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The Growth and Adaptive Capabilities of the Hoof Wall and Sole: Functional Changes in Response to Stress (21-Nov-2003)

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1. Introduction

The hoof wall represents the outer protective structure of the foot and has long been of interest in terms of its growth, structure, and functioning, especially during disease conditions such as laminitis. The incidence of problems related to the hoof wall has been estimated to be 30% or more [1,2]. Although causal factors of some hoof wall abnormalities are obvious, such as direct trauma to the hoof wall and/or grain overload during laminitis, other problems and some forms of laminitis are not. Resolution of hoof abnormalities by corrective trimming or shoeing depends on our understanding of the basic structure and function of the hoof wall as well as its short-term and long-term growth. Many descriptions of the hoof structure and the hierarchical organization of the hoof wall have been presented previously [3,4]. The hoof wall at the toe is thicker than that of the quarters. The hoof wall becomes progressively thinner and more elastic toward the heels, and, then, it increases in thickness as the bars are formed. The structure of the hoof consists of three main layers: the stratum externum, stratum medium, and stratum internum (Fig. 1). Many studies of the hoof wall have focused on the stratum medium. It is the thickest of the three layers, and it seems to be the main load support system, whereas the outer layer serves a "waterproof" function [3-9]. The structural features of the stratum medium are the tubular and intertubular horn [4,8]. The tubular horn is composed of a central medullary cavity that extends the proximodistal length of the hoof wall. In the proximal third of the hoof wall (4 - 6 mm), the central medullary cavity of the horny tubule accommodates the papillae of the corium. The cortex of the tubule is surrounded by keratinized epithelial cells, which gradually merge with the keratinized cells of the intertubular horn. The epithelial cells of the intertubular horn are continuous with the epidermal cells between the papillae of the corium at the coronary band [4]. The horn tubules are generated by the germinal layer of the coronary epidermis underlying the long papillae of the corium, whereas the intertubular horn is formed from the germinal epithelium, located between the tubular projections of the coronet [4]. The relative density of the tubules within the hoof wall seems to vary across the cross section of the hoof; more tubules per unit area are located on the outer regions of the hoof and fewer tubules per unit area are located on the inner hoof wall [5,8,10,11]. This differential density in hoof wall tubules may be related to the different mechanical properties of the hoof wall, which vary according to the demands across the hoof wall [7]. The tubules of the stratum medium have long been thought to contribute to significant mechanical dampening properties of the hoof wall during locomotion [12]. However, the mechanical functioning of the hoof wall may not be as simple as the "load bearing elements" of the hoof wall suggest [4,10], but they may be related to the ability of the fibers to reinforce the hoof wall against propagation of any cracks against the long axis of the hoof [10,13]. Other functions of the hoof wall tubules, such as water conduction and temperature insulation, may also be important [11].

The innermost layer, the stratum internum or stratum lamellatum, is unpigmented and consists of the keratinized laminae and non-keratinized secondary laminae that interdigitate with congruent structures of the corium (Fig. 1). These primary epidermal laminae (PEL) serve to unite the inner hoof wall to the distal phalanx through collagen fibers. There are approximately 600 PEL around the perimeter of the hoof wall that suspend the distal phalanx, and each PEL has about 100 secondary epidermal laminae (SEL) [3,14]. This arrangement increases the surface area immensely, which, in turn, may serve to reduce the tension per unit area on the inner hoof [14]. The PEL originate from the germinal epithelia of the coronet at the inner aspect of the coronet and then pass toward the ground surface [3,6]. Along the lengths of the longitudinal sheets of laminae, there are very few mitotic figures present, indicating that the majority of the cells of the PEL and the SEL are produced at this proximal site [6,15]. In fact, some scientists have emphasized that all hoof wall growth occurs and originates at the coronet [15]. When formed, the PEL consist of mainly partially keratinized single sheets, with an occasional branched

PEL being distributed around the perimeter of the hoof [3,6,14]. This relatively consistent population of individual sheets extends along the entire inner length of the hoof wall between the coronet and the ground [3,6,14]. During the distal descent of the hoof wall, minimal changes in the morphological features of the PEL or SEL occur when they are formed [16]. The relative lengths of the PEL are significantly greater at the distal hoof wall than in a more proximal position [17]. The keratinized portions of the PEL are continuous with the stratum medium of the hoof wall (Fig. 1 and Fig. 2).

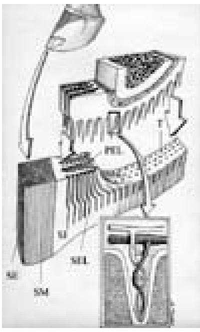

Figure 1. Schematic drawing of the hoof wall (inset) showing the relationship of epidermal laminae to the corium and tubules of the solar corium as adapted from Pollitt [18]. The stratum externum (SE) and stratum medium (SM), which form the majority of hoof wall, are shown outside the stratum internum (SI) with the primary epidermal laminae (PEL) and the attached secondary epidermal laminae (SEL). A corresponding corium of laminae inserts into the accommodating PEL and SEL. On the sole, the solar epidermis forms tubules (T) into which vascular papillae (bottom inset) are located. - To view this image in full size go to the IVIS website at www.ivis.org . -

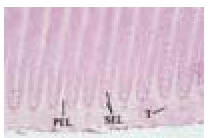

Figure 2. Microscopic section from the stratum internum showing the primary epidermal laminae (PEL), the secondary epidermal laminae (SEL), and the tubules (T) present in hoof wall. This section is consistent with the long-held view that PEL and SEL, once formed, represent a rather homogeneous population of hoof wall structures. - To view this image in full size go to the IVIS website at www.ivis.org . -

