UPDATE ON THE ANATOMY OF THE INNER HOOF WALL

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Research into the structure and function of the hoof wall has proven fundamental to the understanding of how important diseases such as laminitis develop. This article reviews current information on the equine hoof wall and its internal lamellar layer (with notes on the developmental mechanism of laminitis) in the hope that a more unified approach to the rational management of the hoof wall, by both veterinarians, horse owners and farriers alike, will be the outcome.

INTRODUCTION

Members of the mammalian family Equidae represent the extreme result of digitigrade evolution. Single digits, encased in tough, keratinised hooves, on the end of relatively lightweight limbs, have undoubtedly, contributed to the speed and versatility of the equids. But at a price. Immobility and crippling result if the connection between hoof and bone (the lamellar distal phalangeal attachment apparatus) fails. Considerable selection pressure against such failure (otherwise known as laminitis) must exist among wild equids as a foundered animal would quickly attract the attention of predators. Equids are normally mobile and athletic but when they develop laminitis and become crippled we realise, belatedly, how dependent they were on an intact, functional, pain-free lamellar distal phalangeal suspensory apparatus.

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 is composed of hard keratin, is rich in disulphide bonds sole and has great physical strength. The frog and the white zone on the other hand are rich in sulphydryl 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, with molecular weights in the range 40-70 kDa with varying degrees of hardness and sulphur concentration, expressed in hoof tissues in accordance with their functional destiny.

Examination of the hoof capsule, with its contents removed, shows countless thousands of small, circular, holes pocking the surface of the coronary groove (Fig 1).

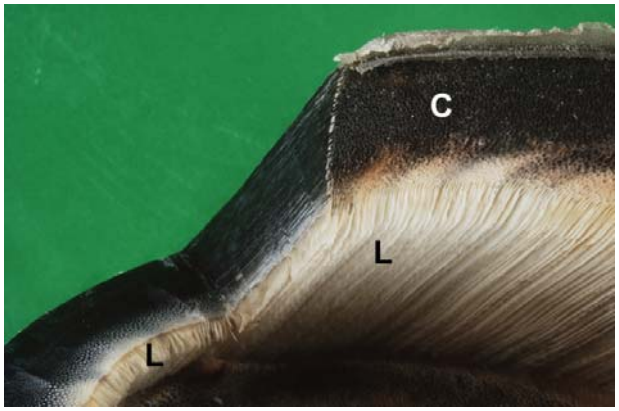

Fig 1. Hoof with contents removed. Countless small holes pock the surface of the coronary groove (C). The 550-600 lamellae (L) of the hoof wall arise on the inner shoulders of the coronary groove.

A sagittal section of the proximal hoof wall shows that the holes continue distally into the body of the wall as tubes, 4-5 mm in length, gradually tapering to a point. A layer of confluent epidermal basal cells covers the surface of the holes and the surface of the coronary groove between the holes.

HOOF WALL GROWTH.

The hoof wall grows throughout the life of the horse. Continual regeneration of the hoof wall occurs at the coronary band where germinal cells (epidermal basal

cells) produce populations of daughter cells (keratinocytes or keratin producing cells) which mature and keratinise, continually adding to the proximal hoof wall. We have used an improved method of cell proliferation detection to show the precise location of basal cells undergoing mitosis and the kinetics of basal cell proliferation in the coronary band region of ponies (Daradka and Pollitt unpublished data). The thymidine analogue (5-bromo-2'-deoxyuridine or BRdU) injected intravenously into living horses was incorporated into all cells undergoing mitosis during the 6h study period. Histological sections of hoof tissue stained immunohistochemically, using monoclonal antibodies against BrdU, showed a high rate of basal cell mitosis in the coronary zones producing intertubular hoof and tubular hoof (Fig 2) and in the proximal lamellar zone. Evidence of basal cell proliferation in the remainder of the lamellar region was lacking. The implications of this finding will be discussed later.

