

The Role of Arteriovenous Anastomoses in the Pathophysiology of Equine Laminitis*

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ABSTRACT

Arteriovenous anastomoses (AVAs) are important components of the dermal lamellar microcirculation of the equine foot. Densely innervated, they are found clustered around axial arteries and number about 500/cm² in each lamella. AVAs can draw 50% of the total limb blood flow and steal blood from neighboring arterioles and capillaries when they are fully dilated. When AVAs dilate, the foot becomes warm. Experimental horses kept in a climate room at 50 °F develop hot feet prior to the onset of foot pain when laminitis is induced by using the carbohydrate overload model. Histopathology shows extensive damage to the lamellar architecture, without evidence of inflammation, edema or intravascular coagulation, at the same time as or before foot pain commences. Because prolonged extensive AVA dilation appears capable of inducing ischemia by stealing blood from more peripheral tissues, it is proposed that AVA dilation is the primary causal event in the pathophysiology of equine laminitis. Thus, laminitis is a failure of the hoof pedalbone bond, occurring because fresh arterial blood is shunted through dilated AVAs instead of through nutrient capillaries that support key epidermal structures within the hoof wall. Prolonged AVA dilation causes stagnation of the nutrient capillary circulation, ischemia of the hoof epidermis and eventual destruction of the lamellar architecture.

INTRODUCTION

In adapting the equine foot to its environment, evolution has seen fit to supply the digital microcirculation with numerous arteriovenous anastomoses (AVAs). Horses and ponies are quite able to survive the harshest of winters while standing for long periods with their feet immersed in ice and snow. They never suffer from frostbite because their digital blood supply is equipped with a dual circulation; one for nutrition and one for local thermoregulation. Blood may be directed through the slow, high-resistance, nutrient capillary circulation to maintain the tissues of the hoof-pedal bone bond, or through low-resistance, high-speed, AVAs to bring hot blood close to the periphery. Switching from one circulation to the other to keep the foot above critically low temperatures implies a degree of central (hypothalamic) control over the circulation of the foot.

In the evolution of the equine hoof, the *lamellar* epidermis (in contrast to the

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coronary, sole, and frog epidermis) seems to have relinquished its proliferative potential in order to develop an almost indestructable hoof-pedal bone bond. Yet it remains an epidermis. It has no circulation of its own and depends on the nearby nutrient capillary bed of the dermal microcirculation for its supply of essential metabolites. Although sometimes labeled the “insensitive” lamellae because of their poor innervation, the cells of the lamellar epidermis are very much alive and from time to time find themselves at the mercy of their mercurial, and sometimes capricious, neighbor, the dermal microcirculation. Our current working hypothesis is that a perturbation of AVA control can explain how the paradox of heat, epidermal ischemia (without inflammation), and a bounding digital pulse can all occur concurrently during developmental laminitis.

Laminitis is a failure of the hoof-pedal bone bond; the bond fails because fresh arterial blood is shunted through dilated AVAs instead of through nutrient capillaries that support key epidermal structures within the hoof wall. Prolonged AVA dilation causes stagnation of the nutrient capillary circulation, ischemia of the hoof epidermis, and eventual destruction of the lamellar architecture.

THE HOOF-PEDAL BONE BOND

The enormous forces that are generated by equine locomotion demand that the bony skeleton of the equine digit remains securely attached to the inside of the hoof wall. Indeed, the surface area of attachment of the inner hoof wall is expanded many times over by a spectacular array of primary and secondary epidermal lamellae, which are aligned along tension lines. The periosteum covering the dorsal surface of the pedal bone is the source of innumerable tough collagen fibers, each with its origin encased, for added strength, in the cortical bone of the third phalanx (reminiscent of Sharpey’s fibers in the tendinous insertion of muscle to bone). To suspend the pedal bone from the inner hoof wall, these collagen fibers orient vertically (dorsopalmar or plantar), subdividing into finer and finer fibrils, penetrating the dermal crypts of each secondary epidermal lamellae (SEL), to finally attach to the basement membrane of the dermoepidermal junction. On the other (epidermal) side of the basement membrane, and permanently attached to it, is the first layer of epidermal cells. A continuous sheet of basal cells, folded many times over to form the secondary epidermal lamellae.

