EQUINE LAMINITIS: A REVISED PATHOPHYSIOLOGY

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Laminitis is the most serious disease of the equine hoof and will be used here to illustrate how pathological changes in anatomy lead to devastating changes in function. The simplest definition of laminitis is: **failure of the attachment between the distal phalanx (coffin bone) and the inner hoof wall**. A horse has laminitis when the lamellae of the inner hoof wall, which normally suspend the distal phalanx from the inner surface of the hoof capsule, fail. Without the distal phalanx properly attached to the inside of the hoof, the weight of the horse and the forces of locomotion drive the bone down into the hoof capsule, shearing and damaging arteries and veins, crushing the corium of the sole and coronet, causing unrelenting pain and a characteristic lameness (Fig 1).

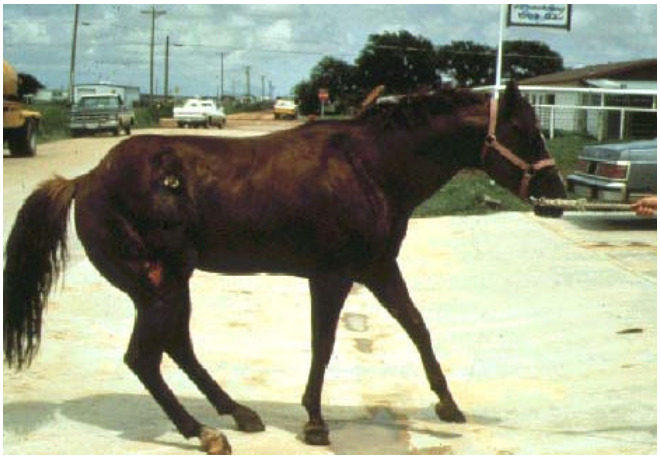

Fig 1. Horse with severe laminitis in both front feet showing typical laminitis gait. The hind feet are placed as far forward as possible before the horse attempts painful shuffling steps in front.

A *developmental phase*, during which lamellar separation is triggered, precedes the appearance of the foot pain of laminitis. This may be as short as 8 -12 h in the case of laminitis caused by exposure to the water soluble toxins of black walnut (Juglans nigra) heartwood shavings (Galey et al 1991) or 30 - 40 h in the case of excessive ingestion of high starch grain. During the developmental phase and prior to the clinical appearance of foot pain the horse or pony usually experiences a problem with one or more of the following organ systems: gastrointestinal, respiratory, reproductive, renal, endocrine, musculoskeletal, integumentary and immune. Multi-systemic aberrations in organs anatomically remote from the foot result in the lamellar tissues of the feet being exposed to factors which lead to separation and disorganisation of lamellar anatomy. The exact nature of the laminitis trigger factors, apparently reaching the lamellar tissues via the circulation, has yet to be elucidated. Sometimes no developmental phase can be recognized: the horse or pony is discovered in the acute phase with no apparent ill-health or inciting problem occurring beforehand. This appears to be the case with grass founder although Longland and Cairns (1998) researching the metabolism of grass, growing when the sun shines in Wales, have shown that the grass founder inciting factor may be a soluble sugar called fructan suddenly reaching very high concentrations in the stem of the plant and triggering a gastrointestinal disturbance when consumed by horses and ponies. The parenteral injection of potent long acting corticosteroid preparations for the treatment of skin disease may precipitate iatrogenic acute laminitis (Eustace and Reddon, 1990).

The *developmental phase* merges into the *acute phase* of laminitis which lasts from the onset of clinical foot pain and lameness at the trot, to the time when there is clinical (usually radiological) evidence of displacement of the distal phalanx within the hoof capsule (Fig 2). After the acute phase, if the horse does not die from the disease process inciting the development of laminitis, it can make an apparent complete recovery or develop palmar displacement of the distal phalanx, the hallmark of chronic laminitis. The *chronic phase* can last indefinitely with clinical signs ranging from persistent,

