease in digital arterial blood flow (Holm et al., 2002b), and in vitro evidence has been shown that ET_A receptors mediate these effects (Katz et al., 2003a), with veins being more sensitive than arteries (Katz et al., 2003a; Holm et al., 2002c). In the pump-perfused extracorporeal digit model, the post-capillary vasoconstriction observed in the developmental stages of experimentally induced laminitis could be reversed using an endothelin receptor antagonist (Holm et al., 2002d). The link between the processes occurring in the hindgut and the release of endothelin is as yet unclear, although following black walnut administration, plasma endothelin levels rise in the digital circulation within about 5 h (Holm et al., 2002e). In some vasospastic diseases in man, such as Raynaud's phenomenon, it has been suggested that endothelin is released following the initiation of vasoconstriction by other causes and is responsible for maintaining and augmenting the vasospasm (Zamora et al., 1990). Whether shown to be a primary or secondary factor in laminitis, endothelin antagonists may potentially be useful for its treatment or prevention in the future.

Counteracting the effects of vasoconstrictor mediators in the equine digit are several vasodilator substances, of which one of the most important is nitric oxide (NO). Basal release of NO from the endothelium appears to be low in the digital circulation (Bailey, 1998), although it is generated in response to acetylcholine, bradykinin or substance P (Elliott et al., 1994; Cogswell et al., 1995; Katz et al., 2003b), mediating a vasodilatory effect (Fig. 4). Intravenous infusion of the substrate for NO production, L-arginine, has been shown to produce vasodilation in the foot of one horse, with an increase in the oxygenated haemoglobin concentration; furthermore, the local topical application of glyceryl trinitrate, a NO donor, gave clinical improvement in 10 ponies with laminitis, with a reduction in systemic blood pressure (Hinckley et al., 1996). However, it has been demonstrated more recently that many of the effects of topical application of glyceryl trinitrate on the pasterns may be mediated systemically rather

Mediator	pEC_{50}	receptor	reference
5-HT	7.55	5-$HT_{1B/D;}$ 5-HT_2	Bailey & Elliott (1998)
noradrenaline	6.86	α_1 ; α_2	Elliott (1997)
endothelin	7.35	ET_A	Katz et al., (2000)
thromboxane A_2	7.45	not determined	unpublished
ATP	3.50	P_{2x}	Soydan & Elliott (1994)

Fig. 3. Endogenous vasoconstrictor mediators with effects on equine digital blood vessels. pEC_{50} values (indicating potency; the higher the value the more potent the mediator) are those relating to the responses of digital veins.

Mediator	pEC_{50}	receptor	reference
adenosine	5.54	A_{2A}	Brady & Elliott (1998)
CGRP	8.89	not determined	Katz et al. (2000)
Substance P	9.02	NK_1	Katz et al. (2002)
bradykinin	9.00	not determined	Elliott et al. (1994)
adrenaline (isoprenaline)	8.46	β_2	Elliott & Soydan (1995)

Fig. 4. Endogenous vasodilator mediators with effects on equine digital blood vessels. pEC_{50} values (indicating potency; the higher the value the more potent the mediator) are those relating to the responses of digital veins.

than locally (Hoff et al., 2002). The efficacy of NO donors in improving digital blood flow does not necessarily imply that its deficiency may play a role in the pathogenesis of laminitis, although a recent study has shown that the response of digital blood vessels to acetylcholine (a NO-dependent vasodilator) may be impaired following black walnut-induced laminitis (Holm et al., 2002a).

Recent evidence, using an isolated perfused hoof preparation, from our laboratory indicates that in addition to NO, endothelium-derived hyperpolarising factor (EDHF) may also play a role in controlling vascular resistance within the equine digit (Berhane et al., 2003). Other vasodilators include prostacyclin, generated by the endothelium in response to endotoxin (Bailey and Elliott, 1999), adenosine generated by ischaemic tissues (Elliott and Brady, 1998) and adenosine diphosphate (ADP) released from platelets, which probably mediates its effects by direct activation of adenosine receptors (Soydan and Elliott, 1994). The effects of some of these mediators are summarised in Fig. 4.

The vasculature of the equine digit, including the dorsal hoof wall, is densely innervated with nerve fibres containing vasodilator substances such as substance P and calcitonin gene-related peptide (CGRP) and vasoconstrictors including neuropeptide Y as well as norepinephrine (Molyneux et al., 1994; Van and Bowker, 2002). These mediators clearly play an important role in the balance between vasoconstriction and dilation of the digital vasculature, and further work is ongoing to examine their possible role in the pathogenesis of laminitis. Thus, knowledge of basic mechanism regulating blood vessel tone in the equine digital circulation has increased enormously over the last 10 years. Extrapolation of these data to small resistance vessels (both pre- and post-capillary vessels) in the digit should probably be made with caution. Nevertheless, these data provide a good basis upon which to build on in the future.

Lamellar explants connected to strain gauges have been used to study factors contributing to the detachment of the dermal and epidermal lamellae brought about by matrix metalloproteinase enzymes (Pollitt et al., 1998). Matrix metalloproteinases are secreted in the pro-form, which requires subsequent activation by other proteases. The induction of MMP-2 and MMP-9 production and secretion is brought about by various cytokines and growth factors, such as IL-1β, tissue necrosis factor (TNF)-α and tissue growth factor (TGF)-β (Li et al., 2001). Their subsequent activation occurs as a result of the actions of proteases such as trypsin, plasmin and cathepsin G, or by reactive oxygen species (Birkedal-Hansen et al., 1993). Therefore, these enzymes are commonly involved in tissue destruction following ischaemia-reperfusion injury (Etoh et al., 2001; Yano et al., 2001). Recently, a number of bacterial proteases have also been found to have this MMP-activating ability (Okamoto et al., 1997). In the equine lamellar explant model, the ability of bacterial proteases (exotoxins) to cause separation has been studied (Mungall et al., 2001). Separation is not directly caused by cytokines, but could be induced by broth supernatants in which various Gram-positive and Gram-negative bacteria from the equine intestine had been grown, including *Streptococcus bovis*. Thermolysin (purified from a bacillus spp.) was subsequently identified as one possible bacterial protease capable of activating MMP-2 and MMP-9 and causing explant separation (Mungall and Pollitt, 2002).

