

THE HORSE'S FOOT – THE INSIDE STORY

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Introduction

Members of the mammalian family Equidae represent the extreme result of digitigrade evolution. Single digits, encased in tough, keratinized hooves, on the end of relatively lightweight limbs, have undoubtedly, contributed to the speed and versatility of the equids. This characteristic makes the horse (and the other Equidae) unique in the animal kingdom. The tough hoof capsule protects the softer, more sensitive, structures within and allows the natural horse to gallop over dry, rocky terrain with apparent impunity. But, at a price: immobility and crippling result if the connection between hoof and bone (the lamellar, distal phalangeal attachment apparatus) fails. Research into the structure and function of the hoof wall and its inner lamellar layer (Fig 1) has proven fundamental to the understanding of how laminitis develops. Considerable selection pressure against laminitis must exist among wild equids as a foundered animal would quickly attract the attention of predators and be eliminated from the gene pool. Equids are normally mobile and athletic but when they develop laminitis and become crippled we realize, belatedly, how dependent they were on an intact, functional, pain-free lamellar distal phalangeal attachment apparatus

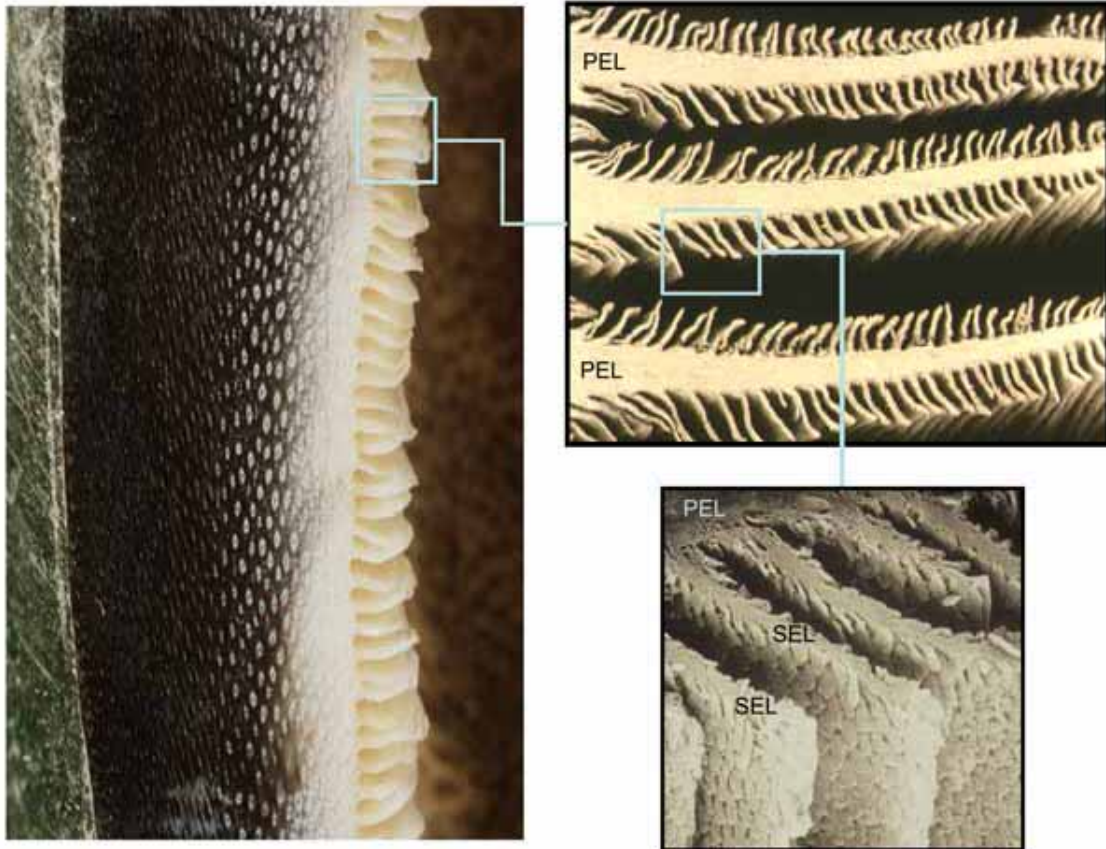


Fig 1. The equine hoof wall and its inner lamellar layer. Secondary epidermal lamellae (SELs) increase the surface area of each leaf-like primary epidermal lamella (PEL). Epidermal basal cells cover the surface of each SEL.

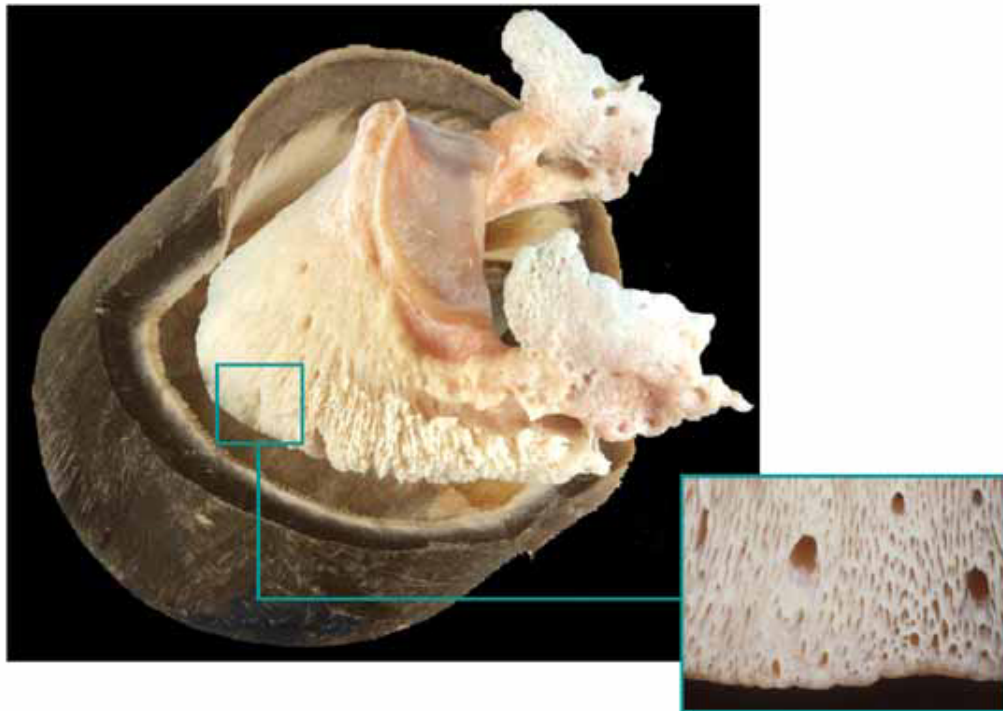


Fig 2. Normally the distal phalanx and the inner hoof wall maintain a constant, spatial relationship with each other attached by a strong yet flexible connective tissue attachment apparatus. The parietal surface of the distal phalanx is perforated by many vascular channels, some large and some small (inset).

Hoof wall keratinisation

The word *keratin* is from the Greek *keratos* for horn, which is appropriate for a discussion on the horse's hoof. Keratin is the main structural protein of the epidermis and is present in skin, hair, nail, claw, wool, horn, feather, scale as well as hoof. The keratins can be loosely grouped into the "soft" keratins of skin and the "hard" keratins of horn and hair etc. The tubular hoof of the wall and sole is composed of hard keratin, is rich in disulphide bonds and has great physical strength. The periople, frog and the white zone on the other hand are rich in sulphhydryl groups but poor in disulphide bonds and have lower physical strength but greater elasticity (Bragulla *et al.* 1994). Non stop production of new hoof makes good the continual loss of hoof wall, occurring at the distal ground surface. The strength, hardness and insolubility of keratin is due to disulphide bonds between and within its long chain fibrous molecules (Priestley 1993). The sulphur containing amino acids methionine and cysteine are incorporated into the keratinocytes in the final stages of its maturation hence the requirement of these amino acids (or their sulphur containing precursors) in the diet. There are in fact dozens of different keratin molecules, some large and some small, with

varying degrees of hardness and sulphur concentration, expressed in hoof tissues in accordance with their functional destiny.

Hoof wall growth

The hoof wall grows throughout the life of the horse to replace hoof lost to wear and tear at the ground surface. Continual regeneration of the hoof wall occurs at the coronet where germinal cells (epidermal basal cells) produce populations of daughter cells (keratinocytes or keratin producing cells) which mature and keratinize, continually adding to the proximal hoof wall. Similarly, mitosis in the proximal hoof primary epidermal lamellae (PELs) also occurs (Leach 1980). Although mitotic figures (MFs) among the basal cells of the proximal lamellar zone are easily observed, there is no convincing evidence that the more distal lamellae proliferate at all. The fundamental question is; how do the inner hoof wall lamellae remain attached to the connective tissue embedded on the surface of the stationary distal phalanx, while one moves over the other? Is it by continuous proliferation of the lamellar epidermis (laminar flow) or by some other remodeling process (that may somehow be involved in laminitis pathogenesis)? Cells in mitosis are rarely, if ever, found in normal lamellae below the proximal, proliferative zone. To determine precisely where in the hoof wall epidermal cell proliferation occurs, a proliferative index (PI) for basal cells of the coronet, lamellae and toe of the dorsal hoof wall of ponies has been calculated (Daradka and Pollitt 2004). To accomplish this we injected ponies with a substance that tricked dividing cells into incorporating a false nucleotide (5-bromo-2'-deoxyuridine or BRdU), into their replicating DNA. In subsequent hoof biopsies we could track cells that had taken up BRdU using anti-BRdU antibody and thus detect all cells that had divided.