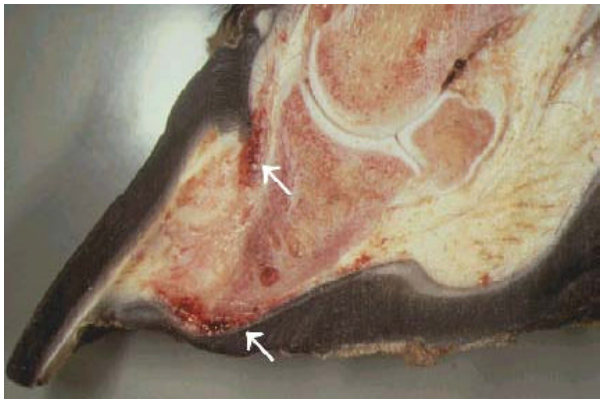

Fig 2. Sagittal section of a horse's foot with severe chronic laminitis. The distal phalanx has separated from its connection to the inner hoof wall and has descended into the hoof capsule causing the sole to bulge downward. Note the haemorrhage and bruising in the corium at the coronet and sole (arrows).

mild lameness, continued severe foot pain, further degeneration of lamellar attachments, recumbency, hoof wall deformation and sloughing of the hooves (Hunt, 1993). It is important to realise that the process initiating the destruction of the lamellar attachment apparatus begins to operate during the developmental phase before the first clinical sign of laminitis, **foot pain**, is apparent. During the developmental phase the specific problems of the horse, often have to be attended to urgently (e.g. acute abdomen, grain overload acidosis, rhabdomyolysis, retained placenta) and unfortunately the feet may not enter into the therapeutic equation until the signs of foot pain appear. By the time foot pain is apparent lamellar pathology is underway. In other words foot pain is the clinical sign that lamellar disintegration is occurring (Fig 3). To wait and see if foot pain is the sequel to a metabolic crisis is to miss the opportunity to prevent or at least ameliorate lamellar pathology.

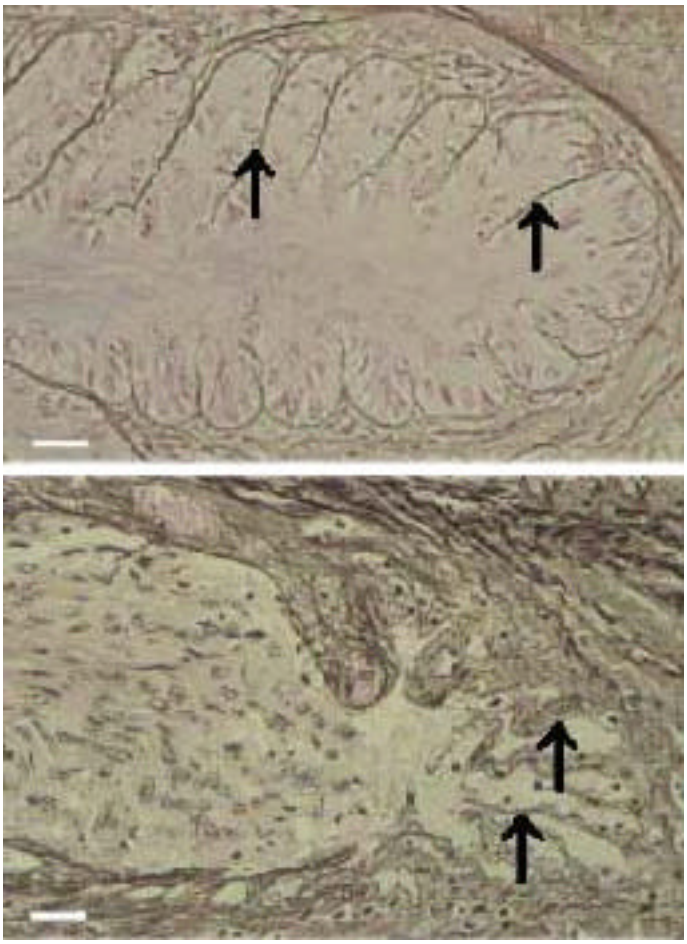

Fig 3. Histology of the tip of a normal epidermal lamella (top) and one affected by acute laminitis of 48h duration (bottom). In the normal lamella the basement membrane (arrows) is firmly attached to the basal cells of the hoof epidermis. When laminitis intervenes the epidermal cells detach from their basement membrane allowing the hoof to separate from the distal phalanx and cause the clinical sign of foot pain (bar=25μm).

The spectacular disintegration of the lamellar attachment apparatus, initiated during the development phase of laminitis, renders a normally robust and trouble free hoof/connective tissue/bone attachment system useless in a relatively short period of time. Logic dictates that somehow, it is a normally tightly controlled metabolic process or structure that is thrown into disarray to cause the lamellar specific lesion of laminitis during its developmental phase.

