Submural histopathologic changes attributable to peracute laminitis in horses

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Objective—To describe submural histopathologic changes attributable to peracute laminitis in horses.

Animals—20 adult horses.

Procedure—A concurrent-control design was used to compare laminar lesions in 10 horses subjected to carbohydrate-induced laminitis with laminar characteristics of 10 sex- and aged-matched control horses with normal feet. Horses in the treatment group were administered an overload of carbohydrate. Tissues were obtained by biopsy 4 to 8 hours after onset of lameness or 72 hours after administration of the carbohydrate overload when lameness did not develop. Sections were stained with H&E, Masson's trichrome, and periodic acid-Schiff stains. Histopathologic changes were analyzed to detect differences between groups and to correlate epidermal changes with severity and duration of lameness.

Results—Analysis indicated that dermal and epidermal lesions were evident despite lack of visible separation of the epidermal basement membrane, can be found in horses without detectable lameness, and were nonspecific and progressive following onset of lameness. Furthermore, severity and location of lesions were associated with severity and duration of lameness.

Conclusion and Clinical Relevance—These observations are consistent with the concept that separation of the laminar epithelial basement membrane is a delayed step in the pathogenesis of acute laminitis, digital vascular hypoperfusion is an underlying cause for laminitis, and the potential for repeated episodes of subclinical laminitis may underlie the development of structural and mechanical changes consistent with chronic laminitis despite lack of clinical signs of acute laminitis. (*Am J Vet Res* 2003;64:829–834)

Gross and histologic changes of the submural tissues have been described for horses with laminitis. Some reports[1,a] have focused on architectural alterations of the laminar interface, such as the blunting and altered axis of the secondary lamina, that are attributed to mechanical failure of the foot secondary to disease, whereas other reports[1,2,b] have focused on the laminar and sublaminar dermal regions and evidence of hemorrhage, perivascular infiltrates, edema, distortions of vascular endothelial cells, platelets, and fibrin emboli. Common findings in the laminar epidermis include cytoplasmic vacuoles, pyknotic nuclei, and loss of cellular integrity as a result of necrosis.[3,a,b] Dermal and epidermal components at the basement membrane separate during the early stages of laminitis.[4,5] It has also been mentioned that histopathologic changes appear to have a regional distribution in which damage is more pronounced at the abaxial extent of the submural laminar interface than at the axial extent.[c] Abaxial refers to that region of the laminar interface that is nearest the stratum medium, and axial refers to that region nearest the sublaminar dermis.

Investigators have attempted to correlate histologic changes with severity of lameness and duration of disease or have used recorded histologic observations to support, refute, or develop hypotheses about the cause of acute laminitis. In this latter regard, some investigators[6,7] have interpreted histologic data to infer support for a metabolic-toxic etiopathogenesis of laminitis. This hypothesis proposes that the initial insult to the foot is directly on the epithelium of the laminar interface. In this scenario, a blood-borne factor (or factors) would act by disrupting a biochemical step that is critical to viability of the laminar epidermis or normal cornification. Advocates of this hypothesis cite the relative severity of the epidermal-to-dermal changes as evidence that the epidermal cells are the target tissues for the inciting blood-borne factor (or factors).

Proposed blood-borne factors have been referred to as laminitis-triggering factors,[3,8] and their actions have been designated as dysfunctional regulation of enzymes responsible for maintaining the normal attachment of the basement membrane to basal epithelial cells. Morphologic evidence of physical separation of the laminar basement membrane was used to support this hypothesis. Advocates of the metabolic-toxic etiopathogenesis of laminitis suggest that other epidermal and dermal changes observed in horses with laminitis are secondary consequences of the inflammatory processes or result from mechanical failure of the affected digits.

The same histologic data have been used to support a hypothesized vascular etiopathogenesis for laminitis.[9-11,c] In this hypothesized paradigm, histologic epidermal changes observed after onset of lameness are considered to be consequences of digital hypoperfusion occurring before the onset of lameness, which is followed by secondary changes induced by an inflammatory response, reperfusion injury, and trauma that is a result of mechanical failure of the digital structure. Vascular endothelial changes, platelets, fibrin thrombi, and hemorrhage are seen as evidence of insult to the digital vasculature.

