

**HISTOLOGIC CHARACTERIZATION OF THE LAMINAR INTERFACE AT  
THE CRENA MARGINIS SOLEARIS IN HORSES**

By:

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## **Abstract**

**Objective:** To characterize the microscopic anatomy of the laminar interface at the distal margin of the distal phalanx in equine forefeet with the presence of a crena marginis solearis.

**Sample Population:** 6 equine cadaver forefeet with radiographic evidence of a crena marginis solearis and 2 equine cadaver forefeet without radiographic evidence of a crena marginis solearis.

**Methods:** Cadaver forefoot specimens were selected by screening for the presence (6) or absence (2) of a crena marginis solearis using radiography. Selected specimens were evaluated grossly for the presence of abnormalities of the hoof wall, white line, and sole in the toe region. Transverse, contiguous tissue sections from each specimen that included the hoof wall, the laminar interface, the dorsodistal margin of the distal phalanx, and sole were obtained, processed, and evaluated by light microscopy. The gross and microscopic findings from specimens with and without a crena marginis solearis were recorded and compared.

**Results:** Gross examination of the hoof capsules of the feet with a crena marginis solearis revealed signs of white line disease in the toe region of all specimens (6/6) and dorsodistal hoof cracks of varying degree in 3 of 6 specimens. Marked disorganization of laminar morphology was observed in all of the specimens with a crena marginis solearis. Both specimens without a crena marginis solearis displayed normal laminar morphology and hoof capsules free of white line disease and dorsodistal hoof wall cracks.

**Conclusion:** There is a distinct aberration of laminar architecture in feet with a crena marginis solearis.

**Potential Relevance:** The disorganization of the laminar interface in specimens with a crena marginis solearis could explain the perceived increased susceptibility to white line disease and dorsodistal hoof wall cracks in horses with a crena marginis solearis.

## Introduction

The horse's distal phalanx is attached to the inside of the hoof capsule by interdigitating dermal (from the distal phalanx) and epidermal lamellae (from the hoof). This connection is commonly referred to as the laminar interface. Normal morphology of the laminar interface consists of approximately 600 primary dermal and epidermal lamellae of approximate equal length interdigitating together, with approximately 100-150 secondary lamellae projecting from each (Stump, 1967 & Pollitt, 1996). The lamellae are arranged parallel to each other and nearly perpendicular to the distal phalanx (Stump, 1967). Dermal and epidermal lamellae are separated by the basement membrane. Nucleated epidermal basal cells line the epidermal side of the basement membrane (Pollitt, 1996). Beyond the basal layer are slender keratinizing cells (parabasal) that thicken and strengthen the bond of the primary to the secondary epidermal lamella (Pollitt, 1996). On the dermal side of the basement membrane, collagen fibers connect the basement membrane to secondary dermal lamellae and form a continuum to the distal phalanx forming the bond of the hoof wall to the distal phalanx (Pollitt, 1995). Throughout the connective tissue of the lamellar dermis are areas of arteries, veins, and capillaries (Pollitt, 1995).

The crena marginis solearis (crena) is a shallow notch in the distodorsal solar margin of the distal phalanx (O'Grady, et al., 2007). One study found that 95% of forefeet and 48% of hind feet had radiographic evidence of a crena (Rendano, Grant, 1978). Another study briefly referenced the histology of the lamellar interface at the level of the crena – describing a disruption of typical architecture of the lamellar interface (Lancaster, et al., 2007). The primary epidermal lamellae appeared to be elongated with no evidence of secondary epidermal lamellae, with the interlamellar space being filled with tubular hornlike material.

White line disease (WLD) is a keratinolytic condition observed on the solar aspect of the equine hoof characterized by a progressive separation of the inner zone of the hoof wall that was first described in 1990 (Redden, 1990). White line disease affects horses of various breeds, ages, and uses. Historically farriers have described an abnormal area on the solar surface of the hoof at white line-sole interface in the toe region that is believed to correspond to the presence of a crena. It has also been hypothesized that this area predisposes the horse to white line disease and dorsodistal hoof wall cracks as a result of a weakened bond between the hoof capsule and the distal phalanx. The purpose of this study was to characterize and compare the histologic morphology of the laminar interface of horses with and without radiographic evidence of a crena. The objective of this study is to determine if the laminar morphology is histologically different among horses with and without a crena.

## Materials and Methods

This study was completed in 2015 and was approved by the Institutional Animal Care and use Committee at Virginia Tech.

Study samples were obtained from the forefeet of horses aged between 4 and 20 years that were euthanized at the Virginia-Maryland College of Veterinary Medicine (VMCVM) for reasons unrelated to the hoof. After recording the horse's age, breed, sex, and reason for euthanasia, the distal forelimbs were removed at mid-diaphyseal level of the third metacarpal bone. Each foot was then examined for the presence/absence of an abnormal area on the solar surface of the hoof at the white line-sole interface in the toe region and dorsodistal hoof wall cracks. Excess hoof wall was then removed using nippers and a rasp, and the sole cleaned using a hoof knife and a wire brush. The foot was next placed in an aluminum 65° dorso-palmar positioning block <sup>a</sup> and a Sound Eclin Mark III <sup>b</sup> digital radiograph machine along with a minXray TR90B <sup>c</sup> x-ray generator set at 80 kVp and 0.12 mA was used to obtain a 65 degree dorsoproximal-palmarodistal oblique radiograph of the distal phalanx. Study populations were created by first determining if there was radiographic evidence of a crena (Figure 1). Those distal phalanxes with the presence of a crena that measured greater than or equal to 5 millimeters in depth at the distal margin were used to create an experimental population of 6. Measurements of the radiographs were performed using EFilm Work Station <sup>d</sup>. Those distal phalanxes with a crena measuring less than or equal to 5 millimeters in depth at the distal margin were discarded. A control population was created by selecting 2 horses without radiographic evidence of a crena along the distal margin of the distal phalanx (Figure 2). All procedures were performed within 4 hours of euthanasia.