Within each SEL epidermal cell is a skeleton of keratin filaments (the tonofilaments of the cytoskeleton). Each epidermal cell is spot-welded to its neighbor by way of numerous desmosomes; the tonofilaments radiate from and are intimately connected to each desmosome. The filaments also orient vertically (dorsopalmar or-plantar), thus continuing, through this epidermal component, the hoof-pedal bone bond. Like epidermal cells at other sites, the cells of the SEL transform directly into corneocytes, migrate peripherally, and merge with the already formed corneocytes of the primary epidermal lamellae (PEL) and the tubules of the inner hoof wall.¹ Keratinization of SEL cells as they approach the PEL occurs abruptly. Autoradiographs of the dorsal hoof wall of horses that are treated with radioactive methionine show positive labeling of the lamellar epidermis. This labeling suggests that lamellar epidermal cell proliferation is more

active than was previously thought.² The epidermal basal cells and the cells adjoining them (the parabasal cells) contain relatively few tonofilaments and desmosomes, and might be the chink in the armor of the hoof-pedal bone bond. For PEL cells to move downward past the immobile SEL basal cells, as they must if the hoof wall is to grow distally past the stationary third phalanx, some of the connecting desmosomes must release from one cell and reattach to a more distal cell.³ This reattachment allows the necessary movement, but places priority on maintaining the integrity of the all-important hoof-pedal bone bond.

AVAS IN THE DERMAL MICROCIRCULATION

The microcirculation of the dermal laminae and papillae of the equine foot was studied by using an improved microvascular casting corrosion technique and scanning electron microscopy.⁴ Casts of veins, arteries, capillaries, and AVAs were readily identified by their characteristic surface morphology. AVAs were found throughout the lamellar circulation but the largest and longest (40 μ m diam) were found clustered close to the origin of axial arteries (Figure 1). A particular characteristic of AVAs in the equine foot was the height and complexity of the endothelial cells. They appeared to be more metabolically active than endothelial cells that line contiguous vessels, and there was evidence that they were involved in the production of potent vasodilators such as substance P, a prostacyclin and endothelium-derived relaxing factor. Direct observation of AVAs in rabbit ear

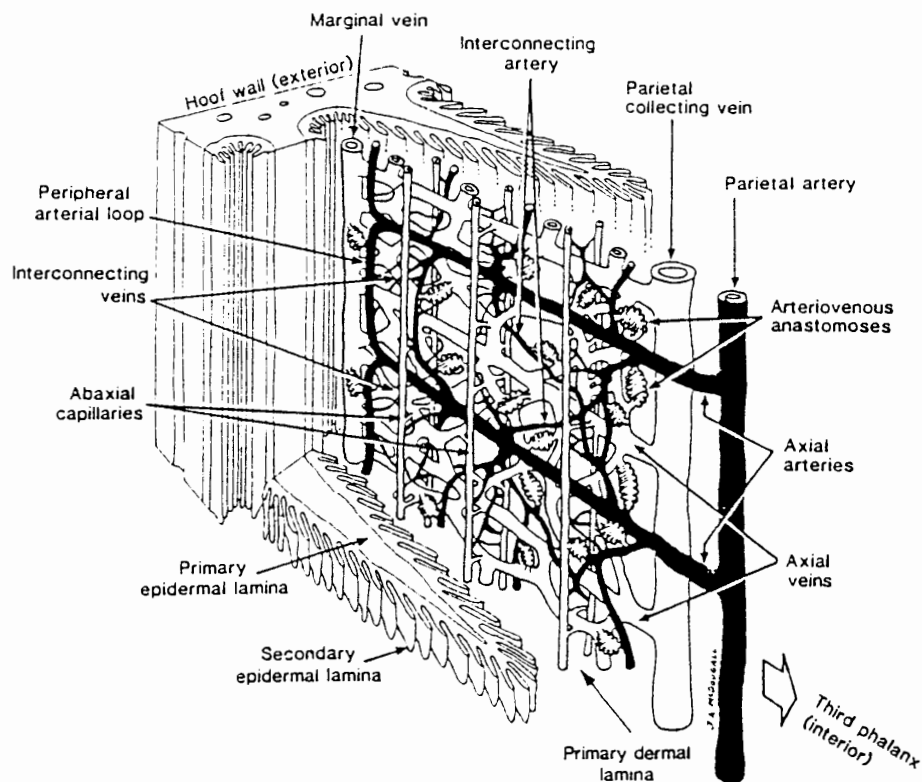


Figure 1. Equine hoof wall lamellar microcirculation. Arteriovenous anastomoses connect artery to vein throughout the lamellar circulation, but are larger and more frequent near the tips of the primary epidermal lamellae.