Received September 13, 2002.
Accepted February 20, 2003.
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Intuitively, some of the interpretative differences of the histologic data could be addressed by a temporal correlation of histologic changes. For example, if the time of appearance and severity of dermal changes clearly precedes those same changes in the epidermis, the vascular hypothesis becomes more tenable. Unfortunately, variability in experimental design used in the aforementioned studies makes it difficult or impossible to determine such correlations. Major differences among studies include definitions used for staging disease, use of severity of lameness as the criterion for determining the times for tissue collection, and inconsistent use of analgesics and nonsteroidal anti-inflammatory agents prior to collection of tissue samples. Thus, submural histopathologic changes during the early phase of acute laminitis have not been clearly defined.

In the study reported here, we chose to describe changes to the dermis, epidermis, and basement membrane during the early phase of acute laminitis. Our hypothesis was that histopathologic lesions of peracute laminitis in horses are characterized by epidermal necrosis, despite a lack of visible separation of epidermal basal cells from the basement membrane.

Materials and Methods

Animals—Twenty adult horses were used in the study. All horses were examined and had normal feet prior to inclusion in the study. Horses were considered to have normal feet on the basis of the following criteria: no history of lameness within 8 weeks of initiation of the study, negative results for lameness examination, and no evidence of pathologic changes to the digits on examination of lateromedial and dorsopalmar radiographs. The experimental protocols used in this study were approved by a university laboratory animal care committee.

Procedure—The study was conducted as a concurrent-control experiment. The treatment group consisted of 10 horses that were serving as nontreated horses with laminitis in other studies, and the control group consisted of 10 sex- and age-matched clinically normal horses. All horses were maintained in a climate-controlled environment (mean ± SD temperature, 19 ± 1°C; relative humidity, 72%) for the 72 to 80 hours required for completion of the study.[11]

Laminitis was induced in the treatment group by administration of a standardized overload of carbohydrate.[12] Subjective evaluation of lameness was completed at 4-hour intervals during a 24-hour period before and for 60 hours after administration of the carbohydrate overload. Careful subjective assessment of lameness included the willingness of each horse to walk from a stall deeply bedded with wood shavings onto a hard rubber mat and then onto a concrete floor. Each horse in the treatment group was placed in a stock with a hard rubber floor and allowed to stand quietly for 5 to 6 minutes; the horse was then reexamined as it walked back to its stall. Willingness of each horse to turn sharply to the left and right was also assessed.

Degree of initial lameness was not graded; rather, we only recorded results as lameness detected or not detected. A horse was considered lame when it was reluctant to come out of the stall, had any change in gait, or displayed stiffness while turning. When an examination yielded a result of questionable lameness, the horse was assessed at the subsequent assessment period (ie, 4 hours later); when lameness was definitely identified at this second time point, the onset of lameness was designated as the preceding time point (ie, the original detection of questionable lameness).

Collection of biopsy specimens—For horses in the treatment group, each biopsy specimen was obtained within 2 hours after lameness was initially observed. In horses in which identification of questionable lameness was followed by confirmation of lameness at the subsequent examination, the biopsy specimen was obtained immediately after completion of the confirmatory examination. If carbohydrate-treated horses did not have detectable lameness, the biopsy specimen was obtained 72 hours after administration of the carbohydrate overload. Immediately prior to biopsy of horses in the treatment group, an Obel grade was recorded to define the degree of lameness of each horse.[13]

A submural laminar biopsy specimen was obtained from 1 randomly selected foot on the forelimb of each horse. Biopsy specimens of control horses and horses with laminitis were obtained following local injection of an anesthetic (carbocaine 1% solution) to create a bilateral abaxial digital nerve block in the selected forelimb. A drill[d] was used to create a small hole (1 to 1.5 cm) in the dorsal wall of the hoof. The center of the hole was located on the dorsal midline of the hoof at a point a third of the distance from the coronet to the bearing surface of the wall. Depth of the hole created in the wall, which involved most of the stratum medium including the inner nonpigmented layer, was subjectively determined. Optimal depth of the hole in the hoof wall was that which did not involve the laminar interface but did allow displacement of the inner wall by mild digital pressure.