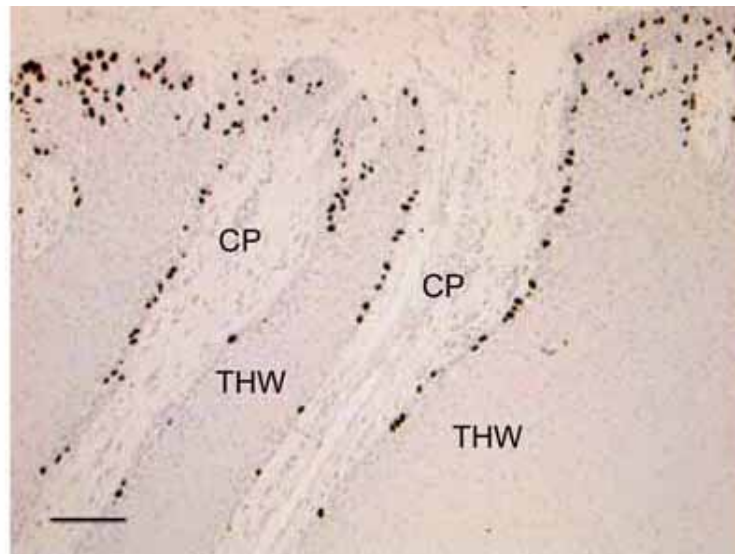


Fig 3. Basal cell proliferation in the coronary band. Longitudinal section of proximal hoof wall (coronary band) immunostained for BRdU that was injected intravenously into a normal pony 60 minutes previously. The positive, brown staining, cells are basal cells that incorporated BRdU as they underwent mitosis during the previous 60 minutes. Both the tubular and intertubular hoof show a high rate of basal cell mitosis. CP = coronary papilla. THW = tubular hoof wall. Bar = 100 μ m.

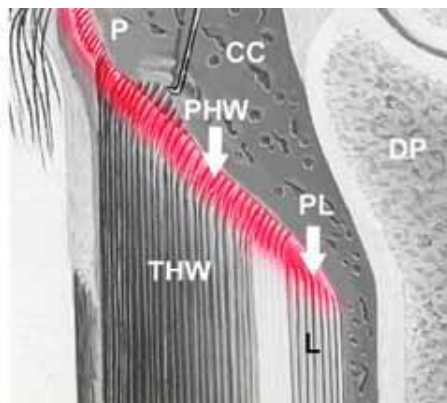


Fig 4. The growth zones of the hoof (highlighted in red) are confined to the top or proximal region of the wall. Basal cells of the tubular hoof wall and periople proliferate non-stop throughout the life of the horse. The proximal lamellae also proliferate at a rate similar to the hoof wall proper, but the rate is near zero in the lamellar regions below this. CC = coronary corium. DP = distal phalanx. P = periople. PHW = proximal hoof wall. PL = proximal lamellae. THW = tubular hoof wall. L = lamellae.

As expected the growth zones of the proximal hoof wall were the coronet and the proximal lamellae (Fig 4). Below the growth zone proliferation in more distal lamellae was almost non-existent. Evidence for a constant supply of new cells in the lamellar region, generating a downward laminar flow, was not provided by this study. The few proliferating cells detected in the main lamellar region had a patchy distribution and were located at the PEL tips, not in cap-horn arcades. A 20-fold decrease in cell proliferation between proximal and more distal lamellae suggests that the majority of the normal lamellae are non-proliferative and their main function is to suspend the distal phalanx within the hoof capsule.

Remodeling within the hoof wall epidermal lamellae, which must occur as the hoof wall moves past the stationary distal phalanx, appears to be a process not requiring epidermal cell proliferation (mitosis). Remodeling of hoof epidermis is now known to involve the controlled release of a class of enzymes called matrix metalloproteinases (MMPs). MMPs have been shown to exist in lamellar hoof (Fig 5) and their uncontrolled activation has been proposed as a mechanism for the pathogenesis of laminitis (Kyaw Tanner and Pollitt 2004; Pollitt *et al.* 1998).

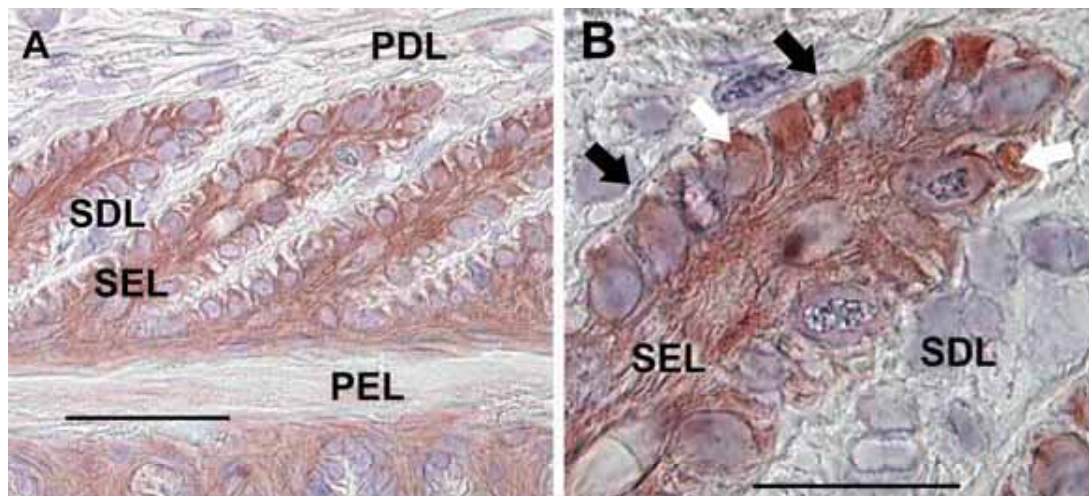


Fig 5. Immunostaining of normal hoof secondary epidermal lamella (SEL) with anti MMP-2. Dark brown, positive cytoplasmic staining was located mainly in lamellar basal and parabasal cells (A). Primary epidermal lamellae (PEL) and primary dermal lamellae (PDL) did not stain. Basal cell MMP-2 of SELs (B) was located in cytoplasm (solid white arrows) adjacent to the basement membrane (solid black arrows). Nuclei of epidermal cells and fibroblasts stained blue by the haematoxylin counterstain. Bar in A = 50 μ m. Bar in B = 20 μ m. (With permission Equine vet. J)

The filamentous connections between hoof cells and the membrane that connects hoof to connective tissue and bone are substrates for MMP activity (Woessner 1991). Thus the mechanistic concept for hoof growth, first proposed in 1983 (Leach and Oliphant 1983); “formation and destruction of hoof cell connections, in a staggered ratchet-like manner” now has a well referenced, biological explanation. Lamellar epidermal cells and their adjacent basement membrane are constantly responding to the stresses and strains of growth and locomotion by releasing MMPs and natural inhibitors (TIMPS) to accomplish whatever cellular reorganisation is required. Since this involves enzymes capable of destroying key components of the attachment apparatus between distal phalanx and inner hoof wall it is clear that triggering this “loaded gun” will have dire consequences for the future health of the foot. Inadvertent or uncontrolled lamellar MMP activation makes horses, with their generic reliance on a single digit per limb, uniquely susceptible to the destructive effects of laminitis.

Hoof wall tubules

Examination of the hoof capsule, with its contents removed, shows thousands of small, circular, holes pocking the surface of the concave, coronary groove (Fig 6). A section through the upper hoof wall shows that the holes continue down into the body of the wall a distance of 4-5 mm, gradually tapering to a point. A layer of living epidermal basal cells covers the surface of the holes and the surface of the coronary groove between the holes.

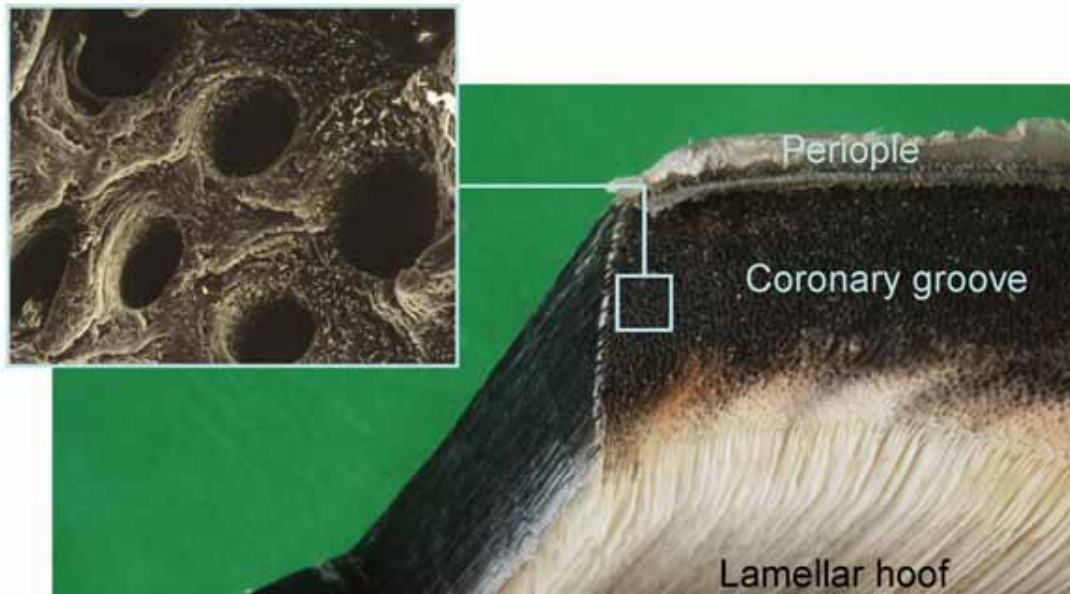


Fig 6. The hoof capsule, with its contents removed, shows thousands of small, circular, holes pocking the surface of the concave, coronary groove. Germinative epidermal basal cells cover the surface of the holes and the surface of the coronary groove between the holes (Inset).

As already shown coronet basal cells undergo mitosis throughout the life of the horse producing hoof wall (*stratum medium*) daughter cells which mature and keratinise undertaking a journey, up to 8 months in duration, in the direction of the ground surface. Maturing hoof cells, arising from basal cells lining the holes, become organised into thin, elongated, cylinders or tubules approximately 0.2mm in diameter (Kasapi and Gosline 1998). In cross-section the keratinocytes of individual hoof wall tubules are arranged around a central hollow medulla in non pigmented, concentric layers (Fig 7).