All selected feet were then processed according to a previously described protocol (Pollitt, 1996). The sole of the hoof was placed on the base plate of the band saw. The first cut was made in a medial-lateral direction just palmar to the hair line of the coronet. With the newly cut surface facing the operator and the sole surface on the base plate a second and a third saw cut was made, on either side of frog, approximately 25 mm apart. Two additional saw cuts were made, the first 1 mm distal to and perpendicular the distal margin of the distal phalanx, the second 4 mm proximal to and perpendicular the distal margin of the distal phalanx. Hoof nippers were used to remove the bulk of the hoof wall and sole. All but less than 1 mm of the inner hoof wall was removed from the block to facilitate successful sectioning of the tissue with a microtome. Sections were placed in a processing cassette and then fixed in 10% formalin for at least one week.

Sections were then batch processed in a vacuum infiltration processor according to the protocol in Table 1. After processing, the sections were placed in a holding chamber and maintained at 61° Celsius until further processing. Subsequently, each section was removed from its processing cassette and placed face down into a warmed, embedding mold containing a small amount of paraffin. The mold (containing the section) was then placed on a cooling plate and filled with more liquid paraffin (enough to cover the entire sample). The bottom portion of the processing cassette was then placed on top of the mold, forming a lid to the tissue - thus creating a block. The completed, filled mold was

then placed on the cold plate at  $-2^{\circ}$  Celsius, securing the paraffin surrounding the tissue into one paraffin embedded tissue block. After approximately 10-15 minutes of cooling, the block was removed from the mold by pulling from the back of the cassette. Excess paraffin was removed using a microtome to achieve a full faced sample. To achieve a more even cut the surface of the block was first treated with a surface decal solution for approximately 30 minutes to 1 hour and then placed in Nair<sup>®</sup> for 3-4 hours. The Nair solution was then removed using cold water and the sample was placed in an ice bath for approximately 30 minutes. Sections 3-5  $\mu\text{m}$  thick were cut using a microtome. The sections were placed into a  $38^{\circ}$  C water bath and picked up onto double charged slides. The slides were first placed into an oven set at  $38^{\circ}$  C for 30 minutes, then transferred to an oven set at  $70^{\circ}$  C for 30 minutes to ensure adherence and drying. After drying the slides were stained with Gomori's one step trichrome. The slides were then examined utilizing light microscopy to describe the lamellar morphology.

## Results

Specimens from the experimental population (crena) ranged in age from 10 to 20 years with a mean age of 13.8 years. Three of the specimens were from thoroughbreds while the other 3 were from warmbloods. Four of the specimens were from geldings and 2 were from mares. Four of the specimens were from left forelimbs and 2 were from right forelimbs. Specimens from the control population (no crena) were both from left forelimbs. One was from a 16 year old warmblood mare and one was from an 18 year old quarter horse mare.

All feet (6/6) that exhibited radiographic evidence of a crena had corresponding abnormalities in the solar surface of the toe of the hoof at the junction of the white line, sole, and non-pigmented stratum medium of the hoof wall (Figure 3) that appeared to be affected with white line disease. The lesions were characterized by lack of a normal hoof wall-white line-sole interface, and soft poor quality horn. 3 of the feet (3/6) with crenas featured a hoof wall with a small crack on the dorsal surface of the hoof (Figure 4). Both feet without radiographic evidence of a crena had a normal junction between the sole, white line, and non-pigmented stratum medium of the hoof wall at the toe (Figure 5) and a dorsal hoof wall free of cracks (Figure 6).

Review of the control specimens at 4X and 40X magnification confirmed previously described morphology of the laminar interface. A layer of tubular horn of the stratum medium connecting to epidermal lamellae of approximate size and shape interdigitated with the dermal lamellae and then connected to a uniform thickness of sublamellar dermis covering the distal phalanx. The opposite side of the distal phalanx connected to solar dermis that connected to uniformly arranged tubules of the sole (Figure 7). The epidermal lamellae were arranged nearly perpendicular to the hoof wall and the dermal lamellae were arranged nearly perpendicular to the distal phalanx. Each primary epidermal lamellae (PEL) had 100-150 secondary epidermal lamellae (SEL) extending from it. The keratinized axes of the PEL were predominately straight and wider at their base while gradually tapering toward the tip. The occasional primary epidermal lamella bifurcated

and had two keratinized axes. The SEL were arranged at an acute angle to the PEL gradually becoming parallel to the PEL at the cap. Each PEL and SEL interdigitated with a corresponding primary dermal lamellae (PDL) and secondary dermal lamellae (SDL) (Figure 8). Each PDL was comprised of two collagen bundles organized parallel to the PEL. There were many arteries and veins located between the two collagen bundles of the PDL. The sublamellar dermis was comprised of many dense collagen bundles. The space between the collagen bundles was filled with several prominent veins and arteries. A single row of nucleated epidermal basal cells with a basement membrane separating the SEL and SDL was apparent. Parabasal cells were found within the SEL. The SEL and SDL were all club shaped at the tip (Figure 9). On occasion SEL bifurcated and contained two axes (Figure 10). The basement membrane was connected to the SDL with fine collagen fibers. Within the collagen tissues of the SDL lamellar capillaries were apparent.