With the improvement of cell culture techniques, it is now possible to study individual processes in individual cell types from the tissues of the lamellae and the laminar vasculature. These techniques will enable the study of how certain cell types may become activated and are involved in the chain of events leading to the onset of structural damage and clinical signs. Cell types studied so far include arterial and venous endothelial cells (Wunn et al., 1999; Bailey et al., 2003b), vascular smooth muscle cells (Rodgerson et al., 2000) and keratinocytes (Wunn et al., 1999). The future study of laminar epithelial cells in culture may provide many insights into the mechanisms by which they become activated to produce MMPs.

6. Factors predisposing individuals to acute laminitis

One of the many intriguing questions about laminitis is why only certain individuals tend to be affected; for

example in a herd of ponies kept on lush spring grass, certain members of the herd suffer recurrently. There is little doubt that any horse or pony can succumb to laminitis, as shown by the results of experimental induction of the disease using large amounts of carbohydrate (Garner et al., 1975). Under field conditions, however, certain managemental, behavioural, phenotypic or genotypic factors may confer resistance or susceptibility.

A major factor in predisposing animals to this disease worldwide may be inappropriate management, leading to obesity and/or the ingestion of excessive amounts of fermentable carbohydrate. However, in many cases, inappropriate management is not a factor, suggesting more inherent traits. These differences in individual susceptibility might also occur at the level of the large intestine, perhaps in the ability of the hindgut contents to buffer changes in pH due to lactic acid production and avoid mucosal damage, or in the bacterial populations responsible for producing laminitis trigger factors. Alternatively, they may lie in the detoxifying capacity of the liver, the responsiveness of peripheral blood vessels or the response of the lamellar tissues to toxic insult or ischaemia.

Epidemiological studies have perhaps been underused in terms of characterising risk factors associated with the condition. Studies to date examining signalment traits in affected animals have shown few significant associations (Polzer and Slater, 1996; Slater et al., 1995). Although a genetic basis for predisposition to *chronic* laminitis has been suggested (Hood, 1999b), no studies have yet shown clear familial or breed-associated links with acute laminitis. Ponies are more commonly affected by laminitis than horses (Coffman and Colles, 1983; Katz et al., 2001), although it is important to note that subclinical laminitis may be of great importance in horses also, including thoroughbreds (Linford et al., 1993).

Increasingly, various metabolic differences are being described between ponies and horses, some of which may have a bearing on predisposition to laminitis. For example, blood platelets from ponies seem to produce considerably more of the vasoconstrictor mediator, thromboxane A_2, when activated in vitro compared to those of horses (McKellar et al., 1990). There are also marked differences in lipid metabolism (Watson et al., 1991). Ponies have been shown to be relatively insensitive to their endogenous insulin compared with horses, and furthermore, obesity and a history of laminitis are also factors associated with relative insulin insensitivity (Coffman and Colles, 1983; Jeffcott et al., 1986). Insulin generally has vasodilator properties (Chen and Messina, 1996), and peripheral vasodilation has even been detected in horses following feeding (Hoffmann et al., 2001b), but interestingly, the chronic hyperinsulinaemic state can also be associated with hypertension and vascular disease in other species (He and Macleod, 2002).

Pars intermedia dysfunction (hyperadrenocorticism) is frequently associated with laminitis (Love, 1993), although the precise mechanistic link between the two conditions is still not known. Glucocorticoids, as well as exacerbating insulin insensitivity, potentiate the vasoconstrictor effects of catecholamines and serotonin on the digital blood vessels (Eyre et al., 1979; Eyre and Elmes, 1980), probably by inhibiting their uptake and clearance from the circulation (Bailey et al., 2003b). In a study by Pass et al. (1998), inhibition of glucose consumption in hoof explants induced lamellar separation, and it was speculated that high circulating corticosteroid levels and/or inhibition of insulin activity may bring about these changes. A third possible explanation for the link between glucocorticoids and the development of laminitis is a steroid-induced increase in intestinal permeability to toxins (Kiziltas et al., 1998), although this has yet to be examined in the horse. Therapeutically administered corticosteroids, either short or long acting, have been considered by many to have the potential to cause laminitis (Slone et al., 1981). The precise risk, however, of inducing or predisposing to laminitis is still the subject of debate, and evidence from controlled prospective studies is not yet available to guide practitioners. However, at present, all corticosteroids licensed for equine use in the UK continue to carry warnings about the risk in their accompanying data sheet.

An increasing number of individual animals are now being recognised which are predisposed to laminitis and are hyperinsulinaemic, but which do not have evidence of pars intermedia dysfunction based on clinical diagnostic tests (Johnson, 2000). Based on similarities with the human condition, the term 'peripheral Cushing's syndrome' has been used to describe these cases. Although a full discussion of this condition is outside the scope of this review and much work is yet to be done in this area, it appears that the local regulation of glucocorticoid vs. mineralocorticoid activity may be imbalanced at the target tissue level, possibly due to excess activity of the enzyme 11β-hydroxysteroid dehydrogenase type I (Johnson, 2000).

7. Implications for treatment and prevention

While man has sought to improve pastures with ever increasing sugar contents to maximise growth rates for sheep and cattle, it is now considered that such pastures may be less appropriate for equines (Longland and Cairns, 1998). Studies suggest that a large proportion of cases in the UK are linked to diet (Hinckley and Henderson, 1996), thus laminitis may be considered a disease of domestication. Work is continuing to understand the pasture and climatic conditions associated with increased laminitis risk in order to give owners better guidance to reduce the incidence of the disease. A more

definitive understanding of the pathways involved in laminitis pathogenesis will be required before an accurate predictive index of an individual animal's risk can be formulated, or for the prodromal stage to be diagnosed.