We believe that it is the enzymatic remodeling of the epidermal lamellae, assumed to be mandatory if the continually proliferating hoof wall is to move past the stationary distal phalanx, that is the target of the laminitis disease process.

Enzymes capable of destroying key components of the lamellar attachment apparatus have been isolated from normal lamellar tissues and in increased quantities from lamellar tissues affected by laminitis (Pollitt et al 1998). The enzymes are metalloproteinase -2 and metalloproteinase -9 (MMP-2 and MMP-9) also found in a wide range of human and animal remodeling tissues such as bone, joints and endometrium as well as in metastasizing malignant tumours (Birkdal-Hansen (1995).

It is assumed that MMP activity is constantly responding to the stresses and strains of normal equine life as well as to constant growth. When called for, sufficient MMP is manufactured locally, to release epidermal cell to cell, and cell to basement membrane attachment, as required, to maintain the correct shape and orientation of the hoof lamellae. From time to time injury to the basement membrane would require its lysis and reconstruction. The controlled release of specific MMP inhibitors keeps this remodeling process in equilibrium and the hoof lamellae and the hoof itself slowly migrate past the stationary basal cells firmly attached to their underlying basement membrane and in turn via connective tissue to the distal phalanx.

The epidermal cells of other species have been shown to readily increase their production of MMP when exposed to cytokines. Cultures of human oral mucosal keratinocytes respond to the addition of tumour necrosis factor (TNF), interleukin -1 (IL-1) and transforming growth factor - 1 (TGF-1) by increasing production of MMP-9 (Salo *et al* 1994). Lamellar tissues affected by laminitis also increase their MMP production especially MMP in its active form (Pollitt et al, 1998) but whether in response to circulating cytokines or some other trigger factor is yet to be established. The lamellar basal and parabasal cells lose their normal shape, become elongated and appear to slide over one another and, as a consequence, the secondary epidermal lamellae become attenuated with tapering, instead of club shaped, tips (Pollitt 1996). The lamellar basement membrane begins to disappear initially at the bases of the SELs where most of the parabasal cells reside (Pollitt and Daradka, 1998). The BM of the remainder of the SEL loses its attachment to the basal cells and sheets of BM peel away to form aggregations of loose isolated BM in the connective tissue adjoining the lamellae. The detachment of BM appears to progress from the epidermal side and the sheets of loose lamellar BM remain attached to the connective tissue. The BM free epidermal cells appear not to be undergoing necrosis, at least initially,

and clump together to form BM free masses on either side of the lamellar axis (Fig 4).