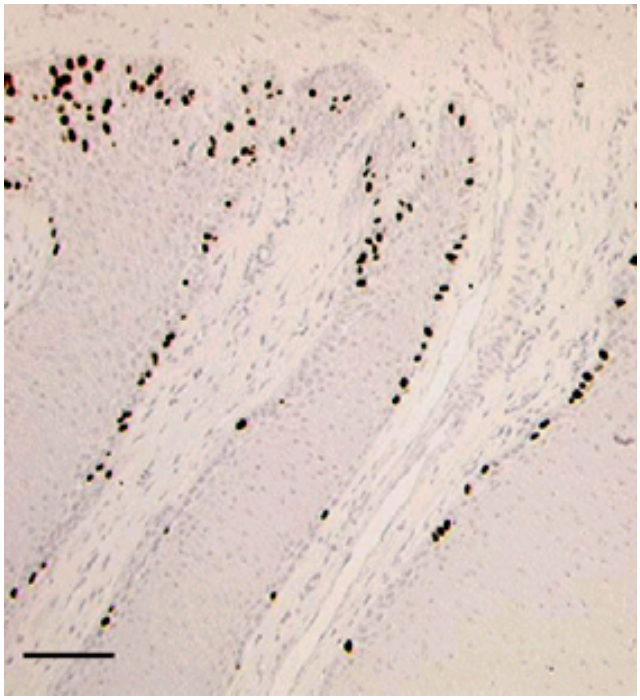

Fig 2. Longitudinal section of proximal hoof (coronary band) stained for immunolocalisation of BrDU that was injected intravenously into a normal horse 6h previously. The positive brown staining cells are basal cells that have incorporated BrDU as they have undergone mitosis in the previous 6 h. Both the tubular and intertubular hoof show a high rate of mitosis (bar = 100μm).

As already shown these coronary basal cells undergo mitosis throughout the life of the horse producing daughter cells which mature and keratinise undertaking a journey, up to 8 months in duration, in the direction of the ground surface. Maturing keratinocytes, arising from basal cells lining the holes, become organised into thin, elongated, cylinders or tubules. In cross-section the keratinocytes of individual hoof wall tubules are arranged around a central hollow medulla in non pigmented concentric layers (Fig 3). Each hair-like tubule is continuous, from its origin at the coronary band all the way to the ground surface (a distance of 5-15 cm depending on the breed). The keratinocytes generated between the holes mature into inter-tubular hoof thus forming a keratinised cellular matrix in which tubules are embedded.

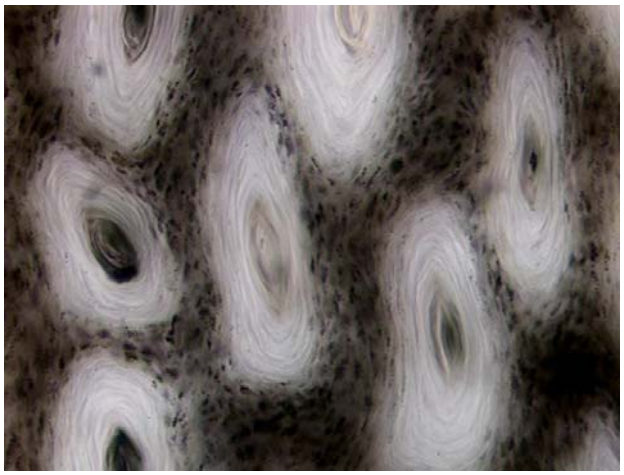

Fig 3. Transverse section of a pigmented hoof wall (unstained). The intertubular hoof is heavily pigmented and is the strongest component of the hoof wall. In contrast the unpigmented tubules of the hoof wall have a hollow medulla and the mature keratinocytes of the tubular hoof are arranged in concentric layers (x 200).

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 (Leach, 1980; Bertram and Gosline 1986). The 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 stratum medium is nonliving tissue but is anatomically constructed to resist stress in every direction and to never require remodelling. During normal locomotion the stratum medium only experiences one-tenth of the compressive force required to cause its structural failure (Thomason et al 1992).

The basal cell daughters, whether destined to be tubular or intertubular hoof, do not keratinise immediately. As the distance between basal cells and their daughters increases (each generation is pushed further away from the basal cell layer by the production of successive generations) the intracellular skeleton of the maturing cells becomes more dense (by the manufacture of more intermediate filaments composed of various keratin molecules). Thus by increasing the number of desmosomes more strong attachment zones are formed between the cell membranes of adjoining keratinocytes. Desmosomes are points of intercellular contact, which function like spot welds between adjacent cells (Fig 4).