The streptogrammin antibiotic, virginiamycin, has been successfully used and marketed to prevent pasture-induced laminitis by preventing the overgrowth of Gram-positive caecal bacteria and production of lactic acid (Rowe et al., 1994), also reducing the bacterial production of vasoactive amines in vitro (Bailey et al., 2002a). Although available for treatment of horses on a named client basis in the UK, virginiamycin has been banned for use as a growth promoter in pigs and poultry in the European Union due to concerns over antibiotic resistance (Aarestrup et al., 2001), and so is unlikely to be granted a full license for use in the horse. Although it may continue to have a role in laminitis prevention in some particular individual cases, dietary and managemental measures will continue to be the mainstays of laminitis prevention, until more specific targets and agents are identified.

The treatment of acute laminitis is still largely an exercise in damage limitation; however, various novel therapeutic strategies are being developed for more effective treatment and prevention. Some older methods are also being re-evaluated; placing the feet in ice or cold water has been advocated for some time as a means of treating acute laminitis, and in a recent study, placing ice around one foot has been shown to prevent experimentally induced laminitis in that foot but not in the other forelimb (Pollitt and Davies, 1998). It was hypothesised that vasoconstriction caused by the cold temperature prevented toxins from reaching the foot; importantly however it is also conceivable that the cold temperature inhibits MMP enzyme activity and furthermore could protect against ischaemia – reperfusion injury (Fascoglione et al., 2000; Verrier, 1996).

Pharmacological agents are also available which block the activation of these enzymes (Pollitt et al., 1998), and these could represent a major step forward in the treatment of laminitis, although at present their cost may prove prohibitive for the treatment of horses. It may also be possible to block the pathogenic pathways higher up the chain, with the selective use of anti-inflammatory drugs or immunisation against toxins.

The phenothiazine derivative class of vasodilators, such as acepromazine and phenoxybenzamine seem to be quite potent and fairly selective in their effects on the equine digit, and have been used in the treatment and prevention of laminitis (Hood et al., 1982). In fact, these agents are not selective in terms of the receptors that they antagonise, blocking 5-HT receptors as well as α adrenoreceptors (unpublished observations). As more detailed knowledge is gained about the exact receptors and pathways involved in the vasoconstriction underlying laminitis, more selective strategies to counteract this could well emerge. With non-selective vasodilator drugs, such as acepromazine and nitric oxide donors, the optimum time of use would rationally be as early as possible in the disease process, although this is rarely feasible since animals are usually presented some time into the acute phase. It is also important to note that the effectiveness of some of these compounds with repeated use, particularly the nitric oxide donors, is relatively short lived (a few days), due to the well-recognised phenomenon of desensitisation of the tissues (Vincent et al., 1992).

Some other novel therapies have recently been successfully tested, and may be of use in the future. A fibrin antagonist, preventing platelet activation and aggregation, was shown to prevent laminitis (Weiss et al., 1998a), and a dihydropyridine calcium channel blocker, thought to increase red blood cell deformability and thereby improve blood flow, decreased the severity of the disease (Hood et al., 2002). Field trials, examining the effectiveness of these agents in naturally occurring cases, will be required to establish their true value in the clinical situation.

In conclusion, a unifying theory for the pathogenic mechanisms causing acute laminitis, drawing on many currently held assertions involving haemodynamic disturbances and metalloproteinase activity, is still justifiable, as at present these two theories are by no means mutually exclusive. There remain, however, some important aspects of this disease which are yet to be definitively clarified, including the significance of the haemodynamic disturbances on tissue oxygenation, and the precise factors which are released from the hindgut to initiate the process. Because of the difficulties in studying the complex tissue structure of the laminae, it is likely that answers will only continue to emerge little by little, from the integration of in vitro, ex vivo and in vivo studies, employing a combination of deductive and inductive reasoning.

References

Aarestrup, F.M., Seyfarth, A.M., Emborg, H.D., Pedersen, K., Hendriksen, R.S., Bager, F., 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrobial Agents and Chemotherapeutics 45, 2054–2059.

Adair, H.S., Goble, D.A., Schmidhammer, J.L., Shires, G.M.H., 2000. Laminar microvascular flow, measured by means of laser Doppler flowmetry, during the prodromal stages of black walnut-induced laminitis in horses. American Journal of Veterinary Research 61, 862–868.

Allen, D., Korthuis, R.J., Clark, E.S., 1988. Capillary permeability to endogenous macromolecules in the equine digit. American Journal of Veterinary Research 49, 1609–1612.

Allen, D., Clark, E.S., Moore, J.N., Prasse, K.W., 1990. Evaluation of equine digital Starling forces and hemodynamics during

early laminitis. American Journal of Veterinary Research 51, 1930–1934.

Bailey, S.R., 1998. A study of the vascular effects and factors regulating the concentration of 5-hydroxytryptamine in the equine digital circulation. Ph.D. Thesis, University of London.

Bailey, S.R., Elliott, J., 1998a. Plasma 5-hydroxytryptamine constricts equine digital blood vessels in vitro: implications for the pathogenesis of acute laminitis. Equine Veterinary Journal 30, 124–130.

Bailey, S.R., Elliott, J., 1998b. Evidence for different 5-$HT_{1B/1D}$ receptors mediating vasoconstriction of equine digital arteries and veins. European Journal of Pharmacology 335, 175–187.

Bailey, S.R., Elliott, J., 1999. The role of prostacyclin and nitric oxide in mediating the response of equine digital blood vessels to endotoxin. Equine Veterinary Journal 31, 212–218.

Bailey, S.R., Cunningham, F.M., Elliott, J., 2000. Endotoxin and dietary amines may increase plasma 5-hydroxytryptamine in the horse. Equine Veterinary Journal 32, 497–504.