chambers⁵ has shown that AVAs are autonomous in action and can open and close vigorously. The amounts of glycogen within the smooth muscle cells of the AVA wall in the equine foot is consistent with such high-energy requirements. The density of the laminar AVAs was estimated to be 500 /cm²; densities greater than this have only been reported in the flipper skin of marine mammals such as the Weddell Seal and the Southern Elephant Seal.⁴ Because over 50% of total limb blood flow can pass through AVAs when they are dilated,⁶ it seems likely that AVAs play a causal role in the development of laminitis. Preferential blood flow through AVAs has effected a decrease in flow to arterioles and capillaries.⁶

The innervation of AVAs in the foot of the horse is more dense than that of the arteries and consists of adrenergic and peptidergic nerves.⁷ Adrenergic nerves are recognized by the presence of typical noradrenergic nerve profiles and by catecholamine fluorescence; they form the great majority of nerves around the anastomoses. Nerve profiles containing large granular vesicles and the presence of immunofluorescence showing neuropeptide Y, vasoactive intestinal polypeptide, calcitonin gene-related peptide, and substance P-like reactivity was evidence of a peptidergic innervation (Figure 2).

We have investigated the physiology of equine AVAs. They appear to be under constrictor control that is mediated by both alpha 1 and alpha 2 receptors, and dilator control that is mediated by beta 2 receptors. Only intravenously administered isoxuprine produced a measurable increase in hoof temperature. The oral dose form of the same drug produced no detectable effect. The effects of isoxuprine appeared to be caused by its alpha antagonist and beta mimetic activity.⁸

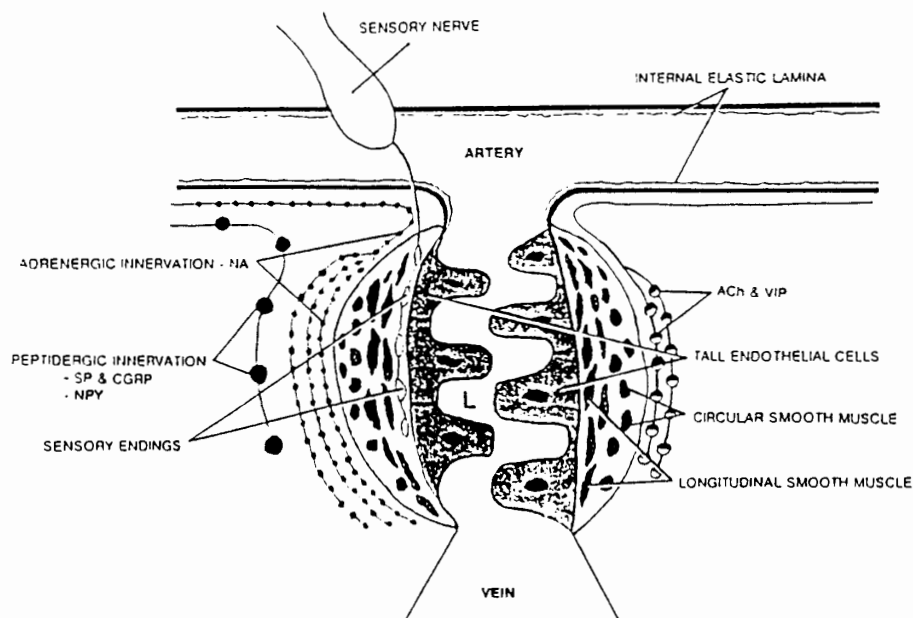


Figure 2. Equine arteriovenous anastomosis (AVA) sectioned at its arterial origin, showing the types and relative positions of the associated nerves. The internal elastic lamina terminates at the origin of the AVA: L, lumen; ACh, acetylcholine; VIP, vasoactive intestinal polypeptide; NA, norepinephrine; CGRP, calcitonin gene-related peptide; SP, substance P; and NPY, neuropeptide Y.

THE ROLE OF AVAS IN LAMINITIS

Arteriovenous shunting of blood was first proposed as the mechanism behind epidermal ischemia, because radioactive, capillary-sized particles failed to lodge in the capillaries of the hoof wall dermis during the developmental stages of the disease.⁹ Studies of the anatomy of the equine digital AVAs^{4,7} have shown that AVA density and innervation are consistent with the proposed role of AVAs in the pathophysiology of equine laminitis. Interestingly, during developmental laminitis, when the epidermal cells are thought to be experiencing ischemia, the lamellar dermis does *not* experience a reduction in total blood flow (which usually increases). This phenomenon was established by Trout,^a who performed angiography with intravascular injections of radionuclide-tagged particles and dyes. Unfortunately, because of technique limitations, he was unable to differentiate between lamellar capillary flow and the flow through AVAs. Thus, conclusive direct proof that epidermal cell ischemic necrosis is preceded by prolonged AVA dilation was not obtained.