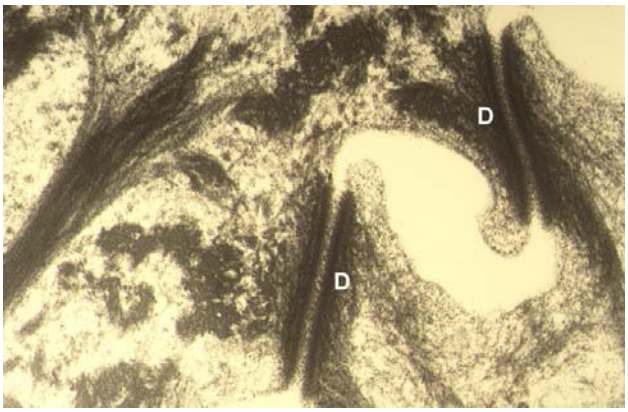

Fig 4. Desmosomes (D) are like spot-welds forming tight junctions between adjacent keratinocytes. Intermediate filaments made of keratin molecules form the internal skeleton of the cell and attach to the inner densely staining attachment plaque of the desmosome. Electron micrograph x 15,000.

Within the cell, keratin intermediate filaments also attach to the desmosome to form the three-dimensional internal skeleton of the cell. Thus the keratinocytes transform, becoming sturdier and more durable to stress and strain. The final stage of keratinocyte maturation is abrupt. The cell nucleus fragments and disappears and the cell is declared officially dead. At this stage hoof keratinocytes will incorporate the fluorescent dye Rhodamine and we have successfully stained the anuclear, fully keratinised layer in the hooves of living horses (Fig 5).

The fully keratinised cells (corneocytes) of the tubular and intertubular hoof, cemented firmly to each other form a continuum; the tough yet flexible stratum medium of the hoof wall. Mature corneocytes, 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 highly vascular dermis, outwards. In addition to acting as a permeability barrier, hoof wall corneocytes, arranged in their specialised tubular and intertubular configuration, have the crucial job of ultimately supporting the entire weight of the horse (Pollitt 1992).

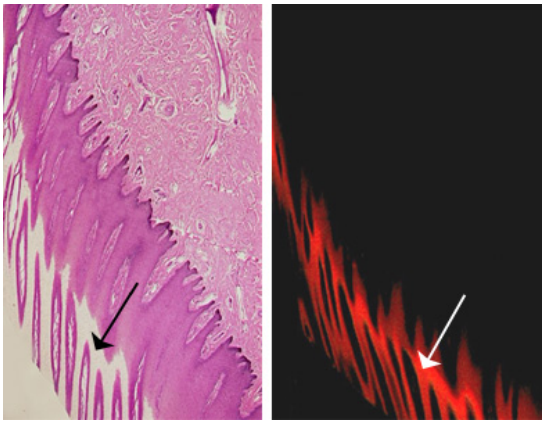

Fig 5. Serial longitudinal sections of the coronary band in the region of greatest hoof wall production. The horse was treated with Rhodamine to detect the zone of final keratinisation. Photographed with UV light (right) the red zone (arrow) shows where Rhodamine was incorporated into keratinocytes as they became anuclear and fully keratinised. For comparison a serial section was H&H stained. The zone of keratinisation corresponds with the anuclear, weakly eosinophilic, zone of hard hoof wall x200.

HOOF WALL TUBULES

The tubules of the equine hoof wall are not arranged randomly. The tubules of the s*tratum medium* are arranged in four distinct zones based on the density of tubules in the intertubular horn (Reilly et al 1996). 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 proximally 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 (Pollitt 1995) and together these factors represent an optimum design for equine hoof wall. Reilly et al (1996) has argued that tubule zonation is also a crack-stopping mechanism. 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 (Thomason et al, 1992). 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 (popularly the "quick") underlies the hoof wall and consists of 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. The corium provides the hoof with nourishment and its dense matrix of connective tissue connects the basement membrane of the dermal-epidermal junction to the periosteal surface of the distal phalanx and thus suspends the distal phalanx from the inner wall of the hoof capsule.

THE CORONARY CORIUM.