Bailey, S.R., Rycroft, A., Elliott, J., 2002a. Production of amines in equine caecal contents in an in vitro model of carbohydrate overload. Journal of Animal Science 80, 2656–2662.

Bailey, S.R., Berhane, Y., Marr, C.M., Elliott, J., 2002b. Effects of vasoactive amines from the equine hindgut on digital blood flow in the normal horse. Journal of Veterinary Internal Medicine 16, 355.

Bailey, S.R., Marr, C.M., Elliott, J., 2003a. Identification and quantification of amines in the equine caecum. Research in Veterinary Science 74, 113–118.

Bailey, S.R., Wheeler-Jones, C., Elliott, J., 2003b. Uptake of 5-hydroxytryptamine by equine digital vein endothelial cells: inhibition by amines found in the equine caecum. Equine Veterinary Journal 35, 164–169.

Berhane, Y., Bailey, S.B., Elliott, J., 2002. Contractile effects on digital arteries and veins of monoamines found in the caecum. British Journal of Pharmacology 135, 291 P.

Berhane, Y., Bailey, S.R., Elliott, J., 2003. Characterisation of endothelium-dependent vasodilatory responses in the equine isolated perfused digital vascular bed. British Journal of Pharmacology, 138, 146 P.

Biondi, M.L., Marasini, B., Bianchi, E., Agostini, A., 1988. Plasma free and intraplatelet serotonin in platelets with Raynaud's phenomenon. International Journal of Cardiology 19, 335–339.

Birkedal-Hansen, H., Moore, W.G., Bodden, M.K., Windsor, L.J., Birkedal-Hansen, B., DeCarlo, A., Engler, J.A., 1993. Matrix metalloproteinases: a review. Critical Reviews in Oral Biology and Medicine 4, 197–250.

Blackwell, B., Marley, E., 1966. Interactions of cheese and of its constituents with monoamine oxidase inhibitors. British Journal of Pharmacology 26, 120–141.

Bunker, C.B., Goldsmith, P.C., Leslie, T.A., Hayes, N., Foreman, J.C., Dowd, P.M., 1996. Calcitonin gene-related peptide, endothelin-1, the cutaneous microvasculature and Raynaud's phenomenon. British Journal of dermatology 134, 399–406.

Burch, G.E., Harb, J.M., Sun, C.S., 1979. Fine structure of digital vascular lesions in Raynaud's phenomenon and disease. Angiology 30 (6), 361–376.

Cerinic, M.M., Generini, S., Pignone, A., 1997. New approaches to the treatment of Raynaud's phenomenon. Current Opinion in Rheumatology 9, 544–556.

Chen, Y.L., Messina, E.J., 1996. Dilation of isolated skeletal muscle arterioles by insulin is endothelium dependent and nitric oxide mediated. American Journal of Physiology 270, H2120–H2124.

Clark, E.S., Moore, J.N., 1990. The effects of slow infusion of a low dosage of endotoxin in healthy horses. Equine Veterinary Journal (Colic Supplement), 33–37.

Coffman, J.R., Colles, C.M., 1983. Insulin tolerance in laminitic ponies. Canadian Journal of Comparative Medicine 47, 347–351.

Cogswell, A.M., Johnson, P.J., Adams, H.R., 1995. Evidence for endothelium-derived relaxing factor/nitric oxide in equine digital arteries. American Journal of Veterinary Research 56, 1637–1641.

Eaton, S.A., Allen, D., Eades, S.C., Schneider, D.A., 1995. Digital Starling forces and hemodynamics during early laminitis induced by an aqueous extract of black walnut (*Juglans nigra*) in horses. American Journal of Veterinary Research 56, 1338–1344.

Elliott, J., 1997. Alpha-adrenoceptors in equine digital veins: evidence for the presence of both $alpha_1$ and $alpha_2$-receptors mediating vasoconstriction. Journal of Veterinary Pharmacology and Therapeutics 20, 308–317.

Elliott, J., Brady, F.E., 1998. Characterization of vasodilatory adenosine receptors in equine digital veins. Journal of Veterinary Pharmacology and Therapeutics 21, 74–81.

Elliott, J., Bryant, C.E., Soydan, J., 1994. The role of nitric oxide in the responses of equine digital veins to vasodilator and vasoconstrictor agents. Equine Veterinary Journal 26, 378–384.

Eyre, P., Elmes, P.J., Strickland, S., 1979. Corticosteroid-potentiated vascular responses of the equine digit: a possible pharmacologic basis for laminitis. American Journal of Veterinary Research 40, 135–138.

Eyre, P., Elmes, P.J., 1980. Corticosteroid-induced laminitis. Further investigations on the isolated, perfused hoof. Veterinary Research Communications 4, 139–143.

Etoh, T., Joffs, C., Deschamps, A.M., Davis, J., Dowdy, K., Hendrick, J., Baicu, S., Mukherjee, R., Manhaine, M., Spinale, F.G., 2001. Myocardial and interstitial matrix metalloproteinase activity after acute myocardial infarction in pigs. American Journal of Physiology Heart Circulation and Physiology 281, H987–H994.

Fascoglione, G.F., Marini, S., Coletta, M., 2000. pH- and temperature-dependence of functional modulation in metalloproteinases. A comparison between neutrophil, collagenase and gelatinases A and B. Biophysics Journal 79, 2138–2149.

Flaherty, J.T., Weisfeldt, M.L., 1988. Reperfusion injury. Free Radical Biology and Medicine 5, 409–419.

Fontaine, G.L., Belknap, J.K., Allen, D., Moore, J.N., Kroll, D.L., 2001. Expression of interleukin-1beta in the digital laminae of horses in the prodromal stage of experimentally induced laminitis. American Journal of Veterinary Research 62, 714–720.

Galey, F.D., Twardock, A.R., Goetz, T.E., Schaeffer, D.J., Hall, J.O., Beasley, V.R., 1990a. Gamma scintigraphic analysis of the distribution of perfusion of blood in the equine foot during black walnut (*Juglans nigra*)-induced laminitis. American Journal of Veterinary Research 51, 688–694.