Review of the specimens in the experimental population at 4X and 40X magnification revealed a distinctly different laminar interface morphology. The center of the crena was filled with, from dorsal to palmar with: PEL devoid of SEL; indistinct keratinized horn; solar like epidermis; and then solar dermis. No normal dermal structures (PDL, SEL, blood vessels) could be identified (Figure 11). Of note was that the margins of the crena also displayed aberrant laminar interface morphology. In one sample there was normal laminar morphology on the medial and lateral sides of the crena that transitioned from normal to abnormal as the crena was approached. As the crena was approached from laterally or medially, the SEL appeared to thicken and fuse (Figure 12). Eventually, SEL, PDL, and SDL could not be recognized. Islands of dermal like structures that contained collagen spheres that contained lamellar capillaries and veins could sometimes be seen (Figure 13). However, due to limitations in specimen width dictated by tissue block and microscope slide dimensions, clear demonstration of a complete metamorphosis from a normal laminar interface, through the transition zone, to the crena was not possible in the other specimens.

## **Discussion**

The results of this study demonstrate that there is marked aberration in the organization of the laminar interface at the distal margin of the distal phalanx in forefeet that have radiographic evidence of a crena. The degree of aberration appears to correlate with the size of crena - with larger crenas displaying greater aberration in morphology than smaller crenas. Further studies though, specifically comparing the size of the crena and the severity of the disorganization of the laminar interface would be needed to confirm/refute this observation. In regards to clinical relevance, it is apparent that the crena creates a relatively large space between the inner hoof wall and distal phalanx that must be filled by tissue in order to avoid a corresponding notch or indentation on the dorsal surface of the hoof wall. The tissue that fills the space created by the crena and how this occurs poses many questions that require clarification.

The results of this study also demonstrate that there appears to be a transition zone on each side the crena (medially and laterally) where the SEL begin to fuse (starting at the

base of the PEL) and decrease in numbers as the crena is approached, until there is no evidence of SEL, PDL, or SDL. In the transition zone there are islands of dermal structures left behind surrounded by remaining collagen fibers similar to the basement membrane. It is this author's theory, that in order to fill the void of the crena and maintain a bond between the distal phalanx and the hoof wall, that the epidermal basal cells of the SEL proliferate and produce keratin (to fill the void). As the epidermal basal cells proliferate in this transition zone, the keratinocytes created stack and mature, reducing the area occupied by the dermal structures. Unfortunately, due to limitations in specimen width dictated by tissue block and microscope slide dimensions, clear demonstration of a complete metamorphosis from a normal laminar interface, through the transition zone, to the crena was only possible in one specimen. It is assumed that this metamorphosis from normal to clearly abnormal, would have been seen in the other specimens if larger tissue blocks/microscope slides could have been utilized.

There are other hypotheses regarding the crena and the corresponding laminar morphology. One is that as the hoof wall is growing distal from the coronet to the distal margin of the distal phalanx there is a normal laminar interface. Then at the margin of the crena the laminar attachment takes on an appearance similar to the white line junction where PEL interdigitate with the sole (Lancaster, et al., 2007). This has been described previously and referred to as ectopic white line by the authors of one study (Kuwano, 2002). The results of the present study support this hypothesis as the crenas were observed to be filled with (from dorsal to palmar): PEL devoid of SEL, PDL, and SDL; indistinct keratinized horn, solar epidermis, and solar dermis.

Another hypothesis of this author is that a small keratoma may be the cause of the crena. The description of the tissue filling the area of the crena in the present study resembles the histological description of a keratoma (Hamir A, et al., 1990). Further studies would need to be performed to confirm or reject this hypothesis. Comparing tissue specimens side by side and mitotic activity of the keratinocytes associated with a keratoma and a crena could potentially help differentiate between the two.

The horses in this study that had radiographic evidence of a crena also suffered from varying degrees of WLD and cracking of the dorsal hoof wall at the toe. The marked disruption of normal laminar architecture confirmed by this study support the hypothesis posed by many farriers that horses with a crena are predisposed to WLD and dorsal hoof wall cracks as a result of a weakened attachment between the hoof capsule and distal phalanx. In practice there are many horses with hooves that contain small areas of WLD only at the toe. When veterinarians and farriers are faced with such cases, 65° dorso-palmar radiographs of the distal phalanx should be performed to determine the characteristics and presence of a crena. If there is radiographic evidence of a crena it is reasonable to conclude that these cases are the result of laminar interface disorganization associated with the crena. If the areas of WLD and secondary dorsal hoof wall cracks are left untreated or are inappropriately managed they could become large areas of WLD that could result in an unstable digit or full thickness hoof wall cracks. To manage these horses, it would be wise to take preventative measures. Affected feet should be trimmed on regular intervals (4-6 weeks) to remove distortion and mechanical levers of the toe

that may exacerbate stress on the weak area. Areas demonstrating signs of WLD should be debrided to healthy appearing tissue and treated with an antiseptic topical solution. However, many of these cases only improve when debrided, treated with topical antiseptic, packed with antiseptic packing, and enclosed with a poly-methyl methacrylate impregnated polymeric fabric patch in conjunction with a horse shoe fitted with a pad (with antiseptic packing [such as an oakum, venice turpentine, copper sulfate mixture] beneath).