Hoof wall growth has been studied by both light and electron microscopy; however, the process is still not completely understood. One hypothesis suggests that the keratinized PEL move past the more stationary, non-keratinized SEL by breaking of desmosomes between these two laminar cell groups (Fig. 3) [15,18]. Another theory suggests that it is the actual surface architecture and intimate contact of the cells, like individual "pieces of a puzzle" composing the primary and secondary laminae, that allows the PEL to move distally during growth [17]. However, exact details of this sliding mechanism of the keratinized hoof wall descending past the remainder of the hoof remain under investigation. Other morphological features of the PEL and the SEL have also been examined in terms of the microanatomy [3,4,6,14,15,18,19]. The basement membrane (BM) separates the corium from the overlying epidermis, and it is crucial to the viability of these two tissues [20-22]. Recent studies have emphasized the importance of the BM in laminitis [20-22]. The BM can be seen histologically with silver stains or by immunohistochemistry with primary antibodies raised to one of several proteins. Several protein markers (laminin, collagen type IV, and collagen type VII) have been used to characterized the BM around the perimeter of the SEL and its adherence to the basal cells of the SEL [20,22]. The stratum germinativum is located on the BM and consists of two cell layers: the stratum basale and the stratum spinosum. The stratum spinosum is next to the beginning zone of keratinization. In the hoof wall, the stratum granulosum and the stratum lucidum layers of epidermis seem to be absent with the formation of the "hard" types of keratins [14].

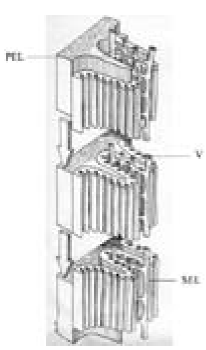

Figure 3. Schematic drawing illustrating the "sliding" concept of growth in which the keratinized and non-keratinized of PEL "slide" past the stationary SEL (adapted from Pollitt [18]). The arrows indicate passage of the hoof wall past the stationary part of the vasculature (V) and SEL. - To view this image in full size go to the IVIS website at www.ivis.org . -

These morphological features of the PEL and the SEL around the perimeter of the hoof wall have formed much of our understanding of the inner hoof wall, its function, and its growth. Many practices and treatments during trimming and shoeing by the farrier and veterinarian have been based on these hypotheses. The concepts of hoof wall growth and its origins from the coronet are well accepted, although they may not be fully understood. However, these current ideas of hoof wall and sole growth do not account for the possibility that the cellular constituents can change and adapt according to the environmental stresses imposed on the foot and, thus, alter their morphological features. Although the possibility of such adaptive changes in the microscopic structure could explain the wide range of hoof conformations, any potential adaptations in the morphology of the PEL and SEL or other hoof wall layers remain unknown. Thus, although our treatments may be formulated according to our current understanding of the growth of the hoof, these latter ideas may be flawed. This, in turn, implies that treatments of the hoof during health and disease may not provide the best care for the equine foot. Our basic understanding of the hoof wall structure and its growth can be formulated into several generalities, based on studies of the hoof wall: (1) The PEL and SEL and their cellular components arise exclusively from the coronet and descend in the stratum internum toward the ground. Once formed, they descend without much change in their morphological features; (2) The PEL consist of primarily single sheets along with an occasional branched PEL around the perimeter of the hoof wall between the toe and quarters, including the bars; (3) Approximately 600 PEL are present around the perimeter of the hoof wall. All having fairly consistent shapes and sizes from horse to horse; and (4) The sole also contains keratinized horn tubules that grow perpendicular to the ground surface. Although these basic anatomical features of the inner and outer hoof wall have been commonly described for hooves considered to be "normal" in the literature, there are a wide range of shapes and sizes of feet of "normal" horses. While these "normal" hooves can vary considerably externally and often are related to the specific breeds, the internal anatomy of the hooves is thought to remain unchanged.

Little discussion has been made of the possibility that there may be morphological differences or variations in the internal features of the hoof wall as a result of the hoof's interaction with the environment and subsequent adaptation to its influences. Under these conditions, the structural features of the internal anatomy of the hoof wall may then correlate with the external hoof conformation. Such findings may provide insight into the locations of zones of loading within a particular foot as well as the areas where the potential stresses were greatest. Such a possible relationship between the external hoof wall and its conformation and the morphological features of the inner hoof (the stratum medium and the stratum internum) is not known. Important questions needing to be asked are "Does the morphology of the hoof wall, with its three different layers of the stratum externum, stratum medium, and the stratum internum, become modified because of the loads being placed on the hoof wall and thereby reflect the potential sites of stress? Are there any morphological and/or biochemical indicators within these three layers of the hoof wall suggesting that they may be capable of responding and adapting to the loads by increasing, decreasing, or somehow modifying the relative features of the PEL or SEL?" Obviously, during certain disease conditions such as laminitis, the hoof wall does respond either as a direct result of the pathology occurring in the support tissues or as an indirect response to other as yet unknown changes present in the hoof tissues. Such changes have been discussed by other knowledgeable scientists [23-27]. Thus, our presentation today seeks to explore the possibility that the internal hoof wall structures are capable of quickly adapting to the stresses of the external (and internal) environmental conditions as evidenced by the changes in its internal morphology during short time periods. These morphological adaptive changes may be occurring even in those feet that seem to be "normal" and "sound" without any clinical evidence of possible lameness and/or pathology. Although adaptive changes within tissues are considered to be a "normal" physiological response, the morphological appearance of these changes is often interpreted to be an indication of pathology at the microscopic level, because these adaptive changes seem different from those normally described in the anatomy texts and journal articles. The clinical importance of these observations is that they provide evidence of the high adaptability of the tissues within the equine hoof wall, especially in the internal regions. Furthermore, as a warning to both farriers and veterinarians alike, these findings suggest that any time that the foot becomes manipulated by trimming, shoeing, or other procedures, it will result in alteration of the stresses applied to the internal tissues. This alteration of the areas of the applied stresses will in effect produce a change in the morphological features of the structures of the inner hoof wall. This will be either to the benefit or detriment of the hoof wall. As our work is constantly evolving, we wish to focus on several adaptable changes that we have detected to date. We believe that these adaptable changes occur "normally," but, during certain diseased states, they are exaggerated and occur at a much faster rate.