Galey, F.D., Beasley, V.R., Schaeffer, D.J., Davis, L.E., 1990b. Effect of an aqueous extract of black walnut (*Juglans nigra*) on isolated equine digital vessels. American Journal of Veterinary Research 51 (1), 83–88.

Garner, H.E., Coffman, J.R., Hahn, A.W., Salem, C., 1975. Equine laminitis of alimentary origin: an experimental model. American Journal of Veterinary Research 36, 441–445.

Garner, H.E., Hutcheson, D.P., Coffman, J.R., Hahn, A.W., Salem, C., 1977. Lactic acidosis: a factor associated with equine laminitis. Journal of Animal Science 45, 1037–1041.

Garner, H.E., Moore, J.N., Johnson, J.H., Clark, L., Amend, J.F., Tritshler, L.G., Coffman, J.R., Sprouse, R.F., Hutcheson, D.P., Salem, C.A., 1978. Changes in the caecal flora associated with the onset of laminitis. Equine Veterinary Journal 10, 249–252.

Gonsalves, A., Johnson, E.S., 1977. Possible mechanism of action of β-phenylethylamine in migraine. Journal of Pharmacy and Pharmacology 29, 646–647.

He, Y., Macleod, K.M., 2002. Modulation of noradrenaline-induced vasoconstriction in isolated perfused mesenteric beds from obese Zucker rats in the presence and absence of insulin. Canadian Journal of Physiology and Pharmacology 80, 171–179.

Hinckley, K.A., Henderson, I.W., 1996. The epidemiology of equine laminitis in the UK. In: Proceedings of the 35th Congress of the British Equine Veterinary Congress, Warwick, p. 62.

Hinckley, K.A., Fearn, S.A., Howard, B.R., Henderson, I.W., 1995. Near infrared spectroscopy of pedal haemodynamics and oxygenation in normal and laminitic horses. Equine Veterinary Journal 27, 465–470.

Hinckley, K.A., Fearn, S.A., Howard, B.R., Henderson, I.W., 1996. Nitric oxide donors as treatment for grass induced acute laminitis in ponies. Equine Veterinary Journal 28, 17–28.

Hoff, T.K., Hood, D.M., Wagner, I.P., 2002. Effectiveness of glyceryl trinitrate for enhancing digital submural perfusion in horses. American Journal of Veterinary Research 63, 648–652.

Hoffmann, K.L., Wood, A.K.W., Griffiths, K.A., Evans, D.L., Gill, R.W., Kirby, A.C., 2001a. Doppler sonographic measurements of arterial blood flow and their repeatability in the equine foot during weight bearing and non-weight bearing. Research in Veterinary Science 70, 199–203.

Hoffmann, K.L., Wood, A.K.W., Griffiths, K.A., Evans, D.L., Gill, R.W., Kirby, A.C., 2001b. Postprandial arterial vasodilation in the equine distal thoracic limb. Equine Veterinary Journal 33, 269–273.

Holm, A.S., Venugopal, C.S., Eades, S.C., Koch, C.E., Holmes, E.P., Moore, R.M., 2002a. Effects of black walnut extract administration on in vitro responses of equine palmar digital arterial and venous rings to endothelin-1, acetylcholine and nitroglycerine. In: Proceedings of the Sixth Colic Research Symposium, Manchester England, p. 140.

Holm, A.S., Eades, S.C., Venugopal, C.S., Koch, C.E., Moore, R.M., 2002b. Endothelin-1 induces vasoconstriction in the normal equine digit. In: Proceedings of the Sixth Colic Research Symposium, Manchester England, p. 106.

Holm, A.S.,Venugopal, C.S., Hosgood, G., Eades, S.C., Moore, R.M., 2002c. Comparison of effect of two endothelin antagonists on in vitro responses of equine palmar digital arterial and venous rings to endothelin-1 in normal horses and those with naturally acquired laminitis. In: Proceedings of the Sixth Colic Research Symposium, Manchester England, p. 141.

Holm, A.S., Eades, S.C., Moore, R.M., 2002d. Evaluation of equine digital Starling forces associated with black walnut extract administration. In: Proceedings of the Sixth Colic Research Symposium, Manchester England, p. 88.

Holm, A.S., Eades, S.C., Moore, R.M., 2002e. Evaluation of jugular and digital venous plasma endothelin-1 concentrations in horses before and after black walnut extract administration. In: Proceedings of the Sixth Colic Research Symposium, Manchester England, p. 126.

Hood, D.M., Amoss, M.S., Hightower, G., McDonald, D.R., McGrath, J.P., McMullan, W.C., Scrutchfield, W.L., 1978. Equine laminitis I: radioisotopic analysis of the hemodynamics of the foot during the acute disease. Journal of Equine Medicine and Surgery 2, 439–443.

Hood, D.M., Stephens, K.A., Amoss, M.S., 1982. The use of alpha- and beta-adrenergic blockade as a preventative in the carbohydrate model of laminitis. American Association of Equine Practitioners Newsletter 2, 142–145.

Hood, D.M., Amoss, M.S., Grosenbaugh, D.A., 1990. Equine laminitis: a potential model of Raynaud's phenomenon. Angiology 41, 270–277.

Hood, D.M., Grosenbaugh, D.A., Mostafa, M.B., Morgan, S.J., Thomas, B.C., 1993. The role of vascular mechanisms in the development of acute equine laminitis. Journal of Veterinary Internal Medicine 7, 228–233.

Hood, D.M., 1995. Endotoxaemia as a direct cause of laminitis. In: Proceedings: American Association of Equine Practitioners, vol. 41, pp. 245–247.

Hood, D.M., 1999a. Laminitis as a systemic disease. Veterinary Clinics of North America Equine Practice 15, 481–494.

Hood, D.M., 1999b. A genetic basis for the development of laminitis. Veterinary Clinics of North America Equine Practice 15, 321–343.