We, however, have strong circumstantial evidence that AVA dilation does indeed precede lamellar degeneration. Assuming that equine hooves will become warm whenever AVA dilation occurs, we have monitored the temperature of them while horses develop carbohydrate-overload laminitis. To measure changes in hoof temperature, the experiments were conducted in a controlled temperature room that was set at 10 °C (50 °F). Normal horses kept under these conditions had hoof temperatures that were close to ambient except when a spontaneous warming episode occurred. During a spontaneous warming, the hoof temperature rose through 18 °C to settle at 30 °C (86 °F) for 3-6 hours. The hoof temperature then fell to near ambient. These episodes of hoof temperature change occurred in one foot independently of the other. For example, in the *normal* horse, the left forefoot could feel warm to the touch while the right could feel cool.

During experimental induction of carbohydrate-overload laminitis, all horses abruptly developed hot (hoof temperature 30 °C) forefeet within 16 hours of dosing. If the feet remained hot for longer than 14 hours, the clinical signs of laminitis foot pain (shifting weight from foot to foot, Obel Grade 1 lameness) appeared a few hours later. Sometimes the feet cooled to near-ambient temperatures before the pain started; sometimes they remained hot throughout. Our early results suggest that the longer the foot remains hot, the more severe the lamellar lesions will be. We propose that the foot becomes hot during developmental laminitis and during normal hoof temperature fluctuations because AVAs dilate. Laminitis results when AVA dilation occurs abruptly for a prolonged period of time. In the face of extensive AVA dilation, blood is stolen from the capillary circulation and flows preferentially from artery to vein, which renders the capillary bed stagnant. We are planning to induce laminitis experimentally and use capillary-sized, radioactive, plastic microspheres and blood-flow meters to measure how much blood is shunted through AVAs, and for how long.

^aTrout DR. Scintigraphic evaluation of digital circulation during the development and acute phases of laminitis. Davis: University of California; 1987. PhD dissertation.

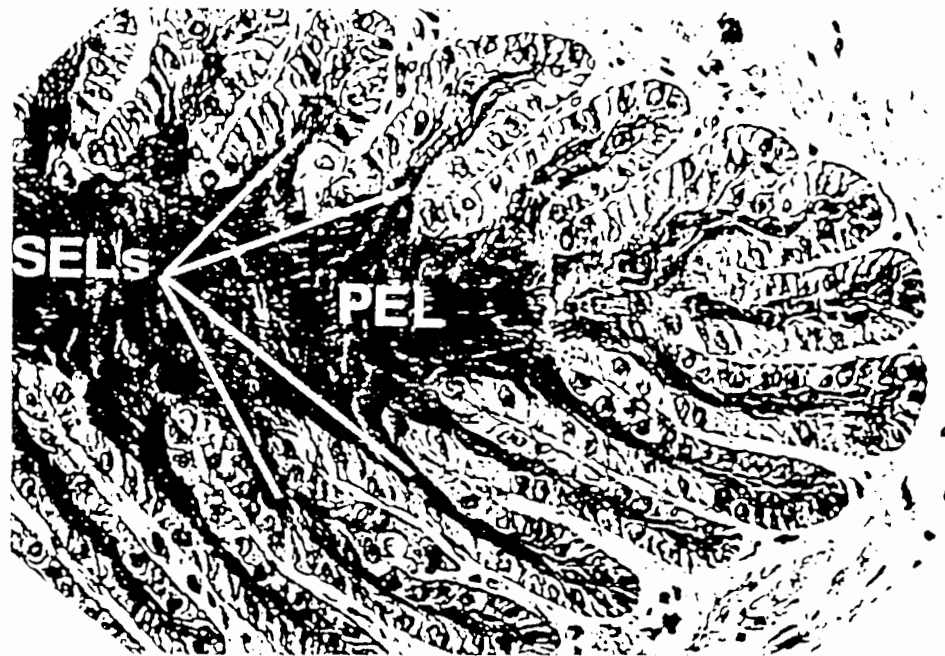


Figure 3. Photomicrograph of normal lamellar structures near the tip of a primary epidermal lamella (PEL). The dermoepidermal junction is clearly defined by the basement membranes of the secondary epidermal lamellae (SELS). Dermal connective tissue penetrates deep into the crypts between adjoining SELs.