Each hair-like tubule is continuous, from its origin at the coronet all the way to the ground surface (a distance of 5-15 cm depending on the breed). It is often stated

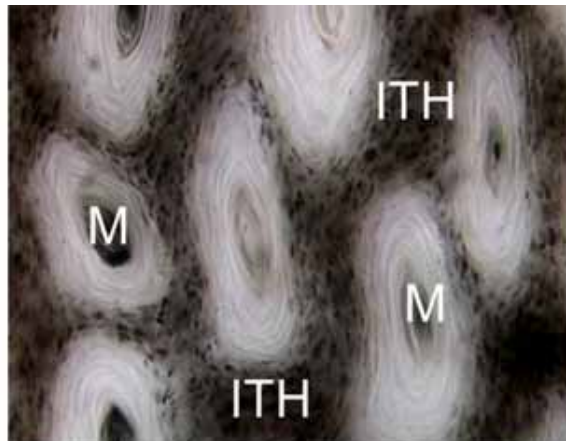


Fig 7. Transverse section of a pigmented hoof wall (unstained). The intertubular hoof (ITH) is heavily pigmented and is the strongest component of the hoof wall. In contrast, the unpigmented tubules of the hoof wall have a hollow, central medulla (M) around which are arranged concentric layers of mature keratinocytes (magnification x 200).

that hoof wall tubules are hollow to transfer water by capillary action either into the hoof from the outside or down the hoof from the coronary papillae. This has given rise to the recommendation that horses be made to stand in water to benefit the hydration status of their hooves. However detailed testing of the water content of the hoof wall has consistently refuted this dogma and in fact tubules at the ground surface act to dehydrate the hoof wall and are unable to transfer water inwards (Kasapi and Gosline 1998). The explanation of why hoof wall tubules are hollow lies with their mechanical (crack redirection) properties rather than their water conducting propensity. Water gradients in the hoof wall are governed by proximity to the dermis; the most highly hydrated areas are adjacent to the corium. Air filled medullary cavities are unlikely to contribute much to the insulating properties of the hoof either. It is estimated that the medullary spaces decrease thermal conductivity by only 7% (Kasapi and Gosline 1998) and indeed dorsal hoof wall temperatures of around 25⁰C (15⁰C above an ambient temperature of 10⁰C) were maintained for 8-10 h in normal horses kept in a controlled environment chamber (Pollitt and Davies 1998).

The keratinocytes generated between the holes mature into inter-tubular hoof thus forming a keratinised cellular matrix in which tubules are embedded. The intertubular horn is formed at right angles to the tubular horn and bestows on the hoof wall the unique property of a mechanically stable, multidirectional, fibre-reinforced composite (Bertram and Gosline 1987). Interestingly hoof wall is stiffer and stronger at right angles to the direction of the tubules a finding at odds with the usual assumption that the ground reaction force is transmitted proximally up the hoof wall parallel to the tubules. The hoof wall appears to be reinforced by the tubules but it is the intertubular material that accounts for most of its mechanical strength stiffness and fracture toughness. The tubules are 3 times more likely to fracture than intertubular horn (Bertram and Gosline 1986; Leach and Oliphant

1983)). The hoof wall proper (*stratum medium*) is considered to have an anatomical design that confers strength in all directions. Unlike bone which is a living tissue and remodels to become stronger along lines of stress the hoof wall non-living tissue but is anatomically constructed to resist stress in every direction and to never require remodelling. During normal locomotion the hoof wall experiences only one-tenth of the compressive force required to cause its structural failure (Thomason *et al.* 1992).

Mature hoof cells, firmly cemented together, form a tough protective barrier preventing the passage of water and water soluble substances inwards and the loss of body fluids, imparted by the adjacent, highly vascular dermis, outwards. In addition to acting as a permeability barrier, hoof wall cells, arranged in their specialised tubular and intertubular configuration, have the crucial job of ultimately supporting the entire weight of the horse (Pollitt 1998).

The tubules of the equine hoof wall are not arranged randomly and form recognisable zones based on tubule density (Reilly *et al.* 1998). The zone of highest tubule density is the outermost layer and the density declines stepwise towards the internal lamellar layer. Since the force of impact with the ground (the ground reaction force) is transmitted up the wall (Thomason *et al.* 1992) the tubule density gradient across the wall appears to be a mechanism for smooth energy transfer, from the rigid (high tubule density) outer wall to the more plastic (low tubule density) inner wall, and ultimately to the distal phalanx. The gradient in tubule density mirrors the gradient in water content across the hoof wall and together these factors represent an optimum design for equine hoof wall. It has been argued that tubule zonation is also a crack-stopping mechanism (Reilly *et al.* 1996). The zones confer on the hoof wall the design properties of a laminated composite; the interface between zones absorbs energy and prevents the propagation of cracks towards sensitive inner structures. In addition the anisotropy (stronger in one direction) of the *stratum medium* ensures that cracks, when they occur propagate from the bearing surface upwards parallel with the tubules ie along the weakest plane. They do not extend to the innermost layers of the hoof wall because in this region the relatively high water content confers high crack resistance (Bertram and Gosline 1986). The hoof wall also has a powerful dampening function on vibrations generated when the hoof wall makes contact with the ground during locomotion. It is able to reduce both the frequency and maximal amplitude of the vibrations (Dyhre-Poulsen *et al.* 1994). By the time the shock of impact with the ground reaches the first phalanx around 90% of the energy has been dissipated, mainly at the lamellar interface.

The corium

The highly vascular corium or dermis underlies the hoof wall and consists of a dense matrix of tough connective tissue containing a network of arteries, veins and capillaries, and sensory and vasomotor nerves. All parts of the corium, except for the lamellar corium, have papillae that fit tightly into the holes in the adjacent hoof. The lamellar corium has dermal lamellae that interlock with the epidermal lamellae of the inner hoof wall and bars (Fig 8). The vascular system of the corium provides the hoof with nourishment. The dense matrix of corium connective tissue connects the basement membrane at the lamellar interface to the periosteal surface of the distal phalanx and thus suspends the distal phalanx from the inner wall of the hoof capsule.

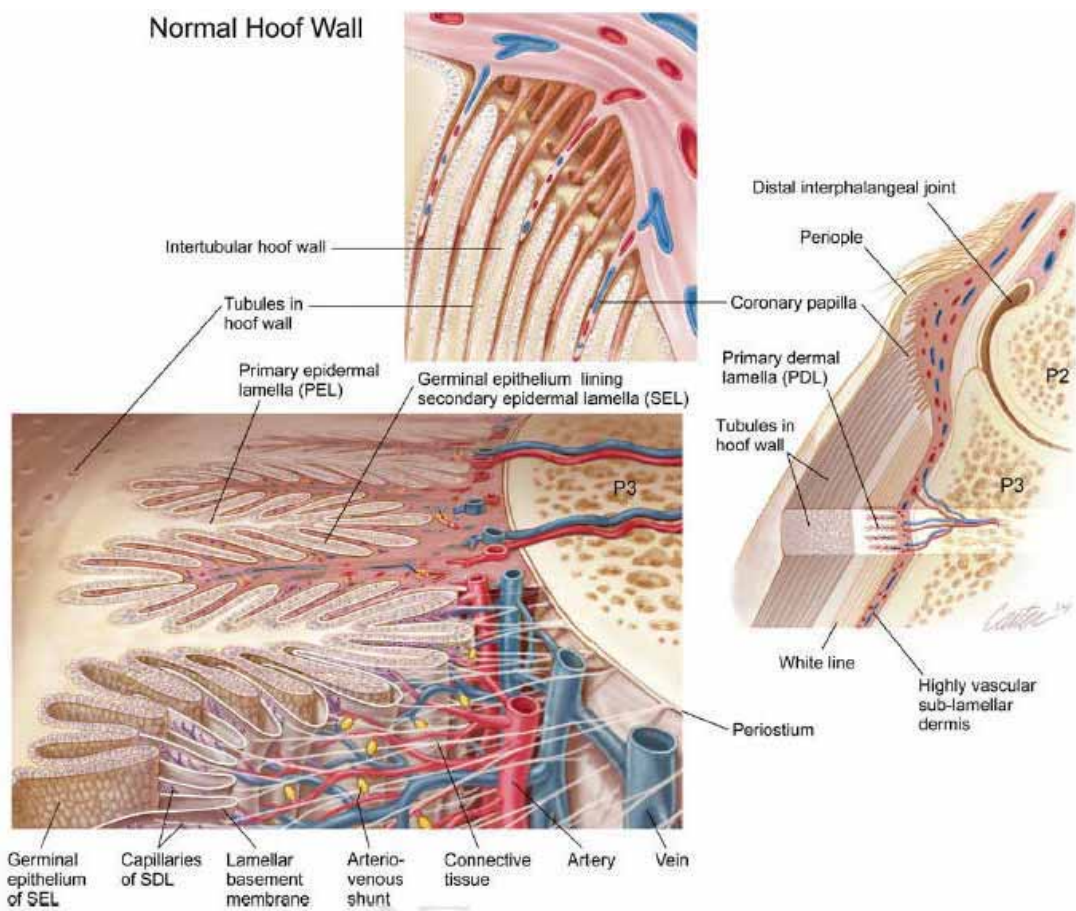


Fig 8. Diagram showing the dermal papillae of the coronet and interdigitating lamellae of the inner hoof wall. The lamellar basement membrane is shown detached to illustrate the underlying epidermal basal cells.

The coronet corium

The coronet corium fills the coronary groove and blends distally with the lamellar corium (Fig 8). Its inner surface is attached to the extensor tendon and the unguis cartilages of the distal

phalanx by the subcutaneous tissue of the coronary cushion. Collectively the coronary corium and the germinal epidermal cells that rest upon its basement membrane are known as the coronet or coronary band. A feature of the coronet corium is the large numbers of hair-like papillae projecting from its surface. Each tapering papilla fits into one of the holes on the surface of the epidermal coronary groove and in life, is responsible for nurturing an individual hoof wall tubule.