An elastic tourniquet was then wrapped tightly around the limb in the metacarpophalangeal region to control hemorrhage, and the foot was prepared for surgery by use of alternating washes with iodophors and alcohol. A No. 11 scalpel blade was used to make 2 vertical, parallel, full-thickness incisions through the remaining stratum medium, laminar interface, and sublaminar dermis. The vertical incisions were typically 4 mm long and 4 mm apart. Two additional incisions were then made to connect the ends of the vertical incisions. A custom-designed, hand-made biopsy knife was then used to remove a small rectangular section of tissue consisting of the remaining stratum medium, entire laminar interface, and a small section of the sublaminar dermis. Typically, biopsy specimens had a proximodistal length of 2 mm, width of 4 mm, and depth of 3 mm.

Following collection of the biopsy specimen, the biopsy site was packed with sterile gauze and bandaged tightly with elastic bandages. The tourniquet was then removed. Postsurgical care consisted of daily bandage changes, until cornification of the biopsy site was evident. At that time, in most horses, the defect was left open to heal, but it was monitored to ensure normal continued growth of the hoof wall.

Histologic examination of biopsy specimens—Collected laminar tissue was rinsed with physiologic saline (0.9% NaCl) solution to remove blood, placed in neutral-buffered 10% formalin for 12 hours, and transferred to alcohol (70%) until processed. All samples were embedded in paraffin and sectioned at a thickness of 6 µm. Mounted sections were subsequently stained by use of H&E, Masson's trichrome, and **periodic acid-Schiff (PAS)** stains.

Stained sections were examined for lesions of the dermis (vascular and nonvascular structures), epidermis, and dermal-epidermal junction. Each histologic variable was assigned a value on a scale of 0 to 4 (0, histologic characteristics similar to those for tissues from control horses; 1, minimal histologic changes; 2, mild histologic changes; 3, moderate histologic changes; and 4, marked histologic changes). Specific changes characterized in the dermis included subjective diameter of the vasculature, hypertrophy of capillary endothelial cells (defined as detection of an enlarged rounded nucleus), perivascular changes (including hemorrhage or inflammation), and interstitial edema.

Epidermal tissues were evaluated to detect changes in morphologic characteristics of basal cells (ie, cytoplasmic vacuoles, pyknosis, or dissolution) and the secondary laminar epithelium (ie, fusion or blunting of epidermal laminae). In addition, regional distribution of histologic abnormalities within the laminar interface of each section was subjectively evaluated and assessed as to whether it was principally restricted to the abaxial (adjacent to the hoof wall), axial (adjacent to the distal phalanx), or midlaminar regions.

Alterations at the dermal-epidermal junction (ie, edema and assessment of an intact or disrupted basement membrane) were also characterized. Separation of basal epithelial cells from the basement membrane was evaluated by use of PAS stain. Separation was subjectively evaluated as to whether it was evident as well as the extent of separation. Separation was scored on a scale of 0 to 3 (0, not detected; 1, involving < 10% of the laminar interface; 2, involving 30 to 50% of the laminar interface; and 3, involving > 70% of the laminar interface).

Statistical analysis—Dermal and epidermal changes between the control horses and horses with induced laminitis were examined by use of a Wilcoxon signed-rank test. Strength of association between the severity of lameness (Obel score) and that of epidermal vacuoles, pyknosis, and dissolution or the degree of separation of the epidermal basement membrane was examined by use of the Spearman rho analysis. A *t* test was used to determine whether the value for the **Spearman rank correlation** (r_s) differed from zero. The same analysis was used to determine whether there was an association between the duration of lameness and changes in the epidermis or basement membrane. A Wilcoxon signed-rank test was subsequently used to test for differences in the means of lesion scores for the epidermis and basement membrane.

Results

Clinical monitoring—Three horses in the treatment group did not have clinical signs of lameness, and biopsy specimens were obtained from those horses 72 hours after administration of the carbohydrate overload. Initial lameness in horses that developed laminitis was subtle, consisting of stiffness of gait and reluctance to step onto hard surfaces. Interval from administration of carbohydrate overload until onset of lameness ranged from 20 to 44 hours (mean ± SD, 33 ± 7.46 hours). After initial detection, lameness became progressively more severe during the next 4 to 8 hours. At the time of biopsy, Obel lameness grades ranged from 0 to 3 (3 horses had a score of 0, 3 horses had grade-1 lameness, 1 horse had grade-2 lameness, and 3 horses had grade-3 lameness).