Figure 4. Photomicrograph of lamellar structures (same region as Fig. 3) from laminitis horse 36 hours after grain overload. The PEL has been torn from its original position and is now at the left of the picture. The SELs at the tip of the PEL are stretched into long, thin strands often consisting of the empty shells of basement membranes. This process is underway before the clinical signs of pain appear.

Pulsation of the digital arteries also occurs *prior* to lameness (by 12-24 hours). Some horses developed pulsation without subsequent lameness. Pulsation of the digital arteries is almost certainly a sign that arteriovenous shunting is occurring.

STRUCTURAL FAILURE

Transmission electron microscopic studies of our horses with induced laminitis, killed at the first sign of pain, concentrated on the objective of defining ultrastructural lesions that were consistent with failure of the hoof-pedal bone bond. In general, our findings agreed with those of Linford,^b who performed sequential biopsies on hooves as the horses developed starch-overload laminitis. The first lamellar alterations were apparent 24 hours prior to the onset of lameness. Initially the epidermal basal cells lost their uniform shape and the dermoepidermal junction became wavy, as did the outline of the SEL. Within each basal cell the cytoskeleton, that is, the tonofilament network, began to collapse; by the time significant lameness occurred lamellar degeneration was well underway.

Basal cell injury was more wide spread and severe, involving the tips of most PELs. Basal and suprabasal cell necrosis occurred and cytoskeletal element and intercellular junctions failed. Surviving cells, under the influence of tensile forces, elongated into long thin sheets, sometimes one or two cells thick. The tonofilaments of suprabasal cells lost their straight (vertical) configuration and became wavy perhaps because tonofilaments detached (internally) from desmosomes or because the desmosomes themselves had broken down. Disruption of intercellular junctions between basal cells and between basal cells and suprabasal cells allowed tensile forces to pull the basal cells in an interior direction; the suprabasal cells tended to stay behind with the PELs. Suprabasal cell tonofilaments had coalesced into hyperchromic wavy masses. The surviving basal cells that formed the long, thin elongated SELs, so characteristic of early laminitis, contained few tonofilaments.

Observing this phenomenon led Obel^c to conclude, erroneously it now seems, that laminitis was caused by a failure of keratinization; that is, a disappearance of the onychogenic substance (tonofilaments, in other words). We now believe that keratinization does not normally occur in this region anyway.

In specimens prepared by perfusion fixation, we have observed breaks and bubbling of the basement membrane at the dermoepidermal junction.

There is clear evidence that the architecture of the equine hoof-pedal bone bond, normally so orderly and resilient, is destroyed in a remarkably short period of time. There is little doubt that ischemia is the initiating factor that precipitates the damage. The damage is characterized by stretching, tearing, and elongation of both epidermal and dermal lamellar elements as tensile forces disintegrate the weakened structural components.

^bLinford R. A radiographic, morphometric, histological and ultrastructural investigation of lamellar function, abnormality and the associated radiographic findings for sound and footsore Thoroughbreds and horses with experimentally induced traumatic and alimentary laminitis. Davis: University of California; 1987. PhD dissertation.

^cObel N. Studies on the histopathology of acute laminitis. Uppsala, Sweden: Almqvist & Wiksells Boktryckeri; 1947. PhD thesis.

PRACTICAL IMPLICATIONS

Unfortunately, by the time pain is evident and veterinary attention is directed to the horse's feet, significant and sometimes overwhelming architectural damage has already occurred. Knowledge of this damage has serious prognostic and treatment ramifications.

Tonofilament-poor basal cells, stretched into thin weak SELs, can never reattach themselves to their original PEL. They have to wait for a new PEL, formed in the coronary band, to grow down from above. In the meantime the basal cells lose contact inhibition and respond to the insults that have befallen them by proliferating, eventually producing a mass of weak flaky horn; this is the laminar wedge. The wedge must be removed or allowed to grow out if rehabilitation of the hoof-pedal bond is to occur.

Epidermal changes, significantly without dermal inflammation, also develop in nonweightbearing appendages such as the chestnut and ergot. Nevertheless the question remains: Would horses suffer failure of their hoof-pedal bone bond if their feet could be relieved of weightbearing (in a flotation tank perhaps)?