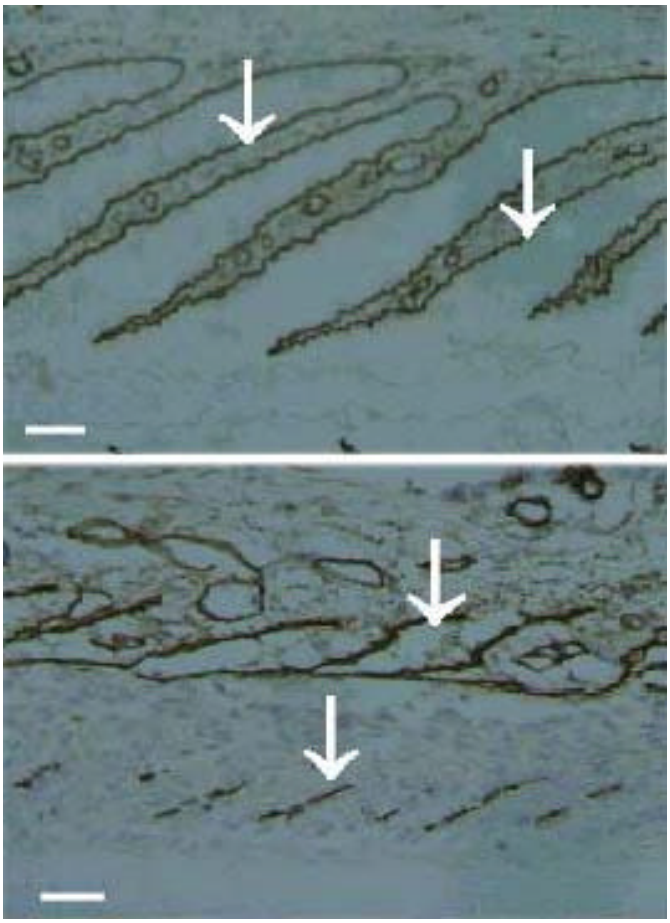

Fig 4. The epidermal lamellar basement membrane (BM) has been immunolabelled using monoclonal antibodies specific for the basement membrane protein laminin (arrows). The top picture shows the basement membrane of normal secondary epidermal lamellae (SELs) and capillaries. The lamellae in the bottom picture are affected by acute laminitis. The BM has separated from the tips and is being lysed by matrix metalloproteinases at the bases of the SELs. Note the decrease in the number of capillaries between the SELs affected by laminitis (bar=100μm).

Since the BM is the key structure bridging the epidermis of the hoof to the connective tissue of the distal phalanx, it follows that the wholesale loss and disorganisation of the lamellar BM inexorably leads to the failure of hoof anatomy so characteristic of equine laminitis.

An additional component of lamellar anatomy to be affected is the lamellar capillaries. As the BM and the connective tissue between the SELs disappears so do the capillaries. The loss of these capillaries may explain why resistance to blood flow was increased 3.5 times (the bounding digital pulse) in horses during early laminitis (Allen et al, 1990) and why blood was bypassing the capillary bed through dilated arteriovenous anastomoses in the horses with acute laminitis studied by Hood et al (1978). Both of these phenonoma are now placed after the triggering of MMP production and occur as a consequence of it.

The enzymatic theory of laminitis aetiology based on lamellar MMP activation challenges the alternative view that laminitis develops because of vascular changes to the circulation of the foot. A current theory is that venoconstriction and high hydrostatic interstitial fluid pressure (compartment syndrome) impede the flow of blood in the lamellar microcirculation to cause ischaemic necrosis of epidermal lamellae (Allen et al 1990). Epidermal cell necrosis, intravascular coagulation and oedema are not recognized by us in sections made from tissue in the early stages of laminitis. The vessels in the primary dermal lamella, even the smallest, are for the most part fully open without evidence of microvascular thrombi. Further, no abnormalities in the systemic coagulation and fibrinolytic cascades are found in horses with carbohydrate induced acute laminitis (Prasse et al,1990). The gross anatomical appearance of freshly dissected laminitis tissue is one of dryness. Sometimes the lamellae peel apart. Tissues affected by a compartment syndrome exude fluid.

How do the trigger factors of laminitis reach the lamellae? There is now strong evidence from three independent experimental sources (Robinson et al, 1976, Trout et al, 1990 and Pollitt and Davies, 1998) that the foot circulation during the developmental phase of laminitis is vasodilated. Laminitis does not occur if the foot is in a state of vasoconstriction during the prodromal phase suggesting that the trigger factors will only cause laminitis if they reach the lamellar tissues via dilated blood vessels at a high enough concentration and over a long enough time period. It follows that therapy aimed at keeping the feet of horses in danger of developing laminitis as cool as possible (and therefore vasoconstricted) is logical. Trials to determine the effect of a slurry of iced water applied to the feet of horses are underway. Preliminary results show that horses, unlike humans, do not regard extremely cold feet as uncomfortable and can tolerate having their feet in iced water for 48h with no ill effect. Scintigraphic studies comparing the circulation of iced feet versus normal shows profound vasoconstriction in the cold feet (Fig 5).

What are the laminitis trigger factors? Since the carbohydrate overload model of laminitis is characterised by endotoxin production it would seem a safe presumption that macrophages in the peritoneal cavity and elsewhere in the body would be subject to endotoxin stimulation just as they are during other acute gastrointestinal diseases (Barton et al 1996). Mononuclear phagocytes express tumour necrosis factor along with other cytokines such as interleukin within minutes of exposure to endotoxin. The cytokine cascade originating from an acute abdomen is responsible for most of the pathological effects of endotoxemia. However laminitis has never been triggered by the experimental administration of endotoxin into the bloodstream or the peritoneal cavity and the actual trigger factors of laminitis remain unidentified. What appears certain in the light of recent research is that the lamellar disintegration of laminitis is mediated by the uncontrolled release of excess MMP.