The biopsy procedure yielded adequate specimens for histologic analysis. We did not detect an obvious increase in lameness following the biopsy procedure in control horses or horses with laminitis. Surface of the surgical site appeared to be cornified within 1 week after biopsy, and there were no apparent long-term complications, such as lameness or cracks or splits in the hoof wall, attributable to the biopsy procedure.

Histologic examination—None of the tissues obtained from horses in the control group had changes attributable to the biopsy or disease. All of the tissue samples obtained from horses in the treatment group had histologic changes. Analysis by use of the Wilcoxon signed-rank test revealed a significant ($P = 0.009$) difference between groups.

Dermal changes that were consistently evident in the laminitis-induced horses included a mild to marked (grade 2 to 4) decrease in luminal diameter of small muscular arteries, which was interpreted as constriction. In contrast, a marked (grade 4) increase in luminal diameter of veins and venules was evident, which was interpreted as vasodilation. These changes were evident in 9 of 10 horses; the specimen from the 1 horse without such changes was obtained 4 hours after onset of lameness.

Other dermal changes that were commonly encountered included mild (grade 2) hypertrophy of capillary endothelium and minimal to mild (rare) perivascular inflammation (grade 1 to 2). Perivascular inflammation was most frequently mononuclear in character with a rare neutrophilic component. Dermal changes that were less consistently detected included minimal (grade 1) perivascular hemorrhage. Dermal edema was not detected in any of the horses in this study.

Epidermal changes included a variable degree of vacuoles, pyknosis, and dissolution in basal cells. Vacuoles were detected in 9 of 10 horses in the treatment group; they were classified as minimal to marked (grade 1 to 4), with a pattern that the more severe changes were from specimens harvested at later (6 to 8 hours) time points after onset of lameness. The 1 horse that did not have vacuoles in basal cells did have marked pyknosis and dissolution in basal cells. The biopsy specimen was obtained from that horse 8 hours after initial onset of lameness and was 1 of the latest specimens collected.

A significant but weak association was detected between severity of lameness (Obel grade) and severity of vacuolated basal cells (r_s, 0.6364) and epidermal dissolution (r_s, 0.5861) but not between severity of lameness and epidermal pyknosis (r_s, 0.4562). Analysis revealed that there was an association between time and severity of basal cell epidermal changes for basal cell pyknosis (r_s, 0.2477) and epidermal dissolution (r_s, 0.3868) but not for severity of vacuolated basal cells (r_s, 0.6453).

Regional (axial, midlaminar, or abaxial) distribution of vacuolated basal cells varied among samples. Pyknotic basal cells were detected in all horses and, similar to the situation for vacuolated basal cells, varied from minimal to marked (grade 1 to 4).

Furthermore, pyknosis was slightly more severe in tissues harvested at later (6 to 8 hours) time points after onset of lameness. One important difference from the vacuolated basal cells was that pyknosis was slightly more severe at the abaxial and midlaminar regions than at the axial region of the laminar interface. Differences in histologic changes were evident in secondary epidermal laminae in the axial region, compared with changes in the abaxial region. Secondary epidermal laminae in the abaxial region had severe pyknosis with early dissolution **(Fig 1)**. In contrast, laminae in the axial region were largely intact, with changes consisting simply of cytoplasmic vacuoles, which are potentially reversible **(Fig 2)**. Dissolution of basal cells was seen in most of the horses in the treatment group. It was similar to pyknotic changes in that