The coronary corium fills the coronary groove and blends distally with tnhe lamellar corium. Its inner surface is attached to the extensor tendon and the 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 coronary band. A feature of the coronary 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. This is shown in Figs 6 & 9). The basement membrane surface of the hoof wall corium was examined with the 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 basement membrane of the coronary and terminal papillae was folded into numerous ridges parallel with the long axis of the papilla. These longitudinal ridges on the surface of the papillae are analogous to the folded secondary dermal lamellae and probably share the similar role of increasing the surface area of attachment between the epidermal hoof and the connective tissue of the distal phalanx. They may also act as guides or channels directing columns of maturing keratinocytes in a correctly oriented proximo-distal correction (Fig 6). The density of coronary papillae is greatest at the periphery and least, adjacent to the lamellae. This mirrors the arrangement of the hoof wall tubules in zones based on tubule density.

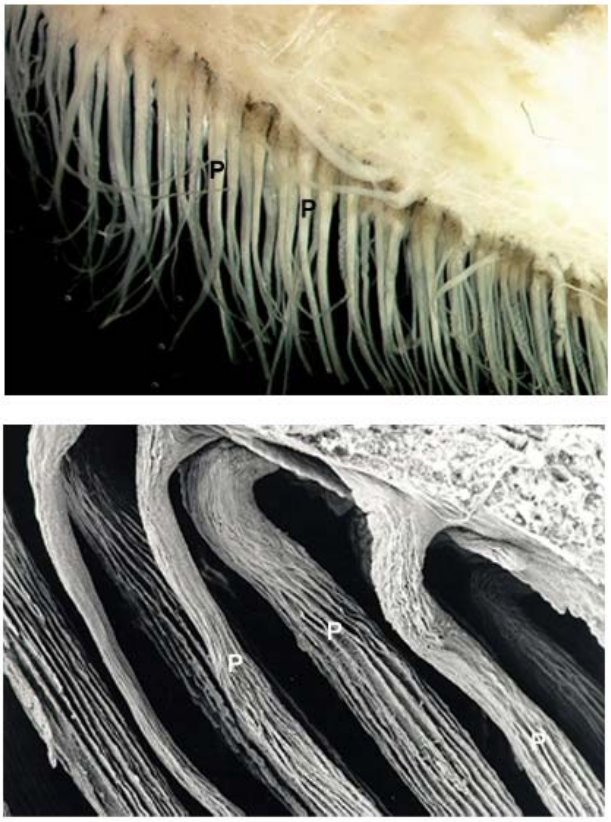

Fig 6. These papillae of the coronary corium have been treated with a detergent enzyme mixture and have been gently teased away from the proximal hoof wall. Normally they fit into long thin tapering holes in the coronary groove. Each papilla is responsible for the nutrition and organisation of an individual hoof wall tubule. The highly magnified electron microscope picture (bottom) shows that the basement membrane of the papillae is folded as if forming parallel channels to act as guides directing columns of maturing keratinocytes in a correctly oriented proximo-distal correction.

THE INNER HOOF WALL

The innermost layer of the hoof wall and bars of horses and ponies is named the *stratum lamellatum* (layer of leaves) after the 550-600 epidermal lamellae (primary epidermal lamellae) which project from its surface in parallel rows. Examination of the hoof capsule, with its contents removed, shows that the lamellae of the dorsal hoof wall are shaped like long thin rectangles approximately 7mm wide and 50mm long. One long edge of the rectangle is incorporated into the tough, heavily keratinised hoof wall proper (*stratum medium)* and the other long edge is free, facing the outer surface of the distal phalanx. The proximal short edge is curved and forms the curved shoulder of the coronary groove. The distal short edge merges with the sole and becomes part of the white zone visible at the ground surface of the hoof.

In common with all epidermal structures the lamellae of the inner hoof wall are avascular and depend on capillaries in the microcirculation of the adjacent dermis for gaseous exchange and nutrients. The epidermal cells closest to the dermis (the basal cell layer, germinal cell layer or *stratum germinativum)* contain little keratin and have the potential to proliferate into keratinising daughter cells. Whereas the epidermal basal cells lining the coronary groove and sole proliferate continuously into keratinising daughter cells to form the tough but flexible hoof wall and sole respectively convincing evidence that the basal cells of normal lamellae proliferate to the same degree is lacking. Proliferating lamellar basal cells are confined to the proximal 10% of the lamellar inner hoof wall and are absent in the rest. Thus, in the same way as the hoof wall proper is subject to a downward force exerted by the proliferating basal cell layer of the coronary groove so to are the lamellae. The primary function of the lamellar hoof is to suspend the distal phalanx within the hoof capsule. It reserves its proliferative potential for the healing of injuries.