We have examined hoof wall papillae with the high-power, scanning electron microscope after treatment of hoof tissue blocks with a detergent enzyme mixture (Pollitt 1994). A clean separation could be made between dermal and epidermal tissues enabling the surface of the dermal basement membrane to be examined in detail. The surface of the coronary and terminal papillae was folded into numerous ridges parallel with the long axis of the papilla. The ridges increase the surface area of attachment between hoof and connective tissue and also act as guides or channels directing columns of maturing hoof cells in the correct downward direction (Fig 9). The density of coronary papillae is greatest on the outside and least, on the inside of the hoof wall, adjacent to the lamellae. This mirrors the arrangement of the hoof wall tubules of the hoof wall in zones based on tubule density (Reilly *et al.* 1998).

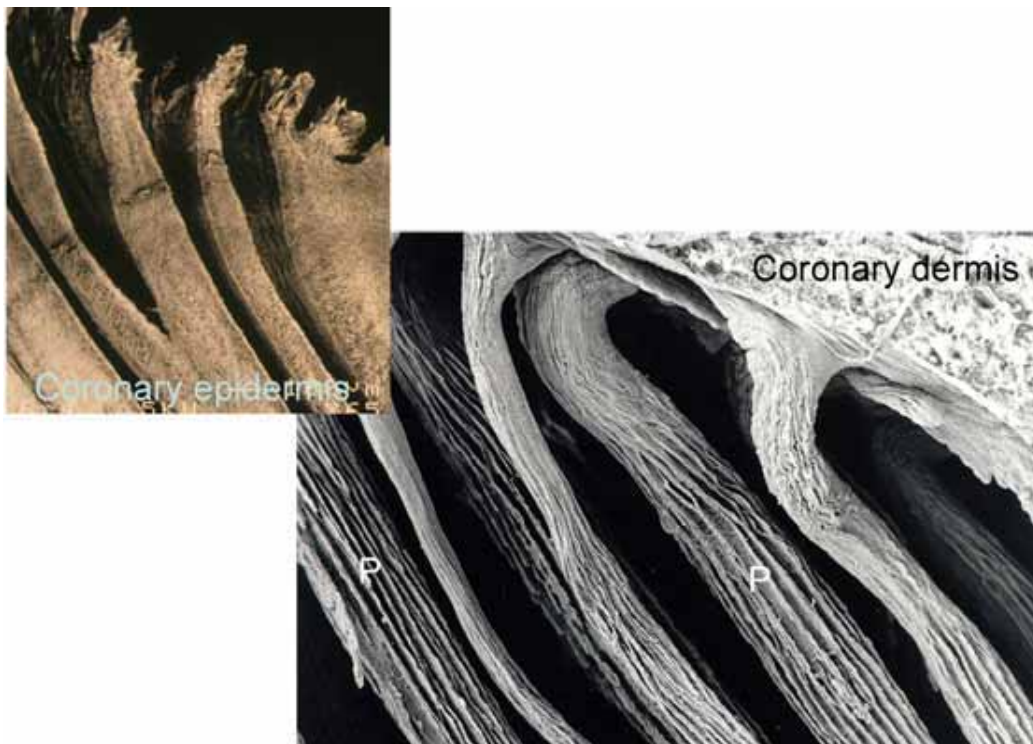


Fig 9. Epidermis and dermal papillae of the coronet. Each papilla (P) is responsible for the nutrition and organisation of an individual hoof wall tubule. The papillae fit into complementary sockets in the coronary groove of the epidermal hoof wall (upper inset).

The sole corium

As in the coronet each dermal papilla of the sole corium fits into a socket in the epidermal (horn) sole. At the distal end of each dermal lamella is a special set of papillae known as the terminal papillae (Fig 10). The epidermis surrounding the terminal papillae is non-pigmented and forms the inner part of the white zone (white line). The white zone is relatively soft and flexible and effectively ‘seals’ the sole to the hoof wall. It is sometimes subject to degeneration and infection, usually described as ‘seedy toe’ or ‘white line disease’. It is of special relevance to farriers because horseshoe nails are driven at the white line and if a nail is set too close pain and infection may result if terminal papillae are penetrated. The colloquial terms for this event are “hot nail”, “quicking” or “pricking”. Immediate antibiotic and anti-tetanus therapy is indicated to prevent serious consequences.

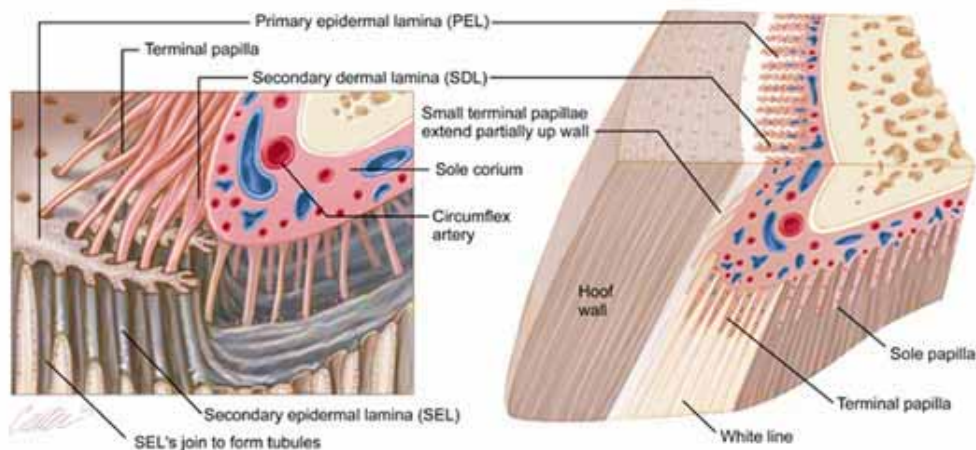


Fig 10. On the distal end of all dermal lamellae are numerous terminal papillae. Germinal epidermis lining the terminal papillae are responsible for generating keratinised epidermal cells which fill the space between the primary epidermal lamellae as they grow toward the ground surface.

The blood supply of the foot

Digital arteries: The medial and lateral digital arteries arise by division of the common digital artery above the fetlock and enter the digit on either side of the pastern (Fig 11).

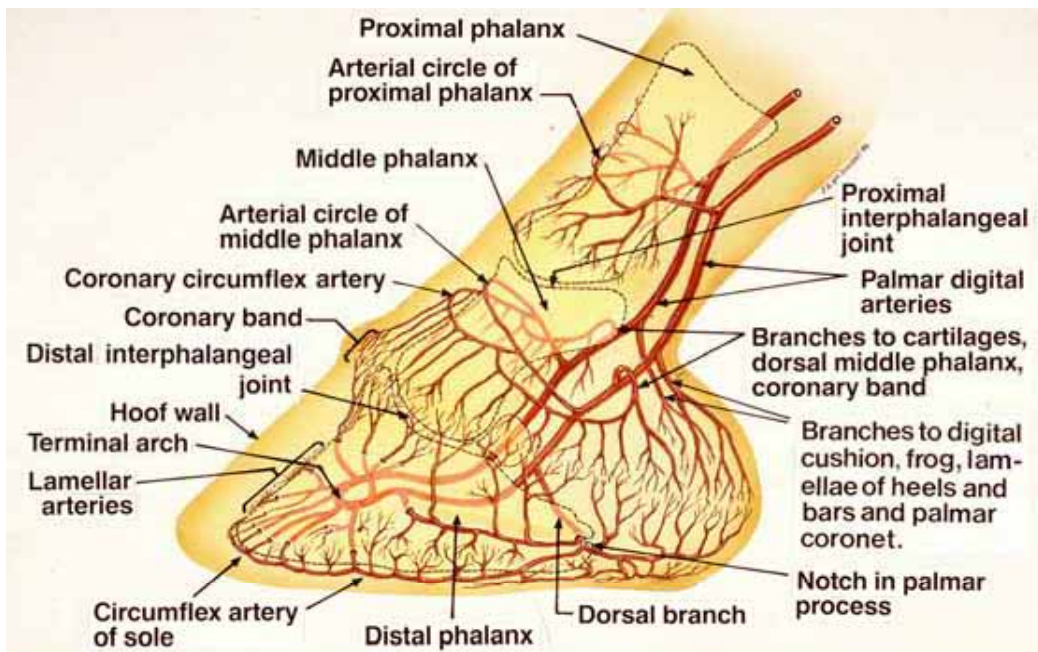


Fig 11. Diagram of the arteries of the equine foot. Design C. C. Pollitt, Artwork J. McDougall.

At the level of the first pastern joint, the digital arteries send major branches to the heels which supply the digital cushion, frog, lamellar and coronet corium of the heels and bars. Opposite the middle of the second phalanx, each digital artery again branches and forms an artery which runs deep to the cartilages and the extensor tendon, and connects with the artery of the opposite side, to form an arterial circle around both the second phalanx and coronary band. This coronary circumflex artery supplies the digital extensor tendon, distal interphalangeal (coffin) joint and supplies numerous branches to the coronary corium and proximal lamellae of the toe and dorsal quarters.

The terminal parts of the digital arteries enter a canal deep in the body of the distal phalanx via a pair of holes at the back of the bone. The arteries unite with each other to form the terminal arch deep within the bone. Branches of the terminal arch radiate outwards through foramina in the dorsal surface of the distal phalanx and supply the lamellar corium and, after forming the circumflex artery, the corium of the sole.

In addition to the 12-15 main foramina, the dorsal surface of the distal third of the distal phalanx is perforated by numerous finer foramina (the bone in this region is very porous) and we have discovered that many of the vessels within these foramina are arranged anatomically to perform counter-current heat exchange, ie a central artery surrounded by a sheath of capillaries and venules (similar to the pampiniform plexus that cools the mammalian testis). This implies that the equine digit is an efficient thermoregulatory organ which is not surprising when the range of equine habitats, from the sub-arctic to the equator, is taken into consideration.