2. Adaptive Changes in the Hoof Wall from the Fetus to the Newborn to the Yearling State

At birth, the hoof wall is covered and protected by soft horn (perionychium), which is rapidly worn away when the foal moves within its new environment. The hard pigmented horn is gradually exposed to provide the external hoof support for the newborn foal. The internal support tissues of the stratum medium and the stratum internum represent a structurally homogeneous population of keratinized sheets on the inner surface of the hoof wall. The term "sterile bed" is often used to describe this environment of the hoof wall, because it grows downward from the coronet [16,17]. Interestingly, although most studies continue to support this view, the external conformation of the hoof wall does not have any consistency of shape and size. In fact, the feet vary greatly from horse to horse. Conformations can range from a long toe and low heel to an under-run heel to an upright hoof wall with high heels, including steep or flared quarters. The resolution of these apparent disparities of wide variation of external hoof wall conformations with the relatively constant internal morphology of the hoof has yet to be addressed.

3. Adaptations of Hoof Wall at Birth

When any mammal is born, many organ systems that functioned during the fetal state undergo specific transformations, thereby enabling the newborn to survive the transition from an in-utero environment to a hostile external environment. The precocious newborn foal must rise to its feet and begin to support its weight to extricate itself from potential predators. The rapid transition from a non-weight-bearing state to full weight support shortly after birth indicates that the musculoskeletal system and the locomotor neural network within the central nervous system (CNS) is primed and ready to respond to the weight support and movement under these new environmental conditions. Whether or not the hoof and foot tissues of the newborn foal respond or adapt to these new weight-bearing conditions from that of the non-weight-bearing fetal state is not known. Thus, in this initial phase of the study by the hoof wall, we examined the stratum internum of the hoof wall of the fetus, the newborn, and the older foal for evidence of any adaptive changes that might be reflected in the morphology of the epidermal laminae. If such changes in the PEL or SEL are detectable, then they might provide support for the idea that these epidermal tissues are capable of responding to outside influences when the laminae are formed at the coronet.

Near term fetuses (n = 10), foals (n = 15; newborn to 2 mo of age), and yearlings (n = 5; 6 mo - 1 yr of age), represented by several breeds, including Arabians, Quarter Horses, Thoroughbreds, Standardbreds, and draft breeds, were obtained at necropsy. The feet were frozen and then sectioned parallel to the ground surface at 4 - 6 mm with a band saw. They were examined macroscopically under the dissecting microscope (Fig. 4). On sections from the distal third of the hoof, quantitative measurements of the interlaminar spacing of the 50 PEL around the perimeter of the hoof wall were obtained by counting the PEL beginning at the toe and proceeding to the medial quarters, lateral quarters, and heel areas in all feet examined. The number of PEL conforming as single sheets or as branched or "Y"-shaped under the dissecting microscope were noted and recorded. Tissue blocks from the toe, quarters, and heels, sections from the middle and distal thirds of the dorsal hoof wall, and sections of the quarters were obtained and placed in buffered formalin for several days. Then, they were either embedded in paraffin and sectioned at 6 microns or placed on a freezing microtome and cut at 60 - 90 microns. The sections were stained using hematoxylin and eosin (H&E) stains and Von Gieson stain for elastic fibers.

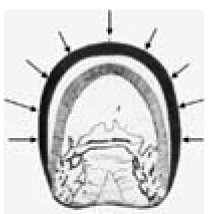

Figure 4. Schematic drawing of section cut parallel to the ground with the arrow at the toe indicating the dorsal midline. The PEL were grouped as 25 PEL on either side of the dorsal midline and then as 50 PEL (arrows) between the toe and heel on the medial and lateral hoof wall. Then, the distances between the groups of 50 PEL were measured to ascertain the interlaminar spacing. - To view this image in full size go to the IVIS website at www.ivis.org . -

The general descriptions of the different regions of the hoof wall at these three age groups revealed that several characteristic features are apparent in the gradual transition of the hoof wall from the fetus to the foal and yearling states. The residual stratum medium and the stratum internum of the hoof wall of the late term fetus were very consistent morphologically in the forelimb and hindlimb specimens. After birth, however, the epidermal laminae changed in the newborn foal and in the yearling as they became more diverse morphologically. These changes included variations in the shapes, sizes (width and length), and relative number of laminae along the length of hoof wall.

In the fetus, the PEL had a uniform distribution around the perimeter of the hoof wall from the toe to the quarters in both forefeet and hindfeet with no significant difference in the interlaminar spacing. Examination of the hoof wall revealed that the PEL of the forefoot were more developed than the PEL of the hindfoot when they were compared in the same horse. In these instances, the PEL of the forefoot were slightly longer and wider when compared with that of the hindfoot. These PEL extended as single sheets from the inner hoof wall with little evidence of branching (Fig. 5). The PEL consisted of a central keratinized core with short secondary laminae (range = 20 - 52 microns) distributed around the PEL at both the toe and the quarters. Less than one branched or "Y"-shaped PEL was observed per 50 PEL in a histological section at the toe with only a rare "Y"-shaped PEL being observed at the quarters. In the late term fetus of the draft breeds, the PEL did have branched PEL at the toe, which contrasted with the PEL in the smaller pleasure horses and ponies. In all fetal specimens examined, the absolute length of the PEL varied across the group of horses, but, within each foot, the population of PEL had a relatively consistent length. Lengths of PEL at the toe (8.23 ± 0.55 mm, 0.324 ± 0.072 in) and those at the quarters (8.31 ± 1.75 mm, 0.327 ± 0.069 in) were not significantly different ($P < 0.05$). The epithelial cells of the SEL consisted of little cytoplasmic volume but contained a prominent nucleus. The SEL located at the apex of the PEL were smaller in length (mean length = 0.038 mm) than those SEL present at the base of the PEL (mean length = 0.050 mm).