SECONDARY EPIDERMAL LAMELLAE.

If the role of the epidermal lamellae is indeed suspensory, then an anatomical specialisation increasing the surface area for the attachment of the multitude of collagenous fibres emanating from the outer surface of the distal phalanx would be expected. The secondary epidermal lamellae are just such a specialisation. During the formation of an epidermal lamella, on the shoulders of the inner coronary groove, the basal cell layer proliferates causing folds (secondary lamellae) to form along the lamellar perimeter. The basal cell proliferation index is high on the shoulders of the coronary groove in the region of secondary lamella formation (Daradka and Pollitt, unpublished data). The folds elongate to form an extra 150-200 secondary lamellae along the length of each of the 550-600 primary lamella (Figs 7 & 9)).

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 suspensory apparatus is subjected. The surface area of the equine inner hoof wall has been calculated to average 1.3m^2 (Daradka and Pollitt unpublished data), 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 interdigiting lamellar architecture helps reduce stress and ensures even energy transfer during peak loading of the equine foot (Bertram and Gosline, 1987). 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.

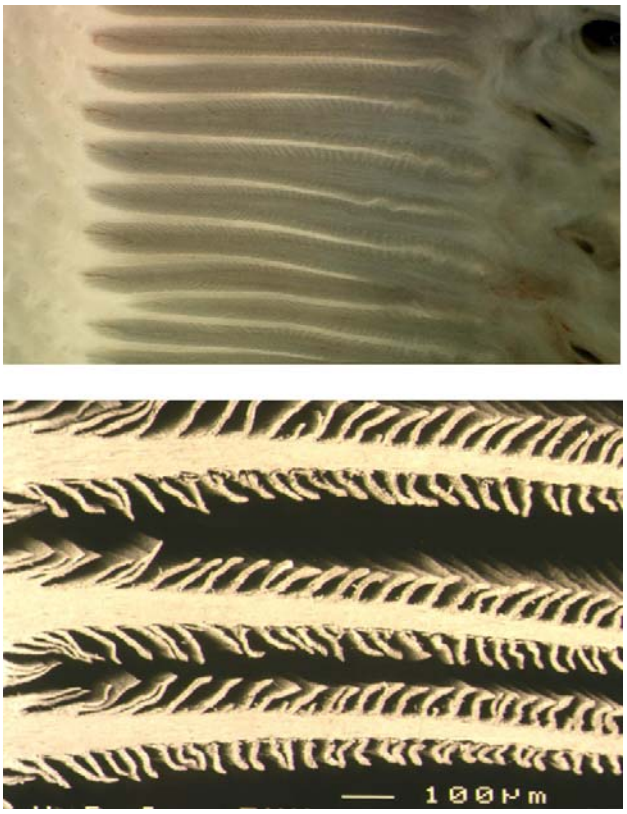

Fig 7. To increase the surface area of the inner hoof wall there are 550-600 primary epidermal lamellae (PELs). The surface area of each PEL is further increased with an extra 150-200 secondary lamellae along the length of each of PEL. The tips of the lamellae (both primary and secondary) all orientate towards the distal phalanx (on the right of each picture but not shown) thus indicating the lines of tension to which the lamellar suspensory apparatus is subjected. The upper picture is an unstained section magnified about x10. The lower picture is an electron micrograph: the bar = 0.1mm. In life each PEL measures about 7 mm when viewed in this plane.

THE BASEMENT MEMBRANE.