The sequential biopsy technique of Linford^b clearly shows that lamellar necrosis, lamellar elongation, and lameness all precede inflammation and edema. Inflammation does not initiate laminitis. When it does appear, after the architecture of the lamellar epidermis is disintegrating, it undoubtedly exacerbates the destruction of the hoof-pedal bone bond and justifies the use of nonsteroidal anti-inflammatory drugs and perhaps antihistamines.

Endotoxemia is often superimposed on the clinical picture of developmental and acute laminitis. Although experimental endotoxin administration has never induced laminitis, the coagulopathy and other vascular changes associated with endotoxemia probably exacerbate the laminitis lesion in its late acute stage. Low doses of flunixin meglumine (0.25 mg/kg) and heparin are often useful in this regard.

Laminitis has occurred in horses following vigorous corticosteroid therapy. After carbohydrate overload corticosteroid administration increases mortality, lameness and the severity of the clinical syndrome. Corticosteroids appear to potentiate the effects of catecholamines on the digital vasculature and are contraindicated in the treatment of laminitis.

Hypertension and peripheral vasoconstriction are coincident with pain. Because lamellar degeneration precedes pain, these vascular changes cannot cause the lesion. However, as pain-induced vasoconstriction exacerbates perfusion failure, the use of adrenergic alpha-blockers such as acepromazine and phenoxybenzamine, as well as pain relief, is rational.

Keeping the feet in warm water (40-45 °C) would physiologically encourage vasodilation of the capillary circulation at the expense of AVA flow; this is a logical treatment modality in the developmental and early acute stage.

Why are the lamellae of the dorsal hoof wall invariably affected most severely? The answer to this question probably lies with the anatomical commitment the horse has made to supplying the lamellar circulation through arteries that emanate from the dorsal surface of the third phalanx. When the hoof-pedal bone bond begins

to fail, break-over and weightbearing forces combine to tear the pedal bone from the hoof wall. The pedal bone descends and is anatomically committed to taking the terminal arch, and hence the lamellar blood supply, with it. The deeper the bone descends into the hoof capsule, the more devastating the shearing trauma to the lamellar blood supply will be. Consequently, every effort should be made to ameliorate the devastating effects of the forces of hoof breakover and weight bearing. Frog support, heel elevation, and soft bedding help in this regard. Forced exercise is definitely contraindicated.

CONCLUSION

Our current working hypothesis is that a perturbation of AVA control explains the paradox of how heat, epidermal ischemia (without inflammation), and a bounding digital pulse can all occur concurrently during developmental laminitis. Drinking cold water during heat stress and the pyrexia of endotoxemia probably upset the central hypothalamic thermoregulatory set point and trigger prolonged AVA dilation, sometimes to the point of laminitis. Our ultrastructural and immunofluorescence studies show the wall of the equine digital AVA to be invested with a surprisingly numerous array of neurological transmitter substances. We aim to understand the pharmacology of these, and their antagonists, and elucidate the fundamental cause of equine laminitis. We intend to help develop therapies for this crippling equine disease that are more efficacious than those currently available.

REFERENCES

1. Kempson SA. The structure of the equine hoof horn: a light and electron microscope study, in *Proceedings*. Annu BI Lam Symp 1991; 46-51.
2. Pollitt CC. An autoradiographic study of hoof growth. *Equine Vet J* 1990;22:366-368.
3. Leach DH, Oliphant LW. Ultrastructure of the equine hoof wall secondary epidermal lamellae. *Am J Vet Res* 1983;44:1561-1570.
4. Pollitt CC, Molyneux GS. A scanning electron microscopical study of the dermal microcirculation of the equine foot. *Equine Vet J* 1990;22:79-87.
5. Molyneux GS. Preferential blood flow through arteriovenous anastomoses. *J Anatomy* 1985;143:217-218.
6. Hales JRS, Molyneux GS. Control of cutaneous arteriovenous anastomoses. In: Van Houte PM, ed. *Mechanisms of vasodilation*. New York: Raven Press, 1988.
7. Molyneux GS, Haller CJ, Mogg KC, et al. The distribution, structure and innervation of arteriovenous anastomoses in the equine foot. *J of Anatomy* (in press) 1989.
8. Mogg KM, Pollitt CC. Hoof and distal limb surface temperature in the normal pony under the influence of drugs used clinically in equine laminitis. *Equine Vet J* (in press) 1991.
9. Hood DM, Amoss MS, Hightower D, et al. Equine laminitis I. Radioisotopic analysis of the haemodynamics of the foot during the acute disease. *Equine Med Surgery* 1978;2:439-444.