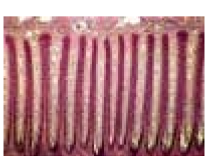

Figure 5. Microscopic section from late term fetal hoof at the toe illustrating relatively consistent lengths and shapes of PEL. The SEL are evenly distributed around PEL. This section is typical of late-term fetal hooves. - To view this image in full size go to the IVIS website at www.ivis.org . -

In the newborn foal during the first 2 wk of life, the PEL were seen to change in terms of the relative density of PEL around the perimeter of the hoof wall and also the basic morphology from those seen in the late term fetal stage (Fig. 6). Quantitatively, the relative number of PEL observed in the microscopic sections obtained from the distal third of the hoof wall increased significantly at the toe from those found in the quarters of the same foot ($P < 0.05$). This relatively increased density (decreased interlaminar spacing) of the PEL at the toe of the forefoot of newborn foals ($n = 15$) was 12.29 ± 1.37 mm/50 PEL, whereas, at the quarters, the relative density was 15.90 ± 1.27 mm adjacent to the distal phalanx and 18.39 ± 0.89 mm/50 PEL overlying the lateral cartilage. In the hindfoot ($n = 10$), a similar observation was seen with the 50 PEL being distributed over a distance of 12.04 ± 1.63 mm at the toe, whereas, at the quarters, the distance for the 50 PEL was 16.71 ± 0.86 mm adjacent to the distal phalanx and 18.56 ± 1.83 mm over the lateral cartilage. The morphology of the PEL at the toe of the several-week-old foal varied more than in the fetus and had two basic shapes extending from the base of the laminae to the apex near the distal phalanx: (1) the single sheet laminae, and (2) the "Y"-shaped or branched laminae interspersed between these single sheets of epidermal laminae. The single sheet laminae with its thick base accounted for the majority of the laminae present along the perimeter of the hoof wall. These PEL were keratinized along the central three-quarters (approximately) of their length, whereas the apical ends were not. In addition, a significant group of branched or "Y"-shaped laminae were also present at the toe. The population of "Y"-shaped laminae represented >5 laminae/50 PEL (5.01 "Y"-shaped PEL/50 PEL) in those foals that survived more than a few days to several weeks. The configuration of the "Y"-shaped laminae varied (Fig. 6 and Fig. 7).

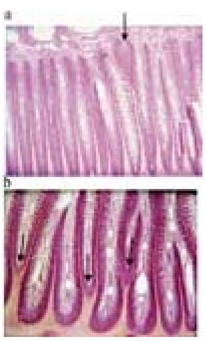

Figure 6. Histological sections from young hooves showing branched PEL (arrows) at toe. In A, the branched PEL has bifurcated near the apex, whereas in B, several bifurcations (arrows) are more complete along the PEL. Observe that the bifurcating PEL are approximately twice as wide at the base as the adjacent single-sheeted PEL. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 7. Microscopic section showing PEL undergoing initial "dimpling" at apex of PEL (arrow) and another branched PEL near base (asterisk). - To view this image in full size go to the IVIS website at www.ivis.org . -

The PEL with a small branch had an indentation at the apex with a relatively long stem, whereas other "Y"-shaped PEL had two long branches and a very short stem. In the "Y"-shaped PEL, the SEL varied in their relative lengths depending on whether they were located on the inside (axial) side or on the outside (abaxial) surface of the branched shaped laminae (Fig. 8). The SEL were shorter on the inside of the "Y"-shaped laminae, especially those present at the vertex of the "Y," than those SEL present on the outside of the laminae. The base or stem part of the "Y"-shaped laminae had a mean thickness of approximately two times the thickness of the adjacent laminae. The morphology of the PEL varied more in the shapes and sizes than those observed in the fetus. At the apical end of the PEL, the cells comprising the SEL increased in cytoplasmic volume from that of the fetus. The SEL had variations in the relative numbers of epithelial cells, which contributed to the overall shape of the PEL at the apex. In those SEL with more epithelial cells, the PEL had a blunted shape versus a more pointed shape in the PEL with SEL of fewer epithelial cells. On each section, examples were seen at the base of the PEL in which the SEL were reduced or absent.

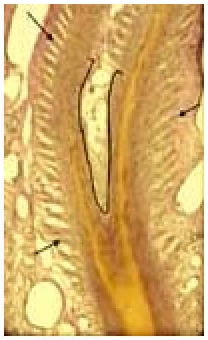

Figure 8. High power view of bifurcating PEL showing reduced heights of SEL on inside of branched PEL. The bracketed line marks region where SEL consists of a cellular layer rather than laminae. Observe that the cells composing SEL form a layer on the inside of the "Y"-shaped PEL rather than the distinct SEL (arrows). - To view this image in full size go to the IVIS website at www.ivis.org . -

In the older foals (6 mo - 1 yr), there were several differences in the morphology of the PEL as well as the relative spacing around the perimeter of the hoof wall. In all of the specimens examined under the microscope, the branching patterns were different than those of the newborn foal; the dimpling and branching of the PEL at the apex was very rare except for at the base of the PEL (Fig. 6b and Fig. 9). In each of the specimens, the relative length of the stem was short, comprising only 10 - 20% of the total length of the PEL. The relative density of the PEL at the toe and the quarters was similar to that of the several-week-old foal; a greater density of PEL was observed at the toe than at the quarters. In addition, there were hints of changes in the relative density of PEL at the quarters in some of the older foals (1 yr). In these instances, the PEL on the medial side of the hoof wall were usually spaced further apart than those PEL on the lateral side of the hoof wall (see below).