We have evaluated the effect of cooling the distal limb of horses by continuously applying an ice and water slurry, to the lower part of one forelimb of 7 horses. The mean temperature of the cooled forelimbs, measured using probes inserted 7 mm deep in the hoof wall, averaged $5.3-0.3^{\circ}\text{C}$ for 46 h after an initial stabilization period of 2 h. A marked reduction ($27.1-0.3^{\circ}\text{C}$) in treated limb hoof temperature compared with the untreated limbs was noted in this study. No adverse clinical effects were noted in the cooled limbs up to 1 year following cessation of the cryotherapy. Cold-induced pain, observed in human patients when cryotherapy was applied at 5°C for 48 h, was not noted in this study. In a second study 6 horses were stood in a circulating cold water bath for 72 h. Thus all 4 limbs were subjected to continuous cryotherapy and when removed from the water bath the horses were observed for 4 days. There was mild transient oedema of the distal limbs but no lameness or skin damage. Histological examination of hoof wall lamellae, taken 7 days after cryotherapy commenced, showed no lesions.

It is clear that the equine distal limb is uniquely resilient to the effects of extreme, continuous cold application. Additionally, horses show no signs of adverse effects in climates where their distal limbs are continuously exposed to low environmental temperatures. Arteriovenous anastomoses (AVAs) or shunts are normal, structures connecting the arterial and venous sides of the circulation. They occur in the peripheral circulation of terrestrial and marine mammals and birds and are important in thermoregulation. Equine AVAs, more densely innervated than contiguous vessels, are located in the equine foot in the dermis of the coronary band, in the coronary and terminal papillae, in neurovascular bundles at the base of the dermal laminae and at the entrance to and along the length of the dermal laminae. The density of AVAs in the foot of the horse has been conservatively estimated at $500/\text{cm}^2$ a value which is significantly higher than in the rabbit ear and sheep leg skin but less than in the skin of the Weddell seal. The number and placement of AVAs in the equine foot indicates their strategic placement to influence peripheral blood flow and perhaps the nutrition and integrity of the dermal-epidermal attachment. The role of cutaneous AVAs in thermoregulation is well established. It is dependent upon the large volume of warm arterial blood that can be shunted into superficial veins, thus promoting heat loss by radiation. In the horse heat loss through the hooves would not be substantial, but AVAs may contribute to the maintenance of tissue metabolism by allowing perfusion of the feet when the horse is in a cold environment, such as standing in cold water or on ice. We have obtained information on the physiological activity of the AVAs in horses' feet and foot circulation in general by measuring hoof temperature in cold environments.

Six mature horses, with clinically normal feet and acclimatized to winter in Norway were kept in outside yards and fed *ad lib* hay. Forelimb hoof temperature and ambient temperature were logged continuously using data logging devices (Tinyview Plus Data Loggers)^a attached to thermistors by 2

m cables. The hoof temperature thermistors were housed in stainless steel probes that were inserted into holes, drilled into the hoof wall 20 mm distal to the coronet of both forelimbs. The data loggers recorded temperature at 5 min intervals. The information stored in each of the four data loggers was downloaded to a computer at the end of the experimental period.

The horses ignored the probes, cables and pouch attached to their feet legs and body. Some probes were dislodged and damaged when the horses lied down and scraped the dorsal hoof surface on to hard packed snow. Probes and cables were also damaged when the horses walked in deep snow that tore the cables away from their attachments. Nevertheless successful continuous recordings were made over periods ranging from 2-3 days. Ambient temperatures ranged from 0⁰C to -12⁰C. Hoof temperatures were continuously fluctuating over a remarkably wide range. Sometimes they were close to zero and at other times they were 30⁰C (Fig 11B). Mostly the left and right hoof temperatures fluctuated together. However sometimes this linkage was lost and a left hoof temperature was close to zero while the right approached 20⁰C. In some recordings a diurnal rhythm was present with lowest hoof temperatures occurring between 6am and midday irrespective of ambient temperature. However sometimes this rhythm was lost and in other horses never occurred at all. High and low hoof temperature did not correlate to changes in ambient temperature.

Horses appear able to exert a fine degree of control over the temperature of their feet probably by a central mechanism. In one horse the right and left hoof temperature was 30⁰C when the ambient temperature was -10⁰C; a gradient of 40⁰C. This suggests that the temperature of horses' feet is controlled by potent mechanisms. The strategic distribution of AVAs in the circulation of the foot and their rich endowment of vasomotor nerves and peptides suggest these structures are the key players effecting this remarkable thermoregulatory control. The horses in the trial at no stage showed signs of foot discomfort that could be attributed to low foot temperature even when foot temperatures of 1-2⁰C were recorded. This confirms a conclusion from our cryotherapy studies that severe cold is not noxious to the horse. Hoof temperature never fell below zero thus preventing thermal damage. Why the high temperatures? The foot removes considerable glucose from its arterial circulation presumably for important energy consuming metabolic functions. Hoof growth and maintenance of the hoof distal phalanx attachment apparatus are two such functions. Frequent warming of the foot no matter what the ambient temperature appears to be a feature of normal foot physiology perhaps to ensure that metabolism and growth are never compromised. Practitioners palpating or thermal imaging either warm or cold horses' feet should not be surprised; both are manifestations of normal foot thermoregulatory control.

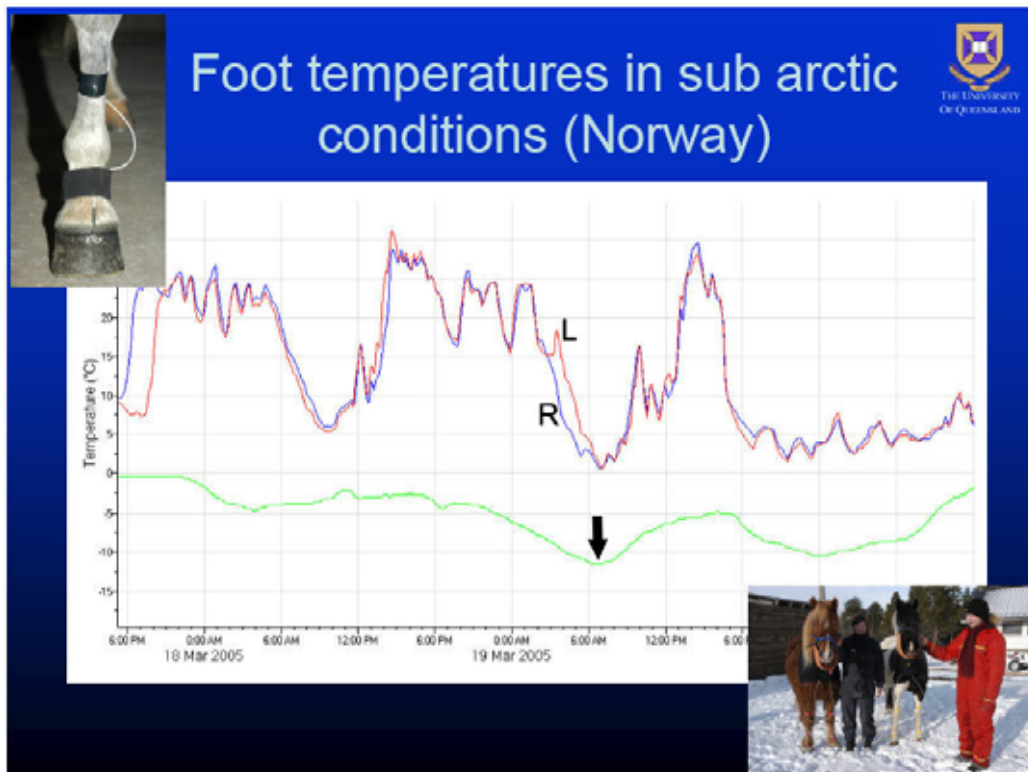


Fig 11B. Horse foot temperatures were continuously recorded, for 3 days in Norway, using probes inserted deep in the upper hoof wall (top inset). Data loggers connected to the probes were carried in pouches worn by normal horses (bottom inset). The environmental temperatures (arrowed) were below freezing at all time points; the lowest was -13°C at 6am March 19, 2005. Foot temperatures fluctuated widely left (L) and right (R) not always varying together. The data showed the range of normal thermoregulatory activity in horse's feet: sometimes the feet were close to freezing at other times they were warm to the touch. At no stage did the horses display any signs of discomfort.

The lamellar corium derives most of its blood supply from the branches of the terminal arch which perforate the distal phalanx. Numerous anastomoses form an arterial lattice beneath and between the epidermal lamellae and blood can flow proximally to the coronary circumflex artery and distally to the solar circumflex artery (Fig 12).

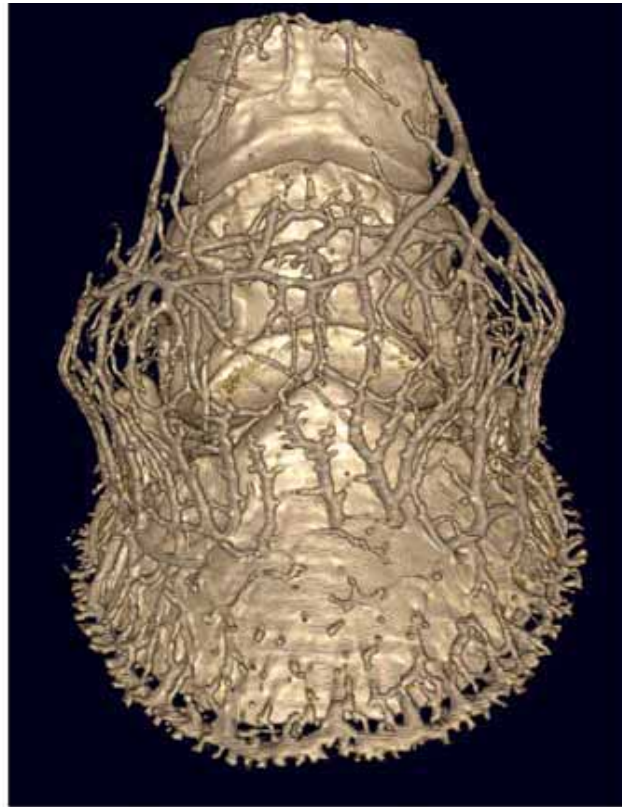


Fig 12. 3D reconstruction of a CT scanned horse's foot with the arterial circulation injected with contrast material. Note how arteries exit through foraminae in the dorsal surface of the distal phalanx and anastomose proximally with vessels of the coronet and distally to form the circumflex artery.