Hood, D.M., Wagner, I.P., Brumbaugh, G.W., 2001. Evaluation of hoof wall surface temperature as an index of digital vascular perfusion during the prodromal and acute phases of carbohydrate-induced laminitis in horses. American Journal of Veterinary Research 62, 1167–1172.

Hood, D.M., Brumbaugh, G.W., Wagner, I.P., 2002. Effectiveness of a unique hydropyridine (BAY TG 1000) for prevention of laminitis in horses. American Journal of Veterinary Research 63, 443–447.

Hunt, R.J., 1991. The pathophysiology of acute laminitis. Compendium of Continuing Veterinary Education 13, 401–407.

Hunt, J.M., Edwards, G.B., Clarke, K.W., 1986. Incidence, diagnosis and treatment of postoperative complications in colic cases. Equine Veterinary Journal 18, 264–270.

Ingle-Fehr, J.E., Baxter, G.M., 1998. Evaluation of digital and laminar blood flow in horses given a low dose of endotoxin. American Journal of Veterinary Research 59, 192–196.

Jeffcott, L.B., Field, J.R., McLean, J.G., O'Dea, K., 1986. Glucose tolerance and insulin sensitivity in ponies and Standardbred horses. Equine Veterinary Journal 18, 97–101.

Jiang, X., Namura, S., Nagata, I., 2001. Matrix metalloproteinase inhibitor KB-R7785 attenuates brain damage resulting from permanent focal cerebral ischemia in mice. Neuroscience Letters 305, 41–44.

Johnson, P.J., Tyagi, S.C., Katwa, L.C., Ganjam, V.K., Moore, L.A., Kreeger, J.M., Messer, N.T., 1998. Activation of extracellular matrix metalloproteases in equine laminitis. The Veterinary Record 142, 392–396.

Johnson, P.J., 2000. Laminitis, ''hypothyroidism'', and obesity: a peripheral cushingoid syndrome in horses? In: Proceedings of the 18th ACVIM Forum.

Katwa, L.C., Johnson, P.J., Ganjam, V.K., Kreeger, J.M., Messer, N.T., 1999. Expression of endothelin in equine laminitis. Equine Veterinary Journal 31, 243–247.

Katz, L.M., Tandy, H., De Brauwere, N., Elliott, J., Marr, C.M., 2000. The prevalence of laminitis in one region of the UK. In: Proceedings of the 39th British Equine Veterinary Association Congress, pp. 211–212.

Katz, L.M., De Brauwere, N., Elliott, J., Marr, C.M., Pfeiffer, D.U., 2001. A retrospective epidemiological study of laminitis in one region of the UK. In: Proceedings of the 40th British Equine Veterinary Association Congress, p. 199.

Katz, L.M., Marr, C.M., Elliott, J., 2003a. Endothelin-1 selectively constricts equine digital veins through ET_A receptors without modulation by the endothelium. American Journal of Veterinary Research, in press.

Katz, L.M., Marr, C.M., Elliott, J., 2003b. Characterisation of the response of equine digital arteries and veins to substance P. Journal of Veterinary Pharmacology and Toxicology, in press.

Kiziltas, S., Imeryuz, N., Gurcan, T., Siva, A., Saip, S., Dumankar, A., Kalayci, C., Ulusoy, N.B., 1998. Corticosteroid therapy augments gastro-duodenal permeability to sucrose. American Journal of Gastroenterology 93, 2420–2425.

Kreuger, A.S., Kinden, D.A., Garner, H.E., Sprouse, R.F., 1986. Ultrastructural study of the equine caecum during onset of laminitis. American Journal of Veterinary Research 47, 1804–1812.

Li, D., Balakrishna, L., Lokeshwa, L., Soloman, A., Monroy, D., Ji, Z., Pflugfelder, S.C., 2001. Regulation of MMP-9 production by human corneal epithelial cells. Experimental Eye Research 73, 449–459.

Linford, R.L., O'Brien, T.R., Trout, D.R., 1993. Qualitative and morphometric radiographic findings in the distal phalanx and digital soft tissues of sound thoroughbred racehorses. American Journal of Veterinary Research 54, 38–51.

Longland, A., Cairns, A., 1998. Sugars in grass-an overview of sucrose and fructan accumulation in temperate grasses. In: Proceedings of the Dodson and Horrell International Research Conference on Laminitis, Stoneleigh, Warwickshire, England, pp. 1–3.

Longland, A.C., Cairns, A.J., Humphreys, M.O., 1999. Seasonal and diurnal changes in fructan concentration in *Lolium perenne*: implications for the grazing management of equines predisposed