A future study is needed to determine the number of front and hind distal phalanxes that have a crena. One study found that 95% of forefeet, and 48% of hind feet had radiographic evidence of a crena (Rendano, Grant, 1978). However, in locating horses that qualified for the present study, these numbers came into question. Though it was not officially documented, finding forelimb distal phalanxes with a crena of at least 5 millimeters in depth was difficult. In the study by Rendano and Grant (1978), a crena was defined as a concavity that was detectable in the dorsal midline solar border of the distal phalanx ranging from a shallow indentation to an easily discernable one, and only 31 feet were evaluated. An updated study needs to be performed utilizing a larger sample population and also utilizing updated digital radiographic technology with a specific measureable depth and width to qualify as a true crena rather than what could be recognized as simply an irregularity in the solar margin of the distal phalanx.

If the current study describing laminar morphology of the crena were to be performed again, it could be improved upon by adding more horses to the sample population. It could also be improved upon by categorizing the sample population into groups according to the width and depth of the crena. In addition, taking samples in both a transverse and sagittal plane, as well as proximal, distal, medial, and lateral to the crena, could further improve the description and understanding of the aberrant laminar morphology associated with crenas.

## **Conclusion**

The results from this study demonstrate clear histologic evidence of aberration of the laminar interface in the region of a crena. The gap between the inner hoof wall and the distal phalanx is filled with (from dorsal to palmar): PEL devoid of SEL, PDL, and SDL; indistinct keratinized horn, solar epidermis, and solar dermis. The attachment of the hoof wall and distal phalanx is potentially compromised as a result, which predisposes the horse to WLD and dorsal hoof wall cracks. Therefore, it would likely be wise for veterinarians and farriers to identify horses with a crena and take preventative measures, such as frequent trimming and application of horse shoes, to discourage this area of the hoof wall from succumbing to WLD and secondary dorsal hoof wall cracks.

## Acknowledgements

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## Manufacturer's Addresses

- a. Redden Navicular X-Ray Block. Nanric Inc., Lawrenceburg KY.
- b. Sound Eklin Mark III. Sound, Carlsbad CA.
- c. MinXray Inc., Northbrook IL.
- d. eFilm Workstation, Version 3.3. Merge Healthcare Inc., Chicago IL.
- e. Nair. Church and Dwight Co. Inc., Ewing, NJ.

## References and Further Reading

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Stump JE. (1967) Anatomy of the normal equine foot, including microscopic features of the laminar region. *J Am Vet Med Assoc* **151**, 1588–1597.

**Table 1:****Delayed Schedule for Tissue Processing**

<b>Station #</b>	<b>Solution</b>	<b>Time (Min)</b>
1	10% Neutral Buffered Formalin	45
2	70% Reagent Alcohol	45
3	80% Reagent Alcohol	45
4	95% Reagent Alcohol	45
5	95% Reagent Alcohol	45
6	100% Reagent Alcohol	45
7	100% Reagent Alcohol	45
8	100% Reagent Alcohol	45
9	Xylene	60
10	Xylene	60
11	Paraffin	60
12	Paraffin	60
13	Paraffin	60
14	Paraffin	60
<b>Total Time (Min)</b>		<b>720</b>



**Figure 1:** 65 degree dorso-palmar radiograph of a distal phalanx with a crena from a study specimen.



**Figure 2:** 65 degree dorso-palmar radiograph of a distal phalanx without a crena from a study specimen. Figures 2, 5, and 6 are of the same specimen.



**Figure 3:** Photograph of the white line from a study specimen with a crena. Note the abnormal horn at the hoof wall- sole interface in the toe region. This specimen exhibited focal white line disease and a dorsodistal toe crack. Figures 3 and 4 are of the same specimen.