Figure 9. Schematic drawing of several PEL in several apparent stages of laminar bifurcation. In A, a single PEL (arrow) with SEL is being distributed around the perimeter. The active zones (act) of PEL are indicated at the apex and base of PEL, whereas the inactive zone (inact) is indicated by line. In B, the initial "dimpling" of PEL at apex (arrow) is followed by a branched PEL in C. The curved arrows indicate the proposed redistribution of SEL and their cells around the perimeter to the inside of the "Y"-shaped PEL during this bifurcation process. - To view this image in full size go to the IVIS website at www.ivis.org . -

Thus, in these initial findings of the fetus, foal, and yearling feet, the microanatomy of the PEL and the SEL revealed definitive changes between when the fetus is born and when the foal develops into a young horse. In the newborn foal, changes in the relative density and morphology of epidermal laminae occur around the perimeter of the hoof wall, beginning within a few days of birth. Shortly after birth, there is a significant increase in the relative number of PEL along the hoof wall length at the toe at the ground level when compared with the quarters of the same foot. In the fetal stage foal, there were equal numbers between the toe and quarter or a greater density of PEL at the quarters than at the toe. There was also a gradual change in the morphological features of the PEL and SEL along the hoof wall. In the yearling, the relative branching of the PEL at the apices was significantly less. We hypothesize that these morphological changes of the relative number and shapes of the PEL along the perimeter of the hoof wall represent adaptive responses by these tissues to the many and varied stresses being imposed on the foot during its interaction with the environment.

The PEL around the perimeter of the hoof wall revealed significant differences in relative numbers and morphology between that of the late term fetus, the newborn foal, and the yearling horse. At the dorsum of the foal hoof, the interlaminar spacing was significantly closer together than that found at the quarters in the same feet in contrast to that found in the fetus. These findings are in contrast to the long-held assumption that the PEL are evenly spaced around the perimeter of the hoof wall [3,4,6,14] and have a relatively constant number, i.e., 600 PEL/foot [3,4,6,14]. Only in a recent study by Douglas and Thomason [28] has any description of the interlaminar spacing reported in the foal or late term fetus suggested otherwise. In their study, a trend was observed. Closer interlaminar spacing of the PEL was seen in the toe than in the quarters, which is consistent with the findings of the present study. However, in the present study, we did not observe any difference between the toe and quarters in the fetus, although we did in the foal that lived for several weeks after it was born. Previously Linford [19] had also observed that, in the adult horse, differences between the interlaminar spacing of the toe and the quarters were evident, although not statistically significant between the sampling sites selected (see below). This increased density of PEL in the foal from that of the fetus may be caused by the increased weight-bearing support needed during locomotion where the toe or dorsum of the hoof appears to a site of high forces transmitted through the hoof wall epidermal-dermal junction. These forces are in part generated by the weight of the animal against the ground surface as well as by the pull of the deep digital flexor muscle at its insertion on the distal phalanx during locomotion [12,28,29]. Such forces during movement are thought to move dorsally from the central solar area and become concentrated at the breakover when the foot starts to leave the ground [30,31].

Interestingly, in the few fetal draft breeds that were stillborn, we did observe the presence of "Y"-shaped PEL and the tendency of the PEL to become more closely spaced than compared with the other horses examined. These findings suggest that the PEL bifurcation may be a normal "time and/or space management" process for creating the PEL by the inner stratum internum at the toe and not having to wait for long time periods for PEL to grow to the ground surface from the coronet. The formation of branched or "Y"-shaped laminae represents an interesting potential mechanism for increasing the surface area for weight bearing, if the occasion should arise. Previously, hoof wall growth has been viewed as occurring exclusively from the coronet [3-5,14,15]. Several studies have presented evidence that cellular mitosis of the basal cells of the stratum germinativum occurs at the coronet and the solar surface of the foot with little being present in association with the stratum medium or stratum internum [15,18]. Mitotic figures and cells have been observed using a bromine marker that can be incorporated into the ribonucleic acid and can be detected with immunocytochemistry [18]. The marker documents the locations of the mitotic cells concentrated in the stratum medium or stratum internum sites. These dividing cells form new laminae on the shoulder of the coronet caudal to the coronary groove and move distally along the inner dorsal hoof wall. Within the stratum internum and distal to the coronet, only an occasional mitotic cell is seen in the laminar epidermis [15,17,18]. Such formation of the laminae has formed the basis for the notion that hoof wall growth originates exclusively from the coronet [3,15]. However, the results of the present study indicate that, within the first few weeks of life, newly formed epidermal laminae appear in the hoof wall at the toe. It is unlikely that these newly formed PEL could have been formed and moved distally from the coronet to reach the sampling site in the distal third of the hoof wall of these young foals. In the late term fetus, only an occasional branched laminae was seen at the toe with nearly a ten-fold increase being seen within the first 2 - 5 wk after birth. Few branched laminae were seen in the quarters. The "Y"-shaped laminae have a range of formations within the same foot from the initial indentation at the apex of the laminae to the nearly complete division of the PEL into two thinner laminae that are similar in width as the adjacent PEL of that hoof wall. This variation in morphology may represent PEL in different stages of bifurcation along its apical-to-base length. Whether this potential process of epidermal laminar bifurcation is a "pre-programmed event" and/or an "inducible event" caused by the environment remains to be explored. However, if similar morphological changes were apparent in the adult foot and in different regions around hoof wall perimeter, then indirect evidence would support the latter notion that these changes would be potentially induced by the external environment. Such possible stimuli facilitating the bifurcation process may be the increased forces at the toe (or other hoof wall region) during movement after birth. Such high stress at the epidermal-dermal junction and at the breakover of the toe may signal the epidermal cells within the PEL [29-31] to become "uncoupled" to initiate the splitting of the PEL. Furthermore, there seems to be softer keratin types in the inner hoof wall. This would be consistent with this bifurcation of PEL, because the epithelial cells would potentially have greater motility than if they were in a more rigid or hard keratin environment [32]. Such mechanisms may occur within the PEL, because the intercellular adhesion of the epidermal cells is believed to be caused by the vast interdigitation of the asymmetrically shaped cells and quite possibly adhesion molecules [15-18]. This possible, normally occurring adaptive response to high stress at this region may represent only one of the several stimuli for signaling the PEL to undergo such changes to increase their numbers at the distal hoof wall. When the PEL has formed the bifurcating PEL, it extends along its entire proximodistal length of the hoof wall. Because the growth of the hoof wall from the coronet takes approximately 8 - 12 mo to reach the ground surface, this long time period would potentially jeopardize the stability of the hoof wall in being unable to adapt rapidly to additional stresses imposed on the foot. The occurrence of an occasional branched or "Y"-shaped laminae in adult horses has also been mentioned in most studies presenting histological findings of the stratum internum [14,15,19]. However, no morphological or clinical significance has been attributed to these "Y"-shaped structures, because they represent only about 5 - 8% of the laminae in any region of the inner adult hoof wall. Presumably, a similar process of PEL division would be occurring as an adaptive response to the external stresses imposed on the various hoof surfaces (see below). Whether such bifurcation of the PEL can be called "growth" may be open for further discussion, because such changes represent only a reorganization of the epithelial cells within the PEL and a possible redistribution of the cells of the SEL around the perimeter of the PEL.