The branches of the terminal arch, that exit the upper surface of the distal phalanx, merge (anastomose) to form the circumflex artery of the sole; a complete arterial loop adjacent to the sharp solar margin of the bone. All of the arterial blood supply of the sole comes from inwardly directed branches of the circumflex artery (Fig 13).

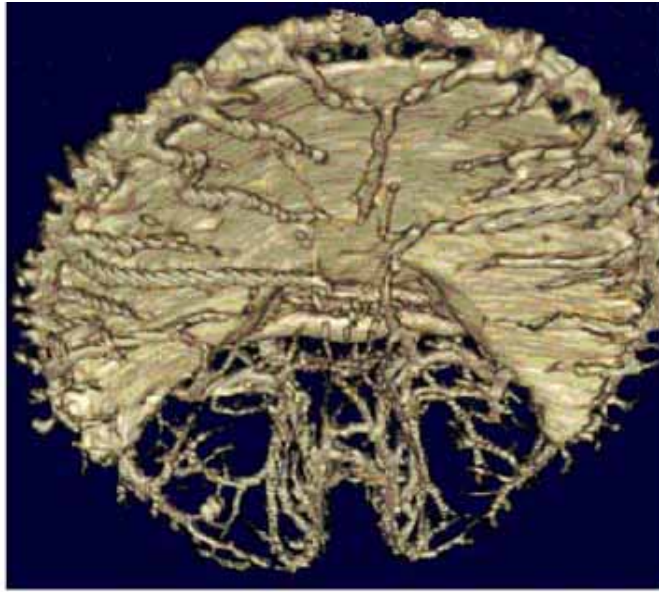


Fig 13. 3D reconstruction of a CT scanned horse's foot with the arterial circulation injected with contrast material. Note how axially directed branches of the circumflex artery supply the sole.

There are no vascular foramina perforating the solar surface of the distal phalanx (except at the back of the palmar processes). This means that almost the entire corium of the sole is dependent upon a blood supply which arises first on the dorsal surface of the distal phalanx and then curls under the margin of the distal phalanx. The solar corium is sandwiched between the epidermal sole and the unyielding solar surface of the distal phalanx and is therefore prone to damage from compressive forces. If a horse is deliberately forced to stand or walk on the soles of its feet (by overzealous trimming of the ground surface wall) the sharp distal rim of the distal phalanx effectively cuts off the blood circulation to the central solar corium and results in severe lameness and, in some cases, necrosis of the sole. Laminitis induced descent of the distal phalanx into the hoof capsule also causes dorsal sole necrosis by a similar mechanism.

Digital veins: The veins of the foot form a large interconnected, valveless venous plexus. (Fig 14). The foot is subjected to a range of weight-bearing and locomotor forces. These forces are believed to cause expansion of the frog, spreading of the heels and compression of all the soft tissue of the hoof, including the digital cushion, the cartilages and especially the thin walled veins. Because the soft tissues of the foot are encased by the hard keratinised wall which cannot expand substantially (Fischerleitner 1974), the internal pressure fluctuations cause venous blood to leave the foot quite quickly. The absence of valves helps evacuation by allowing venous blood to take any convenient path in any direction.

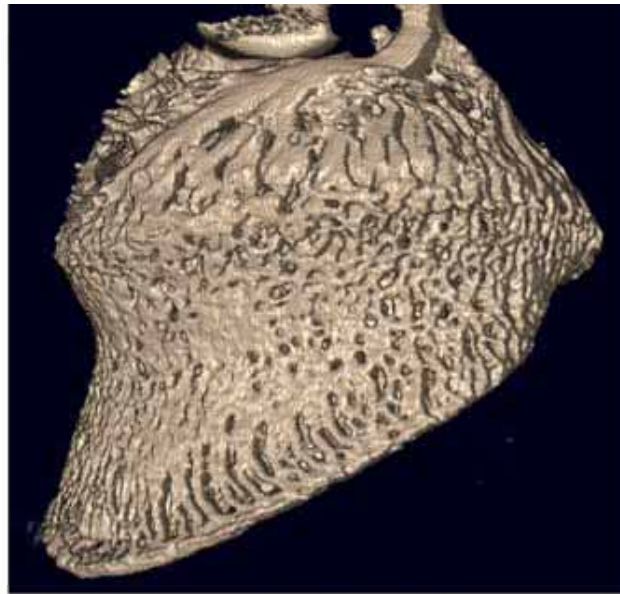


Fig 14. 3D reconstruction of a CT scanned horse's foot with the venous circulation injected with contrast material. Note the extensive degree of anastomosis among the valveless venous plexuses.

Both sides of the ungual (collateral) cartilages are covered by venous plexuses that act like bellows when the foot is loaded. All blood leaves the foot via the medial and lateral digital veins. The presence of the valves in the caudal hoof veins and proper digital veins prevents retrograde blood flow to the hoof and thereby ensures the efficient venous return of blood to the heart (Mishra and Leach 1983a; Mishra and Leach 1983b).

The dermal microcirculation

Numerous ($500/\text{cm}^2$) arteriovenous anastomoses (AVAs) or vascular shunts, connect the smaller arteries and veins of the dermal lamellae (Pollitt and Molyneux 1990). AVAs are present throughout the dermal lamellae (Fig 15). Studies with the transmission electron microscope show that AVAs are richly innervated by autonomic vasomotor nerves and their associated peptidergic nerves, have thick walls of smooth muscle and a specialised, characteristically tall, endothelium (Molyneux *et al.* 1994).

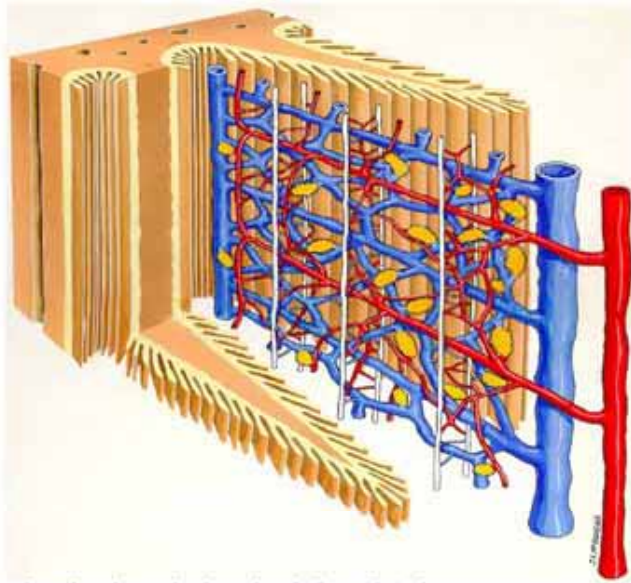


Fig 15. Diagram showing the dermal microcirculation. Arteriovenous anastomoses (short yellow vessels) are numerous and more concentrated near the bases of primary dermal lamellae. SDL capillaries (white) are shown reduced in number for diagrammatic reasons.

Their normal role is in relation to thermoregulation and pressure modulation. Dilated AVAs bring hot arterial blood to the inner hoof wall and can cause rapid and large temperature fluctuations of the hoof wall (Fig 11B). AVAs are equally numerous around the bases of the papillae of the coronary corium and the lamellar terminal papillae.

Secondary epidermal lamellae

The innermost layer of the hoof wall and bars of horses and ponies bears the lamellae or *stratum lamellatum* (layer of leaves) named after the 550-600 epidermal lamellae (primary epidermal lamellae) which project from its surface in parallel rows (Fig 16). Examination of the hoof capsule, with its contents removed, shows that the lamellae of the dorsal hoof wall are organised like a book of long thin pages 7mm wide and 50mm long. One long edge of the rectangle is incorporated into the tough, heavily keratinised hoof wall proper (the spine of the book) and the other long edge is free, facing the outer surface of the distal phalanx (Fig 16).



Fig 16. Hoof with contents removed showing the lamellae of the inner hoof wall.

The role of the epidermal lamellae is suspensory and, not surprisingly, there is an anatomical specialisation increasing the surface area of attachment for the multitude of collagenous fibres required to suspend and attach the distal phalanx to the inner hoof wall. The specialisation, unique to the Equidae, is the 150-200 secondary epidermal lamellae (SELS) incorporated along the length of each of the main or primary lamellae. Normal SELs have a constant histological appearance (Fig 17) that only laminitis alters. A description of hoof lamellar anatomy forms the basis of the histological grading system of laminitis histopathology (Pollitt 1996) and is important if one is to understand the laminitis disease process.