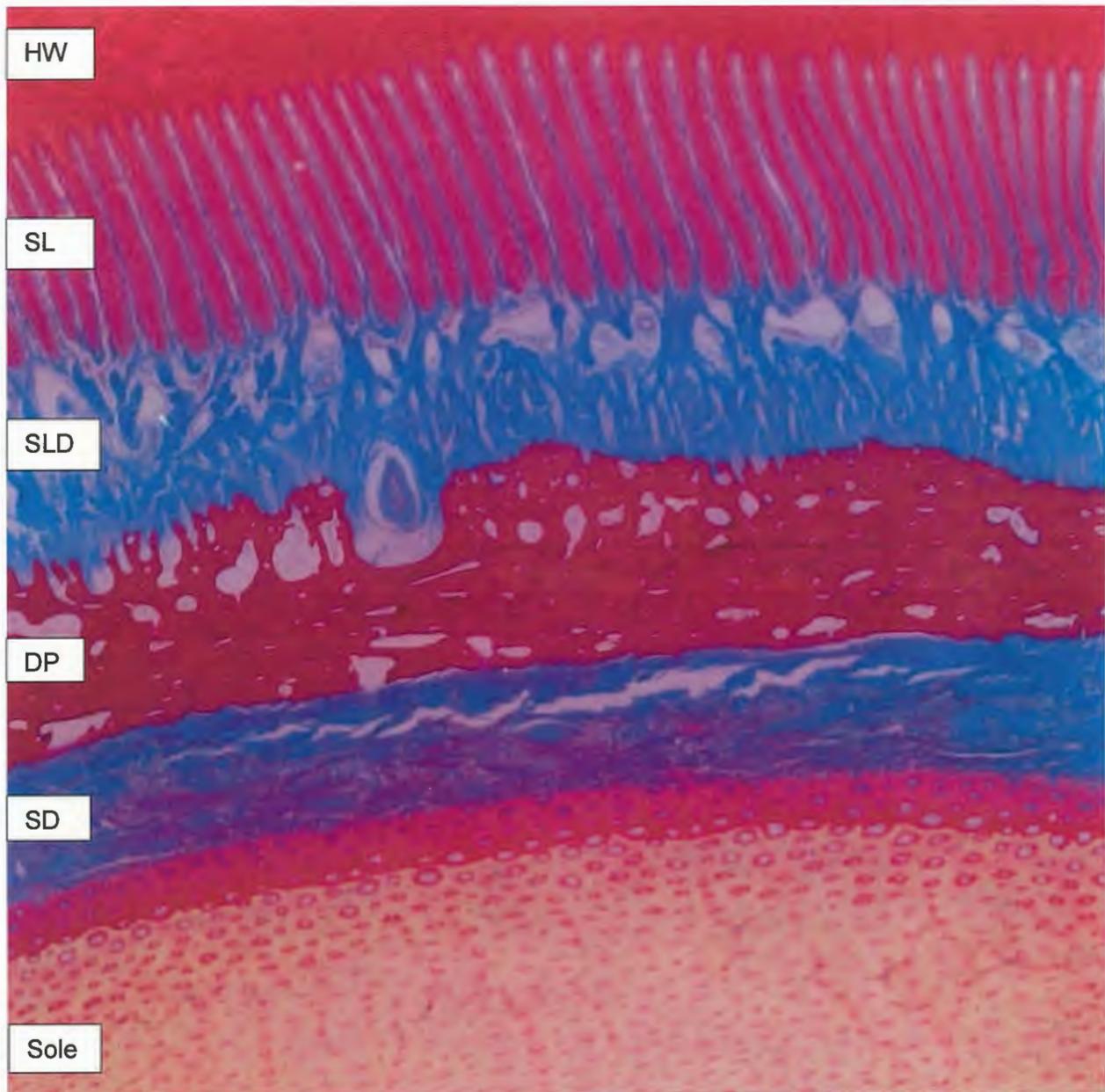
**Figure 4:** Photograph of the dorsal hoof wall from a study specimen with a crena. Note the small dorsodistal hoof wall crack. Figures 3 and 4 are of the same specimen.



**Figure 5:** Photograph of the white line from a study specimen that did not have a crena. Note the normal appearance of the hoof wall-sole interface. Figures 2, 5, and 6 are of the same animal.