In addition to the change in the relative density of the PEL around the perimeter of the inner hoof wall, the morphology of these PEL gradually begin to show changes from that seen in the fetal condition or the early newborn state. In the fetus, the population of PEL represents a rather homogenous appearance with small secondary laminae and very few branched laminae. The absolute length of the PEL can vary from fetus to fetus, but, within a foot, they have relatively constant lengths. However, shortly after birth, the morphological appearances of these epidermal laminae begin to vary from foot to foot as well as within the same foot. These variations from foot to foot may be the result of the influences of the external environment and the stresses affecting the foot, such as weight bearing, ground surface type, and, possibly, unevenness, trimming, etc. Only Linford [19] has described a similar observation that the epidermal laminae in the adult foot are not constant in their shapes and sizes. He indicated that some degree of morphological variation existed and hypothesized that these variations might be related to external stresses applied to the dorsum of the hoof wall. Our laboratory has also observed that the PEL vary extensively in morphology around the hoof wall perimeter in most domestic and feral horses. This wide range of variation seems to be related to the hoof shape and caused by growth and environmental influences (wear, trimming, shoeing, etc.). In the recent study by Douglas and Thomason [28], the orientation of the PEL were found to vary around the perimeter of the hoof wall of adult horses. At the dorsum, the orientation of the PEL was consistently tangential to the outer wall, whereas at the quarters the PEL were oriented with the dermal ends being drawn caudally to the hoof wall. This internal angulation of the PEL at the quarters may be a mechanism to dissipate the shearing forces between the inner hoof wall and the distal phalanx without compromising the laminae themselves. These findings in both the foal and the adult horse suggest that the PEL are responsive to the external forces imposed on the hoof wall and will adapt to minimize such stresses on the internal tissues of the foot.

4. Adaptive Changes in the Adult Hoof: Different Laminar Densities

Another interesting question that is raised is "Do these changes only occur at the transition during birth or do they potentially occur throughout the life of the horse?" If the former were correct, then these changes merely represent a one-time change occurring at this transition period, which suggests that all subsequent growth and potential adaptive changes originate from the coronet. On the other hand, if the bifurcation of the PEL occurred at other times during the life of the horse, then these changes would potentially represent a mechanism for the hoof wall to respond and adapt to "stress changes" in a relatively short period of time as opposed to these changes having to be formed at the coronet and grow distally. Indirect evidence suggests that the potential bifurcation process occurs throughout the life of the horse. This indirect evidence was obtained by quantitatively examining sectioned hooves and observing the differences and variations in the relative numbers of PEL along the perimeter of the hoof wall in relationship to the shape of the hoof wall as well as observing variations in morphological features. In a general population of more than 100 feet examined from various breeds and ages, the conformation of the hoof varied considerably from a round foot to one that was flared to one side. Generally, the medial side of the hoof wall was steeper in approximately 80% of the feet examined with the lateral side being slightly flared. In the remainder of the feet examined, the lateral or outside hoof wall was steeper with the medial side being slightly flared. In a few horses (< 5%), the hooves were rounded and exhibited little or no asymmetries. These asymmetries of the hoof result in the differential distribution of the PEL around the hoof wall perimeter. In virtually all forefeet, the density of the PEL at the toe is significantly greater than at the quarters of the same foot (Fig. 10). In following the PEL pattern around the perimeter of the hoof wall and counting the PEL from the midline at the toe to the quarters, the changes in the interlaminar spacing usually occur abruptly at specific sites rather than gradually around the perimeter of the hoof wall. One such change is at or near the breakover at the toe. At this region of the hoof, the PEL interlaminar spacing increases abruptly (Fig. 11). At the level of the quarters, the interlaminar spacing on the medial and lateral sides at the foot is usually similar in the round or symmetrical feet, but, in asymmetrically shaped feet, the interlaminar spacing of the two quarters differ. In feet with one side steeper than the opposite side, the PEL are not evenly distributed between the toe and the heels but seem to be distributed in accordance with stresses being placed on the foot. When one counts the PEL between the toe and the heels and measures the distances of the PEL around the hoof wall perimeter, there is a greater density (decreased interlaminar spacing) of PEL along the portion of the hoof wall that contains the flare as compared with the non-flared side of the foot. Conversely, on the non-flared side of the foot, the interlaminar spacing increases significantly (decreased density), resulting in fewer PEL.