to laminitis. In: Proceedings: 16th Equine Nutritional Physiology Society, Raleigh, NC, pp. 258–259.
Love, S., 1993. Equine Cushing's disease. British Veterinary Journal 149, 139–153.
Masty, J., Stradley, R.P., 1991. Paneth cell degranulation and lysozyme secretion during acute equine alimentary laminitis. Histochemistry 95, 529–533.
McConnico, R.S., Holm, A.S., Eades, S.C., Moore, R.M., 2002. The effect of Black Walnut extract on equine colonic histopathology and in vitro ion transport. In: Proceedings of the Sixth Colic Research Symposium, Manchester, England.
Mogg, K.C., Pollitt, C.C., 1992. Hoof and distal limb surface temperature in the normal pony under constant and changing ambient temperatures. Equine Veterinary Journal 24, 134–139.
McKellar, Q.A., Nolan, A.M., Galbraith, E.A., 1990. Serum thromboxane generation by platelets in several domestic animal species. British Veterinary Journal 146, 398–404.
Molyneux, G.S., Haller, C.J., Mogg, K., Pollitt, C.C., 1994. The structure, innervation and location of arteriovenous anastomoses in the equine foot. Equine Veterinary Journal 26, 305–312.
Moore, J.N., Allen, D., 1996. The pathophysiology of acute laminitis. Veterinary Medicine 34, 936–939.
Moore, J.N., Garner, H.E., Coffman, J.R., 1981. Hematological changes during development of acute laminitis hypertension. Equine Veterinary Journal 13, 240–242.
Mungall, B.A., Pollitt, C.C., Collins, R., 1998. Localisation of gelatinase activity in epidermal hoof lamellae by in situ zymography. Histochemistry and Cell Biology 110, 535–540.
Mungall, B.A., Kyaw-Tanner, M., Pollitt, C.C., 2001. In vitro evidence for a bacterial pathogenesis of equine laminitis. Veterinary Microbiology 79, 209–223.
Mungall, B.A., Pollitt, C.C., 2002. Thermolysin activates equine lamellar hoof matrix metalloproteinases. Journal of Comparative Pathology 126, 9–16.
Okamoto, T., Akaike, T., Suga, M., Tanase, S., Horie, H., Miyajima, S., Ando, M., Ichinose, Y., Maeda, H., 1997. Activation of human matrix metalloproteinases by various bacterial proteinases. The Journal of Biological Chemistry 272, 6059–6066.
Pass, M.A., Pollitt, S., Pollitt, C.C., 1998. Decreased glucose metabolism causes separation of hoof lamellae in vitro: a trigger for laminitis? Equine Veterinary Journal Supplement 26, 133–138.
Pollitt, C.C., Davies, C.T., 1998. Equine laminitis: its development post alimentary carbohydrate overload coincides with increased sublamellar blood flow. Equine Veterinary Journal 26 (Suppl.), 133–138.
Pollitt, C.C., Pass, M.A., Pollitt, S., 1998. Batimastat (BB-94) inhibits matrix metalloproteinases of equine laminitis. Equine Veterinary Journal Supplement 26, 119–124.
Pollitt, C.C., 2002. Equine laminitis: a new induction model based on alimentary overload with fructan. In: Proceedings of the Sixth Colic Research Symposium, Manchester, England, p. 87.
Polzer, J., Slater, M.R., 1996. Age, breed, sex and seasonality as risk factors for equine laminitis. Preventive Veterinary Medicine 29, 179–184.
Potter, G.D., Arnold, F.F., Householder, D.D., Hansen, D.H., Brown, K.M., 1992. Digestion of starch in the small or large intestine of the equine. Pferdeheilkunde 1, 107–111.
Prasse, K.W., Allen, D., Moore, J.N., Duncan, A., 1990. Evaluation of coagulation and fibrinolysis during the prodromal stages of carbohydrate-induced acute laminitis in the horse. American Journal of Veterinary Research 51, 1950–1955.
Rang, H.P., Dale, M.M., 1995. Other peripheral mediators: 5-hydroxytryptamine and the purines. In: Pharmacology, second ed, pp. 217–242. Chapter 9.
Robinson, N.E., 1990. Digital blood flow, arteriovenous anastomoses and laminitis. Equine Veterinary Journal 22, 381–383.
Rodgerson, D.H., Belknap, J.K., Fontaine, G.L., Kroll, D.L., 2000. Characterization of cultured smooth muscle cells obtained from the palmar digital arteries of horses. American Journal of Veterinary Research 61, 1602–1608.
Rowe, J.B., Pethick, D.W., Lees, M.J., 1994. Prevention of acidosis and laminitis associated with grain feeding in horses. Journal of Nutrition 124, 2742S–2744S.
Shaw, S.W.J., Johnson, R.H., Keogh, H.J., 1978. Oral tyramine in dietary migraine sufferers. In: Greene, R. (Ed.), Current Concepts in Migraine Research. Raven Press, New York, USA, pp. 31–38.
Slater, M.R., Hood, D.M., Carter, G.K., 1995. Descriptive epidemiological study of equine laminitis. Equine Veterinary Journal 27, 364–367.
Slone, D.E., Purohit, R.C., Ganjam, V.K., 1981. Effects of dexamethasone and triamcinolone that may predispose to laminitis in horses. American Association of Equine Practitioners Congress 27, 469–471.
Soydan, J., Elliott, J., 1994. Vasodilation of equine isolated digital veins by adenosine nucleotides does not depend on their metabolism to adenosine. British Journal of Pharmacology 113, 117 P.
Sprouse, R.F., Garner, H.E., Green, E.M., 1987. Plasma endotoxin levels in horses subjected to carbohydrate induced laminitis. Equine Veterinary Journal 19, 25–28.
Stashak, T.S., 1987. Laminitis. In: Adams' Lameness in Horses, fourth ed. Lea and Febiger Publications, Philadelphia, pp. 468–499.
Trout, D.R., Hornof, W.J., Linford, R.L., O'Brien, T.R., 1990. Scintigraphic evaluation of digital circulation during the developmental and acute phases of equine laminitis. Equine Veterinary Journal 22, 416–421.
USDA, 2000. Lameness and laminitis in US horses. USDA:APHIS:VS, CEAH, National Animal Health Monitoring System. Fort Collins, CO, #N318.0400.
Van, W., Bowker, R.M., 2002. Evaluation of tachykinins and their receptors to determine sensory innervation in the dorsal hoof wall and insertion of the distal sesamoidean impar ligament and deep digital flexor tendon on the distal phalanx in healthy feet of horses. American Journal of Veterinary Research 63, 222–228.
Vanhoutte, P.M., 1991. Platelet-derived serotonin, the endothelium and cardiovascular disease. Journal of Cardiovascular Pharmacology 17 (Suppl. 5), S6–S12.
Verrier, E., 1996. The microvascular cell and ischaemia-reperfusion injury. Journal of Cardiovascular Pharmacology 27 (Suppl. 1), S26–S30.
Vincent, J., Kongpatanakul, S., Blaschke, T.F., Hoffman, B.B., 1992. Desensitization of nitrate-induced venodilation: reversal with oral *N*-acetylcysteine in humans. Journal of Cardiovascular Pharmacology 20, 907–912.
Waguespack, B., et al., 2002. Local digital expression of inflammatory and vasoactive mediators during the developmental stage of laminitis. In: Proceedings of the Sixth Colic Research Symposium, Manchester, England, p. 90.
Watson, T.D., Burns, L., Love, S., Packard, C.J., Shepherd, J., 1991. The isolation, characterisation and quantification of the equine plasma lipoproteins. Equine Veterinary Journal 23, 353–359.
Weiss, D.J., Geor, R.J., Johnston, G., Trent, A.M., 1994. Microvascular thrombosis associated with onset of acute laminitis in ponies. American Journal of Veterinary Research 55, 606–612.
Weiss, D.J., Trent, A.M., Johnston, G., 1995. Prothrombotic events in the prodromal stages of acute laminitis in horses. American Journal of Veterinary Research 56, 986–991.
Weiss, D.J., Monreal, L., Angles, A.M., Monasterio, J., 1996. Evaluation of thrombin–antithrombin complexes and fibrin fragment D in carbohydrate-induced laminitis. Research in Veterinary Science 61, 157–159.
Weiss, D.J., Evanson, O.A., McLennahan, D., Fagliari, J.J., Dunnwiddie, C.T., Wells, R.E., 1998a. Effect of a competitive inhibitor of platelet aggregation on experimentally induced laminitis in ponies. American Journal of Veterinary Research 59, 814–817.
Weiss, D.J., Evanson, O.A., Macleay, J., Brown, D.R., 1998b. Transient alteration in intestinal permeability to technetium Tc99m diethylenetriamino-pentaacetate during the prodromal