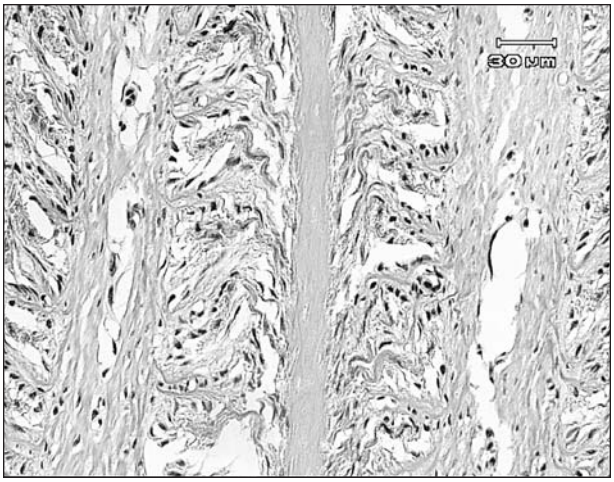

Figure 1—Photomicrograph of a section of secondary epidermal laminae from the abaxial region of the foot of a horse with laminitis induced by administration of an overload of carbohydrate. Notice the widespread epidermal pyknosis and early dissolution of laminar structures. In addition, there are artifactual clefts in the dermis. H&E stain; bar = 30 µm.

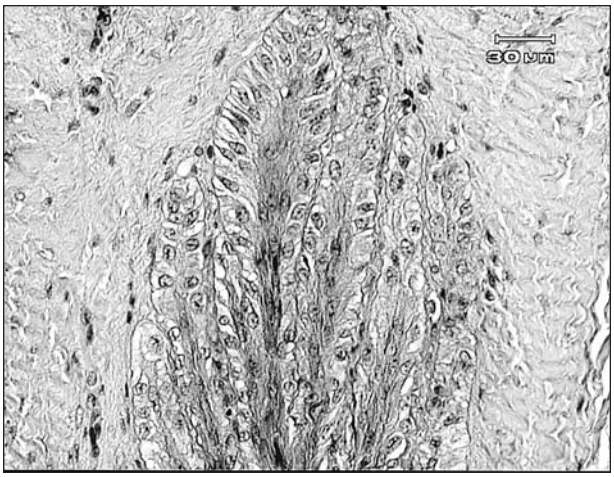

Figure 2—Photomicrograph of a section of secondary epidermal laminae from the axial region of the foot of the horse in Figure 1. Notice that in contrast to the abaxial laminae, this region is relatively normal and features only cells with cytoplasmic vacuoles. H&E stain; bar = 30 µm.

dissolution was more severe at the abaxial and midlaminar regions of the laminar interface.

Analysis revealed a significant association between the axial and abaxial distribution of epidermal lesions and Obel grade (r_s, –0.8833) or time (r_s, –0.8673). These analyses indicated that severity of lameness and duration of lameness were associated with an increase in involvement of the axial regions of the laminar interface.

Dissolution of epidermal laminae often resulted in tearing of the laminar interface; this loss of integrity invariably occurred at the junction between the abaxial and midlaminar aspects of the primary epidermal laminae. Fusion or blunting of secondary epidermal laminae was not detected in tissues obtained for this study.

The basement membrane of all horses was physically intact and attached to basal epithelial cells, despite considerable loss of integrity of the epidermal laminae (**Fig 3**). Analysis indicated that the prevalence

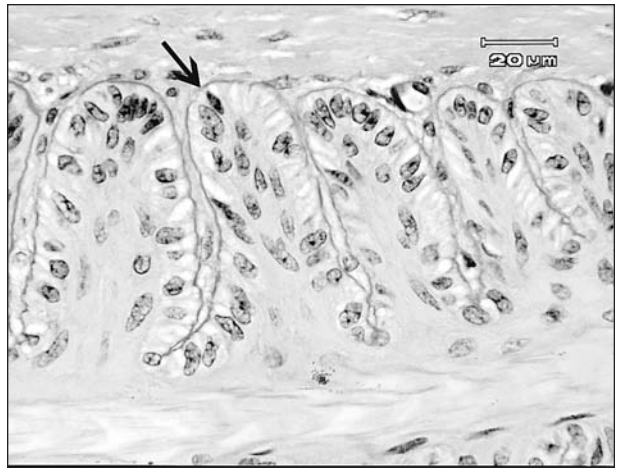

Figure 3—Photomicrograph of a section of tissue obtained from the midlaminar region of the foot of a horse with laminitis and lameness (Obel grade 2) induced by administration of an overload of carbohydrate. Notice the retention of the basement membrane (arrow) despite vacuoles and nuclear pyknosis of basal cells in the secondary laminar epidermis. Periodic acid-Schiff stain; bar = 20 µm.

of separated basement membranes differed significantly from the prevalence of epidermal vacuoles (P = 0.002), pyknosis (P = 0.002), and dissolution (P = 0.019).