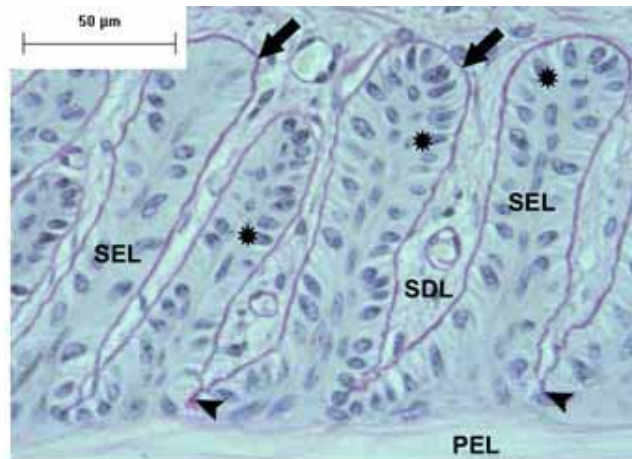


Fig 17. Micrograph of normal hoof lamellae stained to highlight the basement membrane. The basement membrane (arrowed) of each secondary epidermal lamella (SEL) shows as a dark magenta line closely adherent to the SEL basal cells. Between the bases of each SEL the BM penetrates deeply (arrowheads) and is close to the anuclear, keratinised, primary epidermal lamella (PEL). The SEL tips are rounded (club-shaped). The basal cell nuclei are oval in shape (stars) and positioned away from the BM at the apex of each cell. The long axis of each basal cell nucleus is at right angles to the long axis of the SEL. The secondary dermal lamellae (SDL) are filled with connective tissue right to their tips, between the SEL bases. These parameters of hoof lamellar anatomy form the basis of the histological grading system of laminitis histopathology. Stain = PAS. Bar = 10 μ m.

The SEL tips are rounded (club-shaped). The basal cell nuclei are oval in shape and positioned away from the basement membrane (BM) at the apex of each cell (Fig 17). The long axis of each oval, basal cell nucleus is at right angles to the long axis of the SEL. The secondary dermal lamellae (SDL) are filled with connective tissue even at their very tips, between the SEL bases. Special stains such as periodic acid Schiff (PAS) are required to highlight the basement membrane and locate its glycoprotein (sugar) constituents. PAS stained normal hoof lamellae stain the BM of each secondary epidermal lamella (SEL) as a dark magenta line closely adherent to the SEL basal cells. Between the bases of each SEL the BM penetrates deeply (arrowheads in Fig 17) and is close to the anuclear, keratinised, primary epidermal lamella (PEL). There are no white blood cells either in capillaries or in the dermis. The tips of the lamellae (both primary and secondary) all orientate towards the distal phalanx thus indicating the lines of tension to which the lamellar attachment apparatus is subjected.

The surface area of the equine inner hoof wall has been calculated to average 0.8m² (Daradka 2000) about the size of the surface area of the skin of a small adult human (a considerable increase over the inner surface area of bovine hooves which lack secondary lamellae). This large surface area, for suspension of the distal phalanx, and the great compliance of the interdigitating lamellar architecture helps reduce stress and ensures even energy transfer during peak loading of the equine foot. In life, the hoof distal phalangeal attachment apparatus is impressively strong; during peak loading the hoof wall and the distal phalanx move in concert and separate only when laminitis interferes with lamellar anatomy.

Lamellar metabolism

Despite much speculation regarding insulin resistance, glucose metabolism and their links to laminitis there is little information regarding, if, where and how glucose is consumed by the living cells of the equine hoof. To address this we investigated if glucose is consumed by the equine hoof and if glucose uptake into hoof epidermal cells is insulin dependent (Wattle and Pollitt 2006a). Blood glucose concentrations were recorded in seven adult horses by simultaneously taking samples from three blood vessels; an artery, the jugular vein, and a digital vein at the level of the pastern of one of the front legs. Antibody towards glucose transport proteins (GLUTs) and the insulin receptor was used for immunolocalisation of these proteins in the gluteus muscle and in the feet of 7 horses. We found that the foot of a horse consumes more glucose than its head. GLUT1 was the major epidermal cell glucose transporter of the hoof. In contrast to the gluteus muscle, hoof lamellae do not rely on insulin for glucose uptake. Thus in terms of metabolic activity the division of the hoof into sensitive and insensitive layers is no longer tenable. Glucose supplies the energy essential to maintain the integrity of the equine hoof epidermal/dermal interface.

We also measured the lactate concentrations in the same blood samples. The amount of lactate generated by the foot exceeded that of the head indicating that the foot is not only capable of anaerobic metabolism but depends on it to produce energy (Wattle and Pollitt 2006b). Biochemical staining for lactate dehydrogenase showed a strong reaction in the lamellar basal cell layer. Thus lamellar tissues appear to be anaerobic and may be quite indifferent to the oxygen (but not the glucose) status of their blood supply. This argues against theories that rely on lamellar hypoxia to explain laminitis pathogenesis.

The basement membrane

At the interface of the hoof epidermis and the dermis is a tough, unbroken sheet of connective tissue called the basement membrane (BM). This key structure partitions lamellar epidermal cells from the lamellar dermis (Fig 18). On one side of the BM epidermal basal cells of the hoof are firmly attached, while on the other, (dermal or corium) side, tendon-like connective tissue, emanating from the upper surface of the distal phalanx, is tightly woven into the mat-like structure of the BM. The signature lesion of laminitis, failure of the attachment between lamellar dermis and epidermis, occurs at the lamellar dermo-epidermal junction and involves the lamellar BM. The ultrastructure of the equine hoof basement membrane shows a sheet-like, three-

dimensional latticework of fine interconnecting cords. The axial skeleton of the cord network consists of filaments of collagen IV a collagen unique to BMs. The collagen IV filaments are ensheathed with glycoproteins, which together form the electron dense *lamina densa* (Fig 18) of the BM. Innumerable extensions of the *lamina densa* form banded, recurved hooks that intermesh with the connective tissue attached to the distal phalanx.

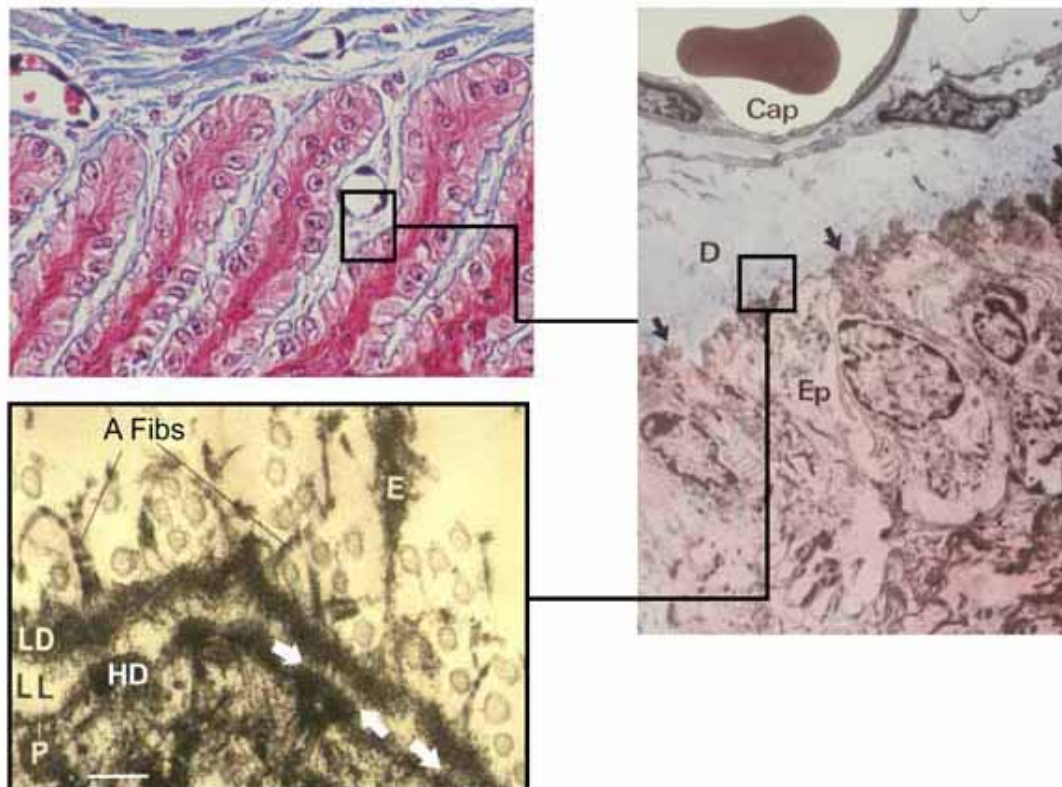


Fig 18. The basement membrane of each secondary epidermal lamella (SEL) stains as a dark blue line closely adherent to the SEL basal cells in light microscopic sections stained by Masson's trichrome. In low magnification transmission electron micrographs (TEMs) the basement membrane is just visible (arrows) and follows the contours of the SEL basal cells (Ep). High magnification TEMs show the lamina densa (LD) of the BM separated from the plasmalemma (P) of the basal cell by a gap; the lamina lucida (LL). Inserted in the basal cell plasmalemma are numerous attachment plaques called hemidesmosomes (HDs). Extensions of the lamina densa (E) and banded anchoring fibrils (AFibs) intermesh with collagen fibrils of the connective tissue of the lamellar dermis (D). Bridging the gap between HDs and the lamina densa are numerous fine anchoring filaments (white arrows). Cap = capillary. Bar = 10 nm

Without an intact, functional, basement membrane, the epidermis, to which it is attached, falls into disarray. Significantly, disintegration and separation of the lamellar basement membrane is a feature of acute laminitis.

Hemidesmosomes

When viewed with the transmission electron microscope (TEM) the BM is dominated by the electron dense *lamina densa* that appears as a dark line following the contours of the epidermal basal cells (Fig 19). The cell membrane (plasmalemma) at the base of each basal cell is attached to the BM by numerous electron dense adhesion plaques or hemidesmosomes (HDs). The various proteins of each HD occur on both sides of the basal cell plasmalemma thus forming a bridge linking the interior of the basal cell to the exterior connective tissue (Fig 19).