At the interface of the epidermis and the dermis is a tough, unbroken sheet of extracellular matrix called the basement membrane (Fig 9). This key structure is the bridge attaching the basal cells of the lamellar epidermis on one side and the fine connective tissue fibrils (type I collagen)
emanating from the dorsal surface of the distal phalanx on the other. The ultrastructure of the equine hoof basement membrane is essentially the same as in other animals but with some important specialisations. It is a sheet-like three-dimensional anastomosing latticework of fine interconnecting cords. The axial skeleton of the cord network consists of filaments of collagen IV. The collagen IV filaments are ensheathed with glycoproteins,

in particular laminin which together form the electron dense *lamina densa*. Innumerable extensions of the *lamina densa* and banded anchoring filaments in the shape of recurved hooks intermesh with the type I collagen fibrils of the connective tissue of the lamellar corium forming an important part of the attachment mechanism between dermis and epidermis. The equine basement membrane has a high density of *lamina densa* extensions and anchoring filaments around the tips of the secondary epidermal lamellae a feature perhaps not surprising in a large ungulate, weight bearing, on single digits (Pollitt 1994).

Laminin, one of the key proteins of the basement membrane, forms receptor sites and ligands for a complex array of growth factors, cytokines, adhesion molecules and integrins. 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. Laminin and collagen IV disappear from the basement membrane which progressively loses its close attachment to the basal cells and strips away from the epidermal lamellae (Pollitt and Daradka, 1998).

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). Two members of the MMP family (MMP-2 and MMP-9) have recently been 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 and, when activated, promptly inhibited by locally produced inhibitors (tissue inhibitors of metalloproteinase of TIMPs) it is MMP activity which is likely responsible for the remodelling of the various classes of epidermal cells between the basement membrane, the secondary epidermal lamellae and primary epidermal lamellae (Fig 8). The protein constituents of the basement membrane (type IV collagen, type VII collagen and laminin), are known substrates of the matrix metalloproteinases MMP-2 and MMP-9 (Salamonsen, 1994). After wounding, surviving keratinocytes, responding to locally produced cytokines detach from the basement membrane and commence the re-epithelialization process. Keratinocytes, responding to trauma or infection, readily synthesise both interleukin-1 and tumour necrosis factor-α (Cork et al 1993). Cytokines such as these upregulate the production of MMPs and an essential first step before keratinocytes can detach from the basement membrane is pericellular proteolysis via the increased production of MMP (Salo et al, 1991). The disorganisation of the epidermal cells of the secondary epidermal lamellae, the wholesale separation of basal cells from the basement membrane and lysis of basement membrane which occurs early in the pathology of laminitis (Pollitt, 1996) are now thought to be caused by the triggering of activation of uncontrolled, excessive MMP production. Since MMPs are now known to be present in the region of the secondary epidermal lamellae, presumably for normal remodelling purposes, this seems a reasonable proposition.

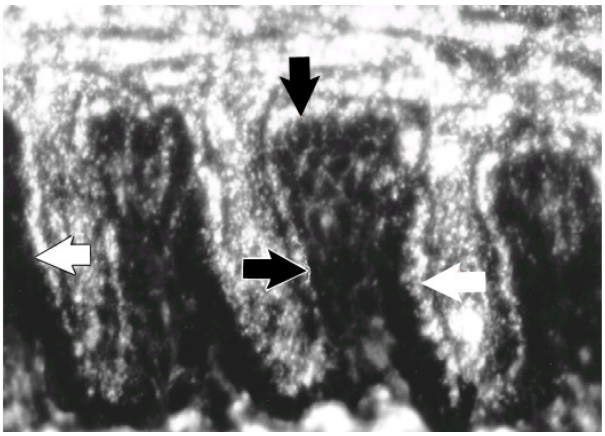

Fig 8. Epidermal lamellae overlayed with a thin film of gelatin (photographic emulsion) show that matrix metalloproteinase (MMP) or gelatinase activity is located in the epidermal basal cells beneath the basement membrane. MMP activity is probably responsible for the remodelling of the various classes of epidermal cells between the basement membrane, the secondary epidermal lamellae and primary epidermal lamellae.