Discussion

The laminar biopsy technique described here allowed consistent collection of samples from the submural laminar interface. This procedure was developed in an attempt to allow serial studies of changes in the laminar interface during acute laminitis and as a potential tool for prognosis of horses with chronic laminitis. All horses in which this procedure was used appeared to recover completely and did not have adverse affects attributable to the biopsy procedure.

Onset of lameness in the experimentally induced laminitis group was consistent with that observed in our previous experiences with the carbohydrate-overload model. Lameness onset in horses with laminitis induced by carbohydrate overload is characterized initially as subtle changes, and it then becomes progressively more severe during the next 8 to 24 hours.[11] During the first 4 to 6 hours of acute laminitis, horses decrease voluntary loading and unloading of the forelimbs, making it difficult to detect lameness.[14] Experience dictates that Obel grades exceeding 2 are rarely reached until at least 6 hours after onset of lameness. Mean interval from onset of lameness until biopsy in the study reported here (ie, 5.7 hours) reflects the duration of detected lameness. Because the horses were evaluated for lameness at 4-hour intervals, it is possible that some horses were lame prior to the time it was first recorded. Thus, histopathologic changes must be viewed as occurring within a time frame of 4 to 8 hours after onset of lameness, rather than at a single point in time relative to lameness onset.

Reported histologic data and interpretations of the data represented only a cross-sectional recording of the changes evident during a fairly narrow range in time. Additionally, the severity and character of observed

changes appeared to be progressive over time. Relative weakness of the r_s, which indicated a significant correlation between time of biopsy and epidermal pathologic changes, was attributed to the small number of horses in this experiment. Consequently, lack of specific lesions (including platelets and fibrin thrombi), some architectural changes (blunting and fusion of the secondary laminar structures), and loss of basement membrane integrity does not preclude that they would have been evident at earlier or later times during the course of laminitis.

The character of dermal and epidermal lesions recorded in this report are generally similar to that reported in other studies.[a-c] Changes observed in the sublaminar dermis during the early, peracute phase of laminitis appear to be largely associated with the vasculature. The decrease in lumen diameter in arterioles, increase in lumen diameter in small veins, perivascular mononuclear infiltrate, capillary endothelial hypertrophy, and sporadic perivascular hemorrhage are consistent with reactions to a local insult. The mechanisms responsible for the changes in vessel diameter cannot be defined from anatomic data alone. It is possible that the circulation at this point in time was responding to endogenous or exogenous factors that were causative for the condition, associated with direct injury to the vasculature, or reflected a vascular response to metabolic factors from the dermal or epidermal tissues. Lack of dermal edema and the increase in venous diameter suggest that compartment injury involving the sublaminar dermis and dermis of the primary dermal lamina is not a major factor during this early peracute stage of the condition. The mechanism or mechanisms responsible for the mononuclear infiltrate could reflect a local response to insult of the dermal vasculature, or it may represent a component of the pathophysiologic response to an epidermal insult.

Changes detected in the laminar interface reflect generalized vacuolization, pyknosis, and dissolution of the basal epithelial cells. Given that the principal tissue examined in this study consisted of highly modified skin, it follows that the histologic changes that were a sequelae to any insult are potentially limited. That is, the histologic responses to ischemia and acute reperfusion that occur secondary to a toxic or other metabolic insult may be similar, thus making it difficult to define which, if either, is responsible for the observed changes. Relative severity of the epidermal changes at this early stage of lameness allows the hypothesis that the epidermal changes were there prior to onset of lameness.

Epidermal (and dermal) changes in those horses that did not have detectable lameness are not surprising. Given that a diagnosis of lameness is a subjective interpretation, it is possible that the horses had some sensation or discomfort that was not detected by the investigators. Alternatively, it is possible that a low degree of epidermal change can exist without inducing discomfort. Regardless, evidence of laminar disease without lameness can be hypothesized to be a potential cause for gross and radiographic changes of chronic laminitis in horses in which pain attributable to acute laminitis has not been documented. Speculatively, internal remodeling of the laminar interface accompanied by failed digital mechanics could be the result of the accumulated damage incurred during repeated episodes of subclinical insults.