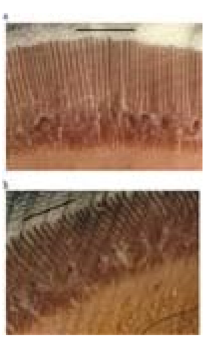

Figure 10. Photographs from the same foot showing interlaminar spacing differences at toe and quarters at the same magnification. A is a photograph of the epidermal laminae at toe of the adult foot, which shows the increased density of PEL as compared with quarters (B); also, observe that no obviously detectable branched PEL were evident in this section. B is a photograph showing increased spacing at the quarters. The bar in the two photographs serves as an internal marker for determining interlaminar spacing. - To view this image in full size go to the IVIS website at www.ivis.org . -

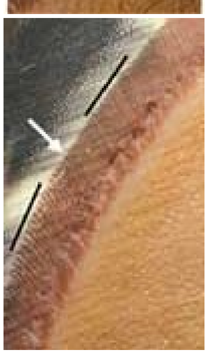

Figure 11. Section from dorsal medial quarters of the adult foot showing relatively abrupt changes (arrow) in interlaminar spacing from higher density at toe to a greater spacing in quarters. The abrupt change corresponds to the callus on the sole. The bars serve as internal markers for determining interlaminar spacing. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 12 is a section from a right front foot that shows a typical pattern of interlaminar spacing of the PEL around the perimeter of the foot. Small needles are inserted along the perimeter of the hoof wall at the base of the PEL of the section. The distances between the needles were measured using calipers, and the sections were radiographed. At the toe, there are 25 PEL on either side of the central needle (arrow), whereas groups of 50 PEL are identified and measured between each of the other needles. A slight flare exists on the dorsal lateral quarter. Notice that the distance between the needles on the right or flared side of the foot (175 laminae between the arrow and needle D) are placed more closely together than the same number of PEL on the non-flared side of the foot (175 laminae between the arrow and needle 4). This example illustrates that the relative distance between PEL is related to the stresses and conformation of the hoof wall.

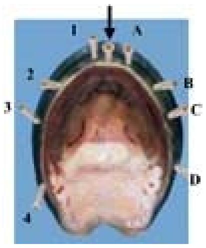

Figure 12. A coronal section through distal third of right front foot with small needles positioned for counting PEL. At the toe (arrow), 25 PEL were marked on either side of the midline followed by intervals of 50 PEL around the flared side (arrow to D) and non-flared side (arrow to 4) of the foot. Between the toe and heels, these needles indicate the same number of PEL. Observe the greater interlaminar spacing on the non-flared side of the foot (arrow to 4) than on flared side of foot (arrow to D). - To view this image in full size go to the IVIS website at www.ivis.org . -

By our current understanding of the growth of the hoof, the PEL around the hoof wall perimeter should be similar or virtually identical on the medial and the lateral sides of the hoof in terms of the interlaminar spacing or density of the PEL along the length of hoof wall. This even distribution of PEL occurs only in rounded and "well-balanced" feet, although the loads applied to the foot are not equally distributed. The medial side of the foot often has greater concentrations of the loads during stance [29,31]. In feet with varying PEL densities along the hoof wall, the flared side will have greater numbers of PEL than the non-flared side of the hoof. In other words, there are significantly more PEL present on the stressed side of the foot than on the non-stressed or less stressed side of the foot. Furthermore, this stressed or flared side of the hoof can be a restricted zone, occupying only a small region of the hoof wall perimeter. This, in turn, will result in a restricted zone of increased density of PEL [a]. An interesting observation is that, on the opposite side of the foot to the flare, there is also an increased density of PEL in the region that we call the "reverse flare" and the hoof wall region that is rolled under the hoof.

The PEL also differ morphologically around the perimeter of the hoof. The PEL and the SEL have many different shapes and sizes around the perimeter of the hoof, which seems to be related to the conformation of the hoof and the stresses imposed on these laminae. Although we do not know, as yet, whether the specific morphological features of the laminae are directly related to specific stresses on the hoof, there does seem to be several general conclusions that can be stated from findings collected to date from the examinations of more than 100 horses. Figure 13 serves as a general example of the wide range of morphological features of the laminae obtained from sound-footed horses. In these sections from several clinically sound horses, the PEL and the SEL can be seen to vary and differ greatly from the structural conformation of the laminae that is commonly described in the many textbooks. In these feet, the PEL can be seen to have few SEL around its perimeter with the apical end of the PEL being overlaid by several layers of cells rather than definitive SEL (Fig. 13B and Fig.13D). This pattern is most commonly seen in sections obtained from the quarters where the PEL often has reduced SEL, although the PEL are completely lined by the SEL at the toe. The structure of the PEL and the SEL can vary with laminae ranging from being blunted to pointed in shape on the same section (Fig. 13A and Fig. 13C). Also, the PEL present on the flared side of the foot are generally thinner than those PEL on the non-flared side of the hoof wall. Correlation of the functional morphology of the PEL to the PEL density may aid in understanding the microcosm of the stresses on the hoof wall and its microanatomical adaptive responses.