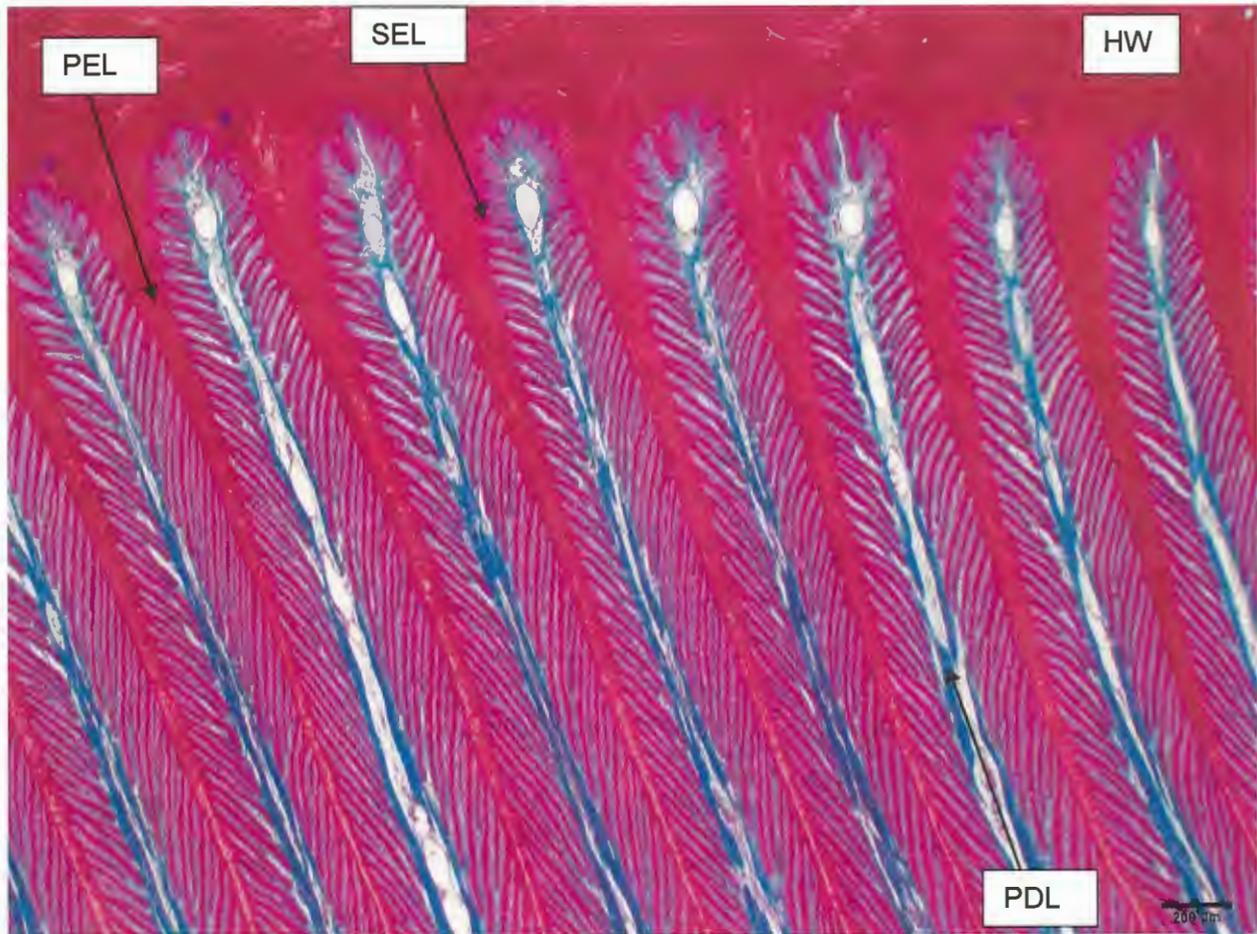


**Figure 6:** Photograph of the dorsal hoof wall of a study specimen. Note the normal appearance of the dorsal hoof wall. Figures 2, 5, and 6 are of the same animal.



**Figure 7:** Photograph of a transverse contiguous tissue section of hoof wall, laminar interface, sublamellar dermis, distal phalanx, solar dermis, and sole from a study specimen without a crena. (Gomori's one step trichrome stain). Keratin structures are stained red while dermal collagen structures are stained blue. Note normal tissue interfaces.

Abbreviations: HW- hoof wall, SL- stratum lamellatum, SLD- sublamellar dermis, DP- distal phalanx, SD- sole dermis.



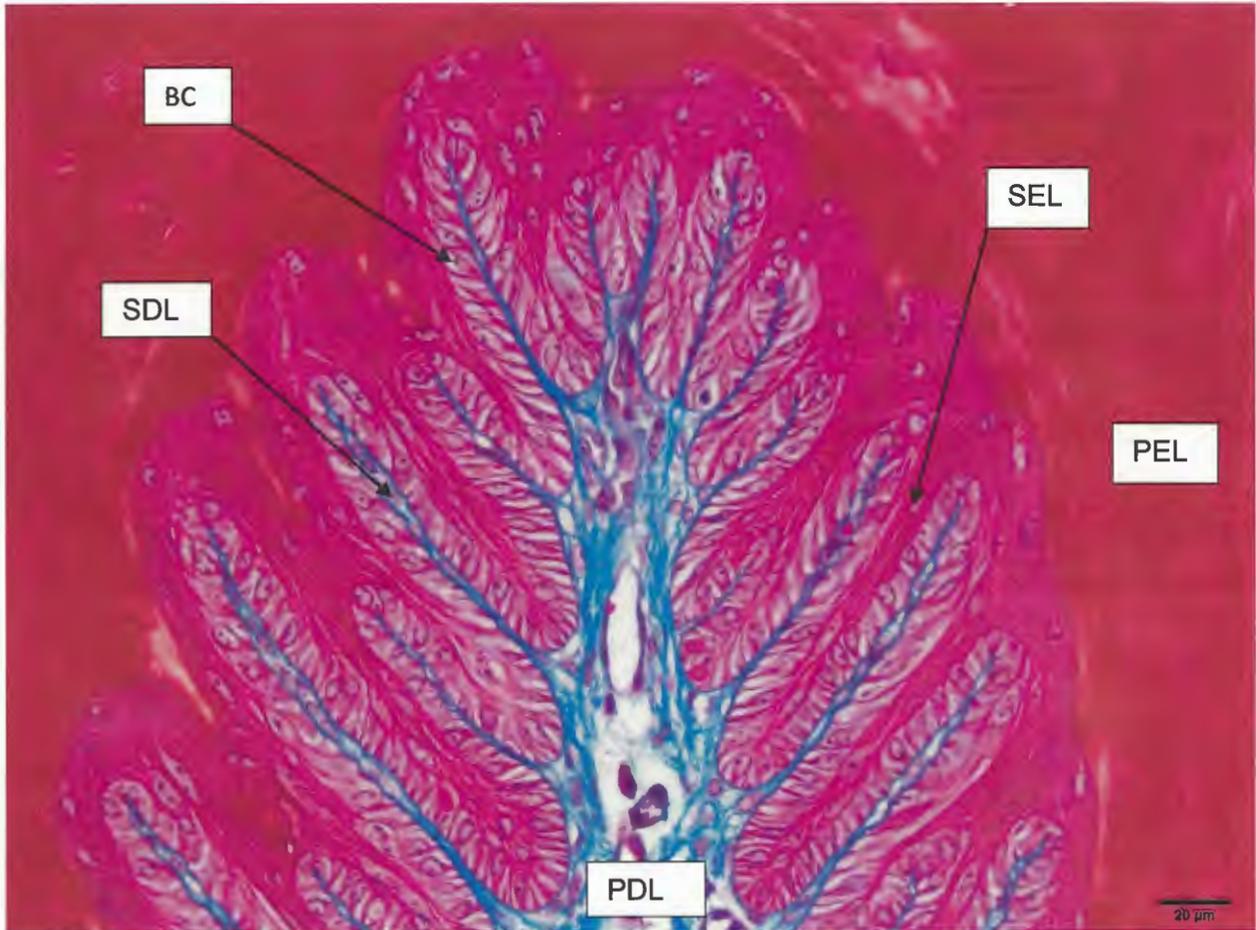
**Figure 8:** Photomicrograph of a transverse section of the laminar interface from a study specimen without a crena (Gomori's one step trichrome stain); 4X magnification; Keratin structures are stained red while dermal collagen structures are stained blue. Note normal lamellar architecture.