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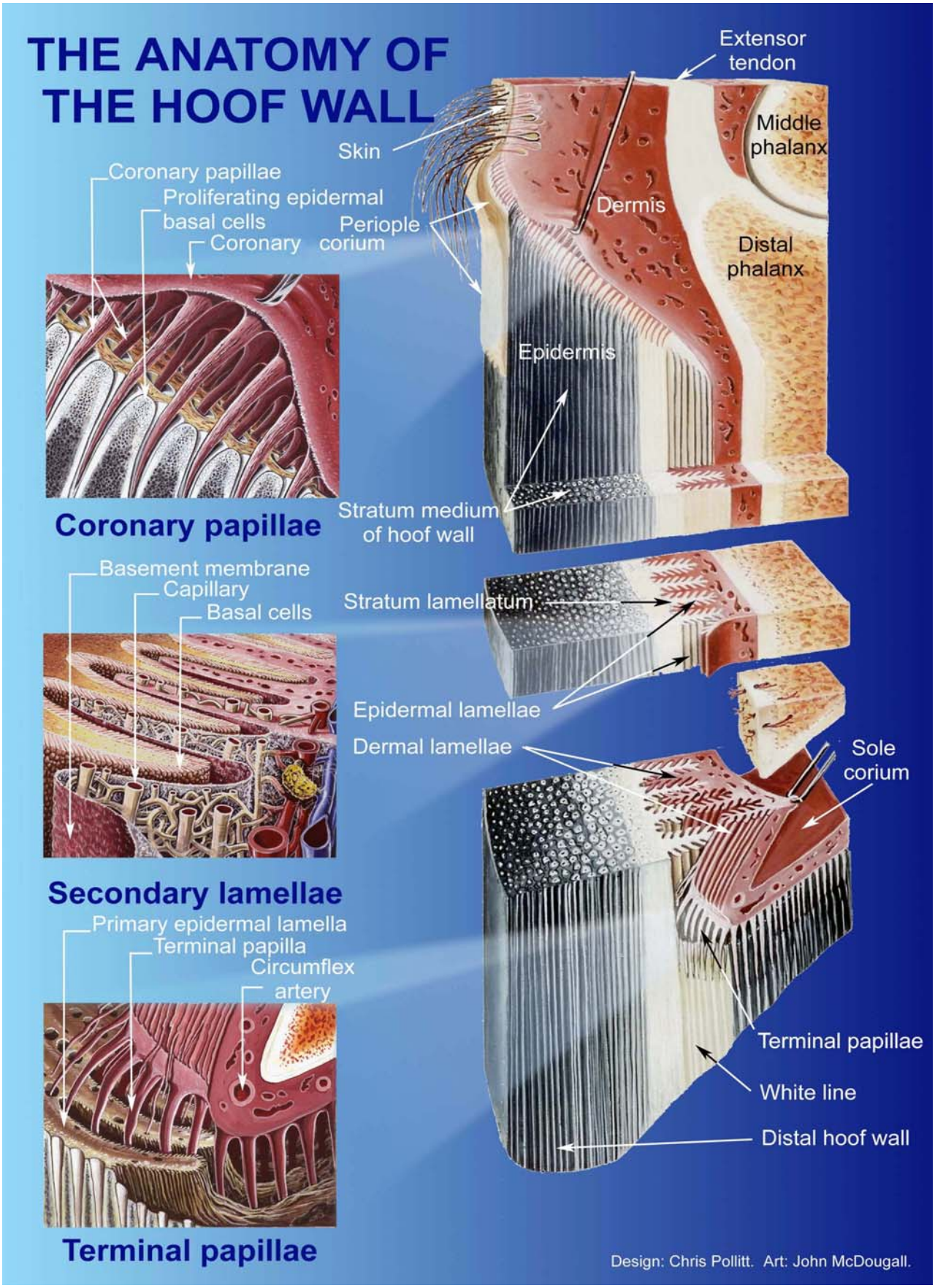
Fig 9. (next page) Diagram of the lamellae and papillae of the inner hoof wall.


THE ANATOMY OF THE HOOF WALL
Extensor tendon
Middle phalanx
Skin
Coronary papillae
Proliferating epidermal basal cells
Coronary corium
Periople
Dermis
Distal phalanx
Epidermis
Coronary papillae
Stratum medium of hoof wall
Basement membrane
Capillary
Basal cells
Stratum lamellatum
Epidermal lamellae
Dermal lamellae
Sole corium
Secondary lamellae
Primary epidermal lamella
Terminal papilla
Circumflex artery
Terminal papillae
White line
Distal hoof wall
Terminal papillae
Design: Chris Pollitt. Art: John McDougall.

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