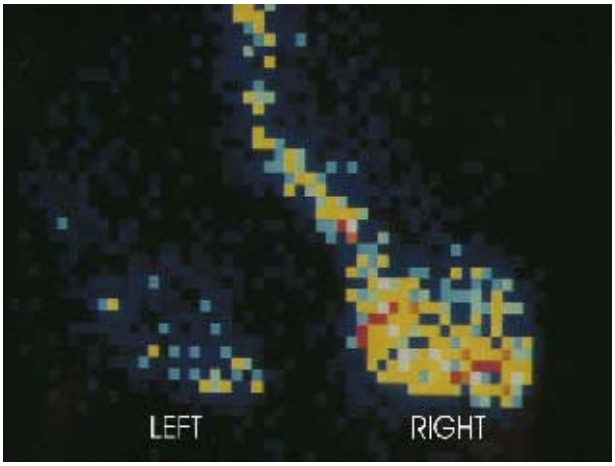

Fig 4. A scintigraphic study of the circulation of both front feet shows that the iced left foot has approximately 10-15% of the blood flow of the normal right foot (Photo Jan. Young).

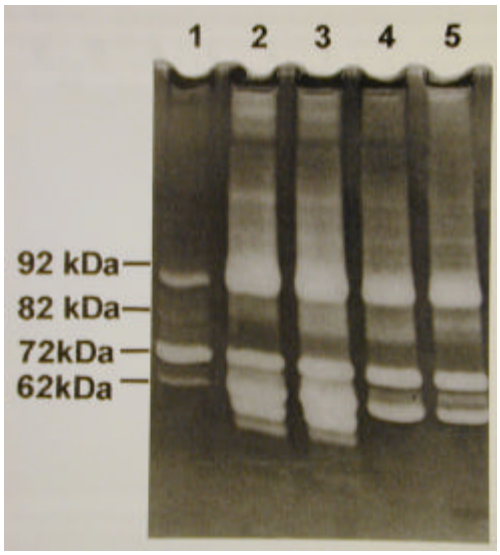

Fig 5. Polyacrylamide gel zymography (gel contains 0.1% gelatin) of lamellar explants from a horse with laminitis. Lane 1 = normal hoof explant supernatant. Lanes 2 & 3 = laminitis fore hoof explant supernatants. Lane 4 & 5 = laminitis hind hoof explant supernatants. Molecular weights are derived from standards (not shown). There is a significant increase is the amount of active MMP 9 (82 kDa) and MMP2 (62kDa). Ref: Pollitt et al, 1998.

We have successfully developed an *in vitro* model (Pollitt et al, 1998) for equine laminitis using small explants of tissue taken from the inner hoof wall of normal, freshly killed, abattoir horses. Each explant consists of stratum medium, the lamellar layer and the sub-lamellar connective tissue. After incubation for 48 h in tissue culture medium, plus the laminitis trigger factor under investigation, each explant is subjected to tension. The force required to separate epidermal from dermal lamellae is recorded. When dermal-epidermal lamellar separation occurs readily (as occurs in field cases of laminitis) we consider the tissue to have developed *in vitro* laminitis. Lamellar explants can be cultured for up to 7 days in normal medium and no lamellar separation occurs. It is virtually impossible to separate normal lamellar explants. One event that readily causes separation of lamellar explants is MMP activation. The addition to the culture medium of the organo-mercurial compound aminophenylmercuric acetate (APMA), a well known non-physiological MMP activator, readily induces explant lamellar separation. Treatment of lamellar explants with APMA is the *in vitro* laminitis control against which naturally occurring laminitis induction factors can be measured. The presence or absence of MMP activation in explant supernatants is detected zymographically using gelatin polyacrylamide electrophoresis and all explant tissues are fixed and examined histologically. Histological sections show a clear zone of complete separation between the basement membrane and the basal cells of the epidermal lamellae. This is a characteristic of *in vitro* laminitis and resembles the basement membrane lesion of natural *in vivo* laminitis.