Abbreviations: PEL- primary epidermal lamellae, SEL- secondary epidermal lamellae, PDL- primary dermal lamellae, HW- Hoof wall.



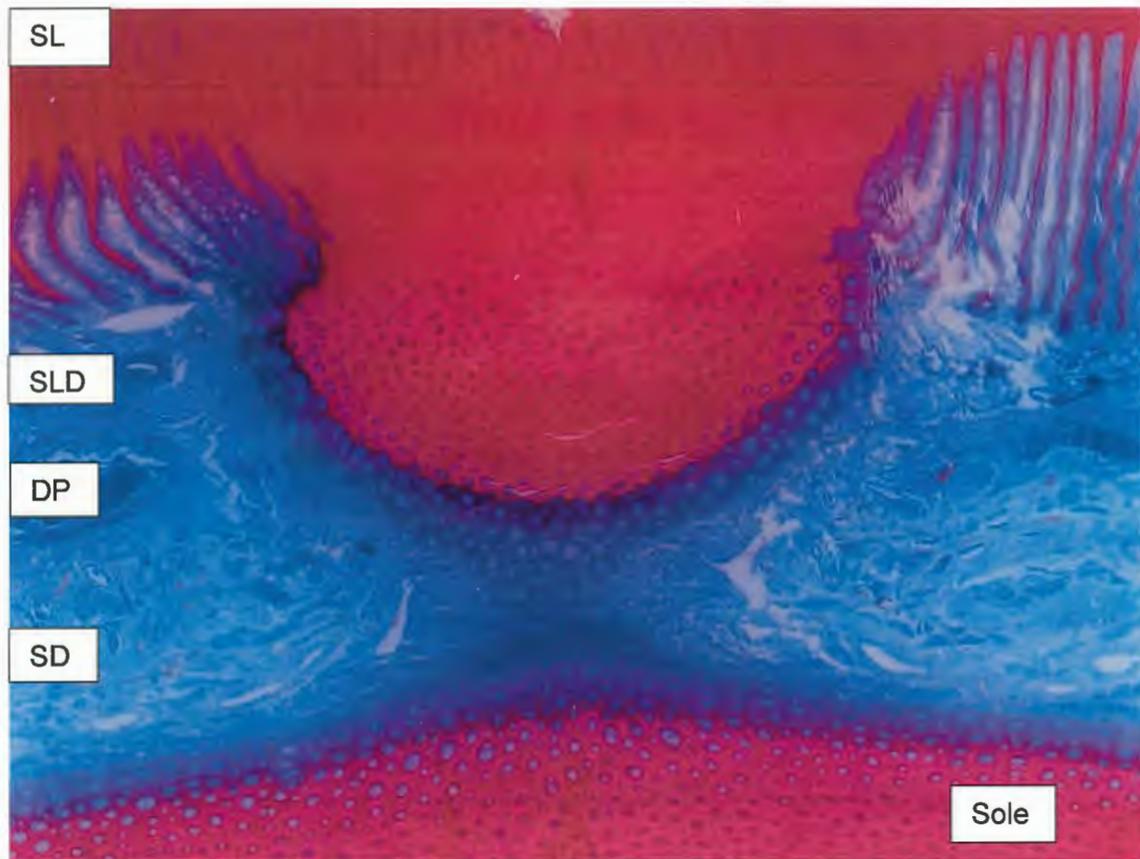
**Figure 9:** Photomicrograph of a transverse section of the laminar interface from a study specimen without a crena. (Gomori's one step trichrome stain); 10X magnification; Keratin structures are stained red while dermal collagen structures are stained blue. Note normal lamellar architecture with caps that are rounded and blunt.

Abbreviations: PEL- primary epidermal lamellae, SEL- secondary epidermal lamellae, PDL- primary dermal lamellae.