Once lameness is detected, its severity is positively correlated with that of epidermal vacuolation, epidermal dissolution, and an increase in axial involvement of the laminar interface. This is presumed to be associated with increases in submural pressure produced by vacuolation, an increasing inflammatory reaction to the damaged cells, and mechanical sequelae associated with collapse of the digit as the supportive function of the laminar interface decreases.

The positive correlation between duration of lameness and increasing depth of axial involvement of the laminar interface is interpreted as evidence that histologic change is progressive during the acute phase of laminitis. Distribution of epidermal pathologic changes within the laminar interface can be interpreted to help illuminate the etiologic mechanisms of laminitis. Although there did not appear to be a distinct regional distribution for vacuoles in the basal cells, cellular pyknosis and dissolution did have a regional distribution. Specifically, these data indicated that detection and severity of these changes in the peripheral (ie, abaxial) aspects of the interface were more severe than in the innermost or axial regions of the interface.

These histologic changes are intuitively consistent with changes expected if it is presumed that there is a vascular etiologic cause for laminitis. Specifically, the damage should be more severe in regions of the laminar interface that are farthest from the primary vascular supply located in the submural dermis. Similarly, the part of the laminar interface closest to the submural dermis should have substantially less damage. It is interesting that this pattern appears to mirror the changes associated with the hypodynamic circulation in intestinal microvilli during shock.[15] However, if it is presumed that a toxic or metabolic defect is the primary etiologic cause for acute laminitis, it has to be further hypothesized that the basal epithelial cells located at the abaxial regions of the interface have a substantially higher metabolic rate or are more sensitive to toxins than are the cells located at the axial regions of the interface. Otherwise, histologic changes associated with a toxin should be more severe at the axial surface, where higher concentrations of laminitis-triggering factors should be encountered.

Detection of substantial epidermal changes without histologic evidence of separation of the basement membrane suggests that the loss of attachment to the basement membrane during acute laminitis is essential to epidermal pathologic changes. Although our experience and reports in the literature[4,5] indicate that there is loss of membrane integrity, physical separation does not become visible until 4 to 8 hours after onset of lameness. Although histologic separation of the basement membrane was not visible in the study reported here, it is likely that biochemical separation of damaged epithelial cells from the membrane may have been initiated.

Various logical scenarios and hypotheses have been offered for the etiologic cause and mechanisms of

separation of the basement membrane during laminitis; however, its occurrence as a consequence of epidermal disease has not been defended. In other species and tissues, separation of the basement membrane is a common consequence of ischemia.[15,16] Given the role of the basement membrane in establishing the normal architecture of the overlying epithelia, it could be assumed that shedding of damaged epithelia is a protective response. In this case, retention of the basement membrane with the relatively undamaged dermis is putatively necessary for the healing epidermis to resume architecture compatible with its function.

In the feet of horses, physiologic loss of pathologic epithelial cells may have additional and important clinical implications. Epidermal cells typically synthesize and store large amounts of cytokines.[17,18] It has been proposed[18] that interleukins are a component of a protective mechanism responsible for promoting an immediate inflammatory response when integrity of the skin is lost. Retention of the epithelial cells with their cytokines in close association with the dermis in the feet of horses could be catastrophic. Any swelling associated with an inflammatory response could lead to an increased likelihood of substantial compartment injury as a result of the encapsulating effects of the relatively ridged hoof wall. This is particularly true when considering the amount of swelling in the secondary epidermal laminar epithelial cells. Thus, it can be hypothesized that separation of the basement membrane from the basal epithelial cell layer is beneficial to the long-term recovery of some horses, and forced retention of this structure may have adverse effects.

Histologic changes of peracute laminitis include dermal and epidermal lesions but do not include visible separation of basal epithelial cells from the basement membrane. It was also evident that the epidermal changes observed were nonspecific, progressive, have a distinct abaxial-to-axial distribution, and were found in horses without detectable lameness. These observations are consistent with the recorded separation of the laminar epithelial basement membrane being a delayed step in the pathway of acute laminitis, underlying digital vascular hypoperfusion as a cause for laminitis, and the possibility that repeated episodes of subclinical laminitis may underlie the development of structural and mechanical changes consistent with chronic laminitis without clinical signs of acute laminitis.

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