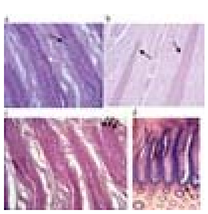

Figure 13. Series of photomicrographs showing morphologic of variations of PEL, presumably caused by hoof wall stresses in clinically sound feet. In A, the SEL have a range of shapes from elongated arrow shapes (black arrow) to blunted SEL (white arrow). In B, the section obtained from quarters shows only the epithelial cells in layers at the apical ends of PEL and short SEL. In C, the SEL have elongated slender shapes. D, from toe near ground surface, illustrates that PEL can have short SEL and apical ends having no SEL (brackets). The arrow indicates the formation of laminar derived tubules. - To view this image in full size go to the IVIS website at www.ivis.org . -

In addition to the bifurcation of the PEL beginning at the apex to form two adjacent laminae, the cells composing the SEL must somehow become redistributed around the PEL to the internal parts of the now-branched PEL. This is because there is little or no mitotic activity observed in the SEL, at least in the area distal to the coronet. Otherwise, there will be laminae without any SEL or epithelial cells. We hypothesize that the redistribution of the SEL around the internal parts of the branched or "Y"-shaped PEL can occur, because these SEL are not keratinized and, thus, would facilitate motility of cells within stratum internum. Such a refilling of the surfaces of the newly forming PEL would explain the observations that the SEL on the inner surface of the branched PEL are consistently smaller and may exist only as a layer of epithelial cells. Such a proposed mechanism would support the idea that the inner hoof of the stratum internum and the stratum medium are highly adaptive and responsive to any potential forces impinging on the hoof wall.

5. Active Zones of the PEL

The two potential processes involving the epidermal laminae, i.e., the bifurcation of the PEL and the redistribution of the SEL around the PEL, enable the hoof wall to rapidly adapt and respond to the many stresses applied to the equine foot. This mechanism occurs at the apical end of the PEL. One question is "Is there another adaptive process at the basilar end nearest the stratum medium of the hoof wall PEL?" Such possible mechanics would enable the stratum internum to respond to stresses by being capable of "producing" greater keratinized horn without cellular division. We hypothesize that the adaptive process occurring at the basilar end of the PEL is the formation of laminar derived tubules (LDT) that merge with the tubules of the stratum internum and the stratum medium of the hoof wall. These two processes, the bifurcation of the PEL with the secondary redistribution of the epithelial cells around the PEL and the formation of LDT at the basilar end of the PEL, suggest the notion that the PEL has at least two active zones where adaptive changes may be initiated or occur as an early response site to the applied stresses on the hoof wall (Fig. 9). First, the apex of the PEL is where an initial dimpling of the PEL occurs and progresses to form the branched PEL. The other active zone is at the base of the PEL where the LDT form and merge with the stratum internum and stratum medium of the hoof wall. The middle part of the PEL may be relatively inactive as suggested by other works [15,17,18]. In these electron microscopic and light microscopic studies, the central PEL region has little indication of any cellular activity, such as mitosis or further keratinization.

6. LDT

Tubules derived from the PEL form an adjunct mechanism of hoof wall growth to the formation of the hoof wall from the coronet. These LDT occur along the hoof wall and are derived from the region of the stratum internum between two adjacent PEL. In examination of this region, small tubules appear to be "budding off" from the region between two adjacent PEL with the formation of a hoof wall tubule that merges with the inner wall (Fig. 14). Often, as one carefully removes the hoof wall during dissection, these vascular papillae can be seen when they are removed from the central core of the LDT. The vascular papillae originate from the corium and pass into the hoof wall to become accommodated by the keratinized tubules. In some sections, there appears to be a series of the LDT that merge with the wall and extend for a considerable, but variable, distance into the wall (Fig. 14A, Fig. 14B, Fig. 14C, Fig. 14D). These LDT are aligned and course through the hoof wall at different angles, depending on their locations along the inner hoof wall. In certain regions, they extend the full thickness of the hoof wall, whereas, in other regions, they pass for approximately 20 - 30% of the full thickness of the wall before they appear to blend in with those tubules derived from the coronet. Their locations and the arc that they form suggest that their movement within the inner layers of the hoof is determined in part by the physical forces placed on the hoof. However, further documentation of this idea is necessary. With greater stress on the hoof wall, the tubules may either move further into the stratum internum and stratum medium of the hoof wall thickness or remain very superficial in the inner parts of the stratum internum of the hoof wall. At the toe region, these LDT move toward the dorsal surface of the hoof before they begin to merge with the tubules derived from the coronet.

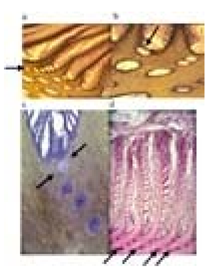

Figure 14. Photomicrographs showing formation of LDT through inner hoof wall. A is a low power view showing LDT (arrow) from the base of the PEL. B is a higher power view showing the short SEL (arrow) at the base of the PEL. C shows LDT "budding off" (arrows) from the base of PEL, and they seem to course into the internal hoof wall. D is a low power view showing the area of LDT at quarters (arrows). - To view this image in full size go to the IVIS website at www.ivis.org . -

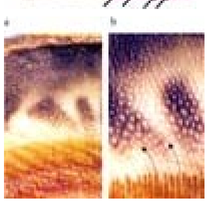

Figure 15. Photographs showing LDT at toe contributing to the thickness at the hoof wall. A is a low power view that shows the hoof at the toe. In B, the curved arrows show the path of LDT into the hoof wall. - To view this image in full size go to the IVIS website at www.ivis.org . -

In shod feet having toe grabs, the LDT extend significantly farther into the hoof wall than in those shod feet without toe grabs (Fig. 15A and Fig. 15B). These LDT appear to contribute to the increased thickness of the dorsal hoof wall in horses shod with toe grabs [b]. In the region of the dorsal quarters, these LDT form an arc toward the dorsal surface at the toe. In the palmar quarters, they move in a caudal direction and extend farther into the thickness of the hoof wall of thickened quarters. At the heels, the LDT extend to the hoof wall surface (Fig. 16). When using immunohistochemistry with antibodies raised to the BM proteins (laminin and collagen IV), the "budded off" LDT is seen to have incorporated "pinched off" BM from the region between two PEL. The BM can be followed in the formation of the new tubules when the BM becomes apparent in the newly formed tubules, and the BM is similar to the lining PEL and SEL along the surface of the stratum internum (Fig. 