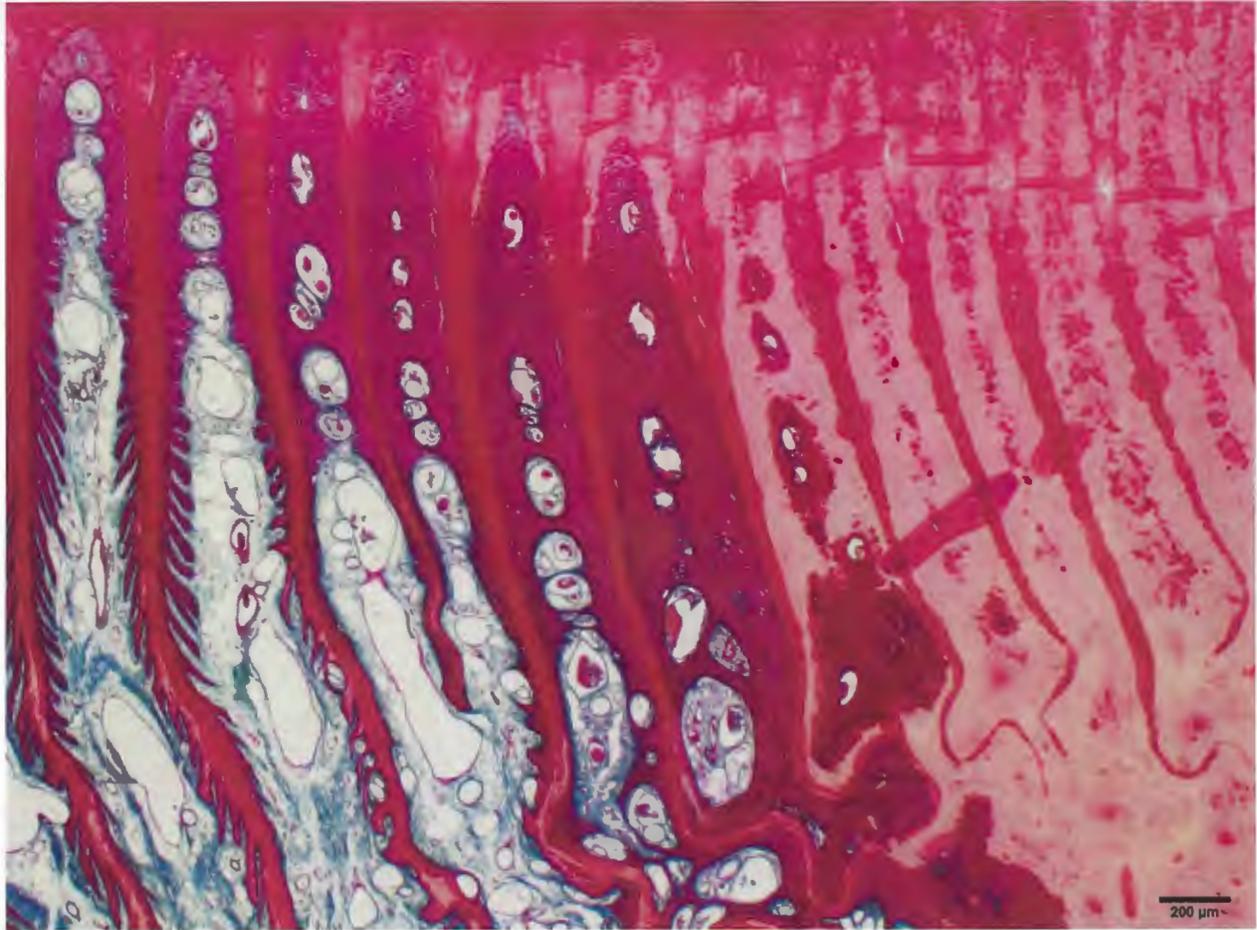
**Figure 10:** Photomicrograph of a lamellar cap from a study specimen without a crena. (Gomori's one step trichrome stain); 40X magnification. Keratin structures are stained red while dermal collagen structures are stained blue. Note normal lamellar architecture.

Abbreviations: PEL- primary epidermal lamellae, SEL- secondary epidermal lamellae, PDL- primary dermal lamellae, SDL- secondary dermal lamellae, BC- epidermal basal cell.



**Figure 11:** Photograph of a transverse section of the laminar interface, sublamellar dermis, distal phalanx, solar dermis, and sole from a study specimen with a crena. (Gomori's one step trichrome stain) Keratin structures are stained red while dermal collagen structures are stained blue. Note the distinct aberration of lamellar architecture at the crena.

Abbreviations: SL- stratum lamellatum, SLD- sublamellar dermis, DP- distal phalanx, SD- sole dermis.



**Figure 12:** Photomicrograph of a transverse section of the lamellar interface from a study specimen with a crena. (Gomori's one step trichrome stain); 4X magnification; Keratin structures are stained red while dermal collagen structures are stained blue. Note the transition from recognizable primary epidermal lamellae, secondary epidermal lamellae, primary dermal lamellae, and secondary dermal lamellae to only recognizable abnormal primary epidermal lamellae at the crena.



**Figure 13:** Photomicrograph of a transverse section of the lamellar interface from a study specimen with a crena. (Gomori's one step trichrome stain); 10X magnification. Keratin structures are stained red while dermal collagen structures are stained blue. Note the sequestered islands of dermal like structures in the transition from more normal laminar attachment to the crena.