phase of alimentary laminitis in ponies. American Journal of Veterinary Research 59, 1431–1434.
Weiss, D.J., Evanson, O.A., Green, B.T., Brown, D.R., 2000. In vitro evaluation of intraluminal factors that may alter intestinal permeability in ponies with carbohydrate-induced laminitis. American Journal of Veterinary Research 61, 858–861.
Wigley, F.M., Flavahan, N.A., 1996. Raynaud's phenomenon. Rheumatic Diseases Clinics of North America 22, 765–781.
Wunn, D., Wardrup, K.J., Meyers, K., Kramer, J., Ragle, C.A., 1999. Culture and characterization of equine terminal arch endothelial cells and hoof keratinocytes. American Journal of Veterinary Research 60, 128–132.
Yano, M., Omoto, Y., Yamakawa, Y., Nakashima, Y., Kiriyama, M., Saito, Y., Fujii, Y., 2001. Increased matrix metalloproteinase activity and mRNA expression in lung ischemia-reperfusion injury. The Journal of Heart and Lung Transplantation 20, 679–686.
Zamora, M.R., O'Brien, R.F., Rutherford, R.B., Weil, J.V., 1990. Serum endothelin-1 concentrations and cold provocation in primary Raynaud's phenomenon. The Lancet 336, 1144–1147.

Book review

Veterinary drugs: synonyms & properties. G.W.A. Milne (Ed.) Aldershot, Ashgate Publishing, 2002. 254 pp. £95 (hard) ISBN 0566085003.

The title describes perfectly the content of the book. Names, synonyms, physical and chemical properties of most of the compounds used in veterinary medicine, either approved as veterinary or human medication, but employed in veterinary practice, are summarised. The book is made up of three main sections.

Part I, termed *Main Entries*, contains over 750 short monographs divided into 118 therapeutic or biological activity categories. Each monograph or entry comprises the main record name of the drug and its corresponding Record Number, Chemical Abstracts Service (CAS) Registry Number (RN), *Merck Index* number (corresponding to the monograph number from the 12th Edition of the *Merck Index*), European Inventory of Existing Commercial Chemical Substances (EINECS) number, formula, molecular formula, chemical name, list of synonyms, pharmacological activity and physical properties. When available, the regulatory status, other biological activities and acute toxicity, usually *per os* in laboratory animals, are indicated. In the case of FDA-approved veterinary drugs, the monograph is completed by a list of trade names of veterinary products with the name of the manufacturer or sponsor along with the New Animal Drug Approval (NADA) number.

Part II, termed *Indexes*, contains the four indexes cited in each monograph, but is quite complicated to read. The list of indexes allows the reader to find the Record Number and the main entry for each compound on the basis of its CAS Registry Number, EINECS Number or NADA Number, respectively. The Name and Synonyms Index section may be very useful in finding the corresponding main entry, when a name or a synonym of the veterinary drug is known. Moreover, this Index contains the names of the manufacturers and suppliers, the medicinal products manufactured or marketed by the company and the relative therapeutic categories.

Part III, termed *Manufacturer and Supplier Directory*, consists of an alphabetical list of the names, addresses and contact information (telephone and/or fax number and sometimes web site) of the companies that supply, manufacture or sponsor veterinary medicinal products. Four useful additional chapters, *Glossary of Terms*, *Glossary of Units*, *Abbreviations and Symbols* and *Table of Therapeutic Categories* are inserted before Part I.

The monographs are easy to read and supply essential information on drugs used in veterinary practice. Unfortunately, the practical information is limited to a brief description of the mechanism of action or biological activity and to the general therapeutic category or field of application, and these characteristics are not reported for all the drugs listed in the book. No dosages, route of administration, specific applications or side effects are given. The list of trade names of the veterinary drugs reported in the book should be helpful, particularly for American and British practitioners. Finally, the book may be considered as a sort of *Merck Index*, for drugs used in veterinary practice, allowing the reader, starting from a synonym, a trade name or an international identification number, to find essential information on a particular compound. The book may be directed at anyone involved in the various fields of veterinary medicine.

Giovanni Re
Division of Pharmacology and Toxicology
Department of Animal Pathology
University of Turin
via Leonardo da Vinci 44, I-10095
Grugliasco (TO), Italy
E-mail address: re@veter.unito.it

doi:10.1016/S1090-0233(02)00255-1