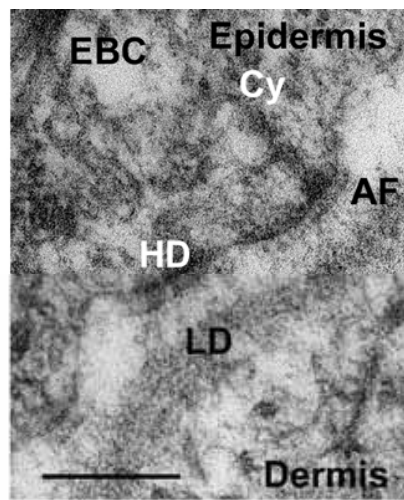


Fig 19. Hemidesmosomes at the dermo-epidermal junction. The electron dense lamina densa (LD) is the major structural component of the basement membrane. HDs are attachment plaques that serve to keep the lamina densa of the BM firmly adherent to all lamellar epidermal basal cells (EBC). Each HD is constructed of several proteins that stain darkly when viewed by TEM. The internal skeleton or cytoskeleton (Cy) of the basal cell is constructed of fine keratin filaments that attach to the intracytoplasmic dense plaque of HDs and interconnect to desmosomes and the nucleus. Bridging the gap between the dense plaque of the hemidesmosome (H) and the lamina densa are numerous submicroscopic anchoring filaments (AF). Bar = 10 nm

Importantly, HDs are maintained and assembled by glucose-consuming phosphorylation reactions. The filaments that bridges the gap between the HD and the lamina densa are constructed of laminin-5 (French and Pollitt 2004a; Spirito *et al.* 2002). TEM resolves laminin-5 as innumerable fine anchoring filaments spanning the *lamina lucida*, the space between the basal cell and the *lamina densa* (Fig 20). The essential nature of hoof lamellar HDs and anchoring filaments is illustrated by horses that inherit mutations in the genes expressing HD proteins.

Within the Belgian horse population, an autosomal, recessive mutation within the gene encoding laminin-5 has been identified. The mutation causes a premature termination codon and consequently expression of the laminin-5, anchoring filament protein is absent (28) resulting in

widespread dermo-epidermal separation in skin and hoof lamellae. Belgian foals afflicted with this hereditary,

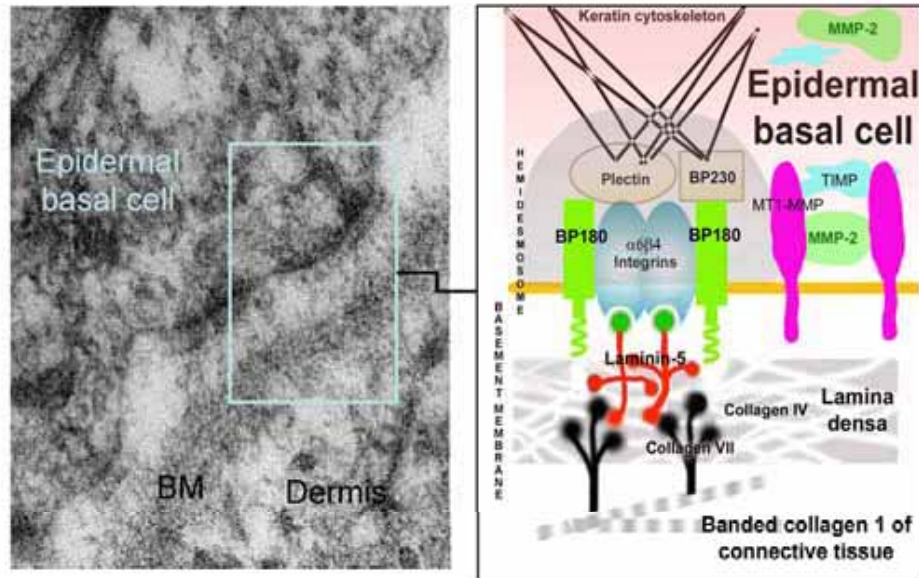


Fig 20. Diagram of hemidesmosome, the key structure anchoring the basal cells of the SEL to the BM. The intracytoplasmic plaque consists of the proteins plectin, BP180 and integrin $\alpha6\beta4$. Keratin intermediate filaments of the cell cytoskeleton connect to plectin which in turn communicates with laminin5 anchoring filaments via integrin $\alpha6\beta4$. Integrin $\alpha6\beta4$ and BP180 have domains on both sides of the plasmalemma and form part of the extracytoplasmic, sub-basal dense plaque of the HD. The anchoring filaments are incorporated into the matrix of the BM.

junctional epidermolysis bullosa suffer hoof loss and generalised skin lesions at pressure points and die or are euthanased within a few days of birth (Shapiro and McEwen 1995). Loss of plectin from the hemidesmosome adhesion complex of a Quarterhorse foal is a second example of congenital, lamellar dysadhesion (French and Pollitt 2004b). The foal had fragile skin and hoof pathology that resembled both acute and chronic laminitis. The distal phalanx had sunk into the hoof capsule and had perforated the sole. Interestingly although the loss of plectin was ubiquitous in hooves and skin only the forefeet showed lesions; the anatomy and histology of the hindfeet was normal thus illustrating how closely the lesions of laminitis follow foot load distribution. If clinicians could do more to lessen the impact of weight bearing during the developmental stage, the destructiveness of laminitis pathology in adult horses could be diminished.

A firm attachment between epidermal basal cells and the dermis is essential in weight bearing hoof lamellae. Any defect, acquired or inherited, that weakens the links of this chain also weakens epidermal-dermal adhesion.

Lamellar remodelling enzymes

Connective tissue and keratinocytes are now known to remodel and continually upgrade their spatial organisation by the tightly controlled production of a specific class of enzymes known as matrix metalloproteinases (MMPs). MMPs are a group of zinc-dependent enzymes that, when activated, degrade connective tissue and basement membrane (BM) components. The activity of MMPs correlates strongly the degree of malignancy and invasiveness of tumours (Giannelli *et al.* 1997; Stetler-Stevenson 1990). Two members of the MMP family (MMP-2 and MMP-9) can be isolated from homogenised normal hoof wall lamellae and from normal lamellar explants cultured in tissue culture medium (Pollitt *et al.* 1998). Secreted as inactive proenzymes MMP activity is responsible for the remodelling of the various classes of epidermal cells between the basement membrane, the secondary epidermal lamellae and primary epidermal lamellae (Fig 21).

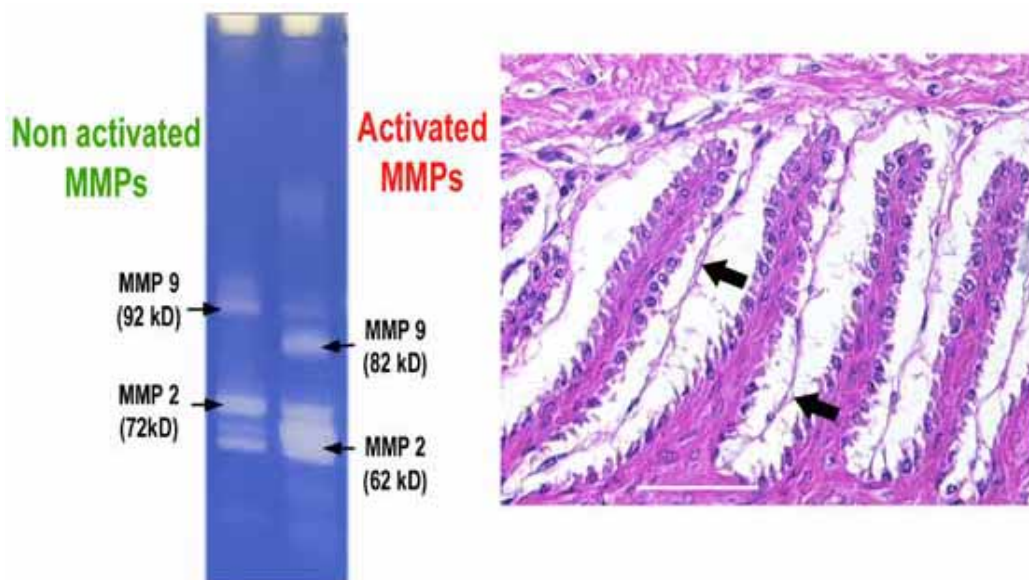


Fig 21. Zymography of normal lamellar explants. The tissue culture fluid, in which explants were cultured, was applied to lanes in a polyacrylamide gel containing 0.1% gelatin. After electrophoresis and overnight incubation, the gel was stained for protein with Coomassie Blue G-250. Because the gel contains protein (the soluble collagen gelatin) the entire gel stains blue, except where gelatin has been digested by MMP activity. Thus the clear areas reveal the existence of MMP-9 and MMP-2 in lamellar hoof tissue. Proteins of known molecular weight (not shown) are electrophoresed at the same time to determine MMP molecular weights in kiloDaltons (kD). Lane 1 shows the MMPs contained in a normal explant. There is pro-MMP-9 but no active MMP-9, a large band of pro-MMP-2 and some active MMP-2. Lane 2 shows the effect of MMP activation with APMA. The pro-MMP-9 has been converted to active MMP-9 and a similar conversion of pro-MMP-2 has occurred. Cleavage of a 10 kD fragment from pro-MMP 9 and 2 activates the enzyme. The micrograph shows a lamellar hoof explant after APMA activation of its constituent MMPs. The BM (arrows) of the secondary epidermal lamellae is no longer attached to the basal cells. Activation of lamellar MMPs causes this *in vitro* lesion that resembles natural laminitis. H&E stain. Bar = 10 µm.

The protein constituents of the basement membrane are known substrates of the hoof MMPs. In addition, laminin-5, the main component of the anchoring filaments bridging lamellar basal cell to the basement membrane is cleaved by MMP activity during laminitis development. The disorganisation of the epidermal cells of the secondary epidermal lamellae, the wholesale separation

of basal cells from the basement membrane, cleavage of laminin-5 and lysis of basement membrane which occurs early in the pathology of laminitis (French and Pollitt 2004a; Pollitt 1996; Pollitt and Daradka 1998) are now thought to be caused by the triggering of activation of uncontrolled, excessive MMP production (Kyaw Tanner and Pollitt 2004). MMPs, present in the cells of the hoof wall lamellae, presumably for normal remodeling purposes, appear to be important players in laminitis pathogenesis.

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