We have used the *in vitro* laminitis explant model to investigate most of the proposed causes of equine laminitis. The equine lamellae have tested resistant to virtually all known cytokines, tissue factors and prostaglandins. Gram negative bacterial endotoxin, extract of black walnut (Juglans nigra) and even anaerobic culture conditions fail to induce lamellar separation or significant MMP activation. There is one notable exception however. A factor present in the supernatant of cultures of *Streptococcus bovis* isolated from the equine cecum activates equine hoof MMP-2 and causes lamellar separation. During grain overload *S. bovis* is the principal microorganism responsible for the rapid fermentation of carbohydrate to lactic acid in the equine hindgut. In the presence of virtually unlimited substrate its population explodes exponentially. We are currently investigating the role of the *S. bovis* MMP activator in natural cases of equine laminitis (Mungall et al 1999). If it crosses the mucosal barrier of the hindgut and enters the circulation it may be a “cause” of laminitis (at least in the carbohydrate overload model) that has escaped previous consideration.

The activity of tissue MMPs has recently been shown to correlate strongly with the degree of malignancy and invasiveness of lethal human tumours such as malignant melanoma, breast and colon cancer. Research in this field has generated a wide range of chemical agents capable of inhibiting MMP activity both in vitro and in vivo. We have shown that one of these (Batimastat or BB-94, British Biotech, Oxford) blocks the activity of the laminitis MMPs in vitro and has the potential to be a useful tool in the prevention and management of acute laminitis (Pollitt et al, 1998). Trials to test whether MMP inhibitors can prevent or ameliorate field cases of laminitis are currently underway in the Australian Laminitis Research Unit at The University of Queensland.

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REFERENCES

Allen, D. et al (1990). Evaluation of equine digital Starling forces and hemodynamics during early laminitis. Am J Vet Res. **51**: 1930-1934.

Barton. M,H, et al (1996). Endotoxin induced expression of tumour necrosis factor, tissue factor and plasminogen activator inhibitor activity by peritoneal macrophages. Equine Vet J. **28**:382-389.

Birkedal-Hansen,H. (1995). Proteolytic remodeling of extracellular matrix. Current opinion in cell biology. **7**:728-735.

Eustace, R.A. and Reddon, R.R. (1990). Iatrogenic laminitis (letter). Vet Rec. **126**: 586.

Galey, F.D. et al (1991). Black walnut (Juglans nigra) toxicosis: a model for equine laminitis. J Comp Pathol. **104**:313-326.

Hood D M et al (1978). Equine laminitis 1: Radioisotopic analysis of the hemodynamics of the foot during the acute disease. J Equine Med Surg **2**:439-444.

Hunt. R,J, (1993). A retrospective evaluation of laminitis in horses. Equine vet J. **25**: 61-64.

Longland, A and Cairns A (1998). Sugars in grass-an overview of sucrose and fructan accumulation in temperate grasses. In: proceedings of the Dodson and Horrelll International Research Conference on Laminitis. Stoneleigh, Warwickshire, England. September 1998;pp1-3.

Pollitt, C.C. (1996). Basement membrane pathology: a feature of acute laminitis. Equine vet. J. **28**: 38-46.

Pollitt, C.C. and Daradka, M. (1998). Equine laminitis basement membrane pathology: loss of type IV collagen, type VII collagen and laminin immunostaining. The Equine Hoof. Equine vet. J. Supplement **27**.

Pollitt. C,C, and Davies. C,L, (1998). Equine laminitis: its development post alimentary carbohydrate overload coincides with increased sublamellar blood flow. . The Equine Hoof. Equine vet. J. Supplement **27**.

Pollitt, C.C., Pass, M.A. and Pollitt, S. (1998). Batimastat (BB-94) inhibits matrix metalloproteinases of equine laminitis. . The Equine Hoof. Equine vet. J. Supplement **27**.

Prasse, K.W. et al (1990). Evaluation of coagulation and fibrinolysis during the prodromal stages of carbohydrate induced acute laminitis in horses. Am J Vet Res **51**: 1950-1955.

Mungall, B.A., Kyaw-Tanner, M. and Pollitt, C.C. (1999) In vitro evidence for a bacterial pathogenesis of equine laminitis (In preparation).

Robinson NE et al, 1976. Digital vascular responses and permeability in equine alimentary laminitis. Am J Vet Res **37**:1171-1174.

Salo, T., Lyons, J.G., Rahemtulla,F. Birkedal-Hansen, H. and Larjava,H. (1990). Transforming growth factor –1 up-regulates type IV collagenase expression in cultured human keratinocytes. J. Biol.Chem. **266**:11436-11441.

Trout TR et al (1990). Scintigraphic evaluation of digital circulation during the developmental and acute phases of equine laminitis. Equine vet J**. 22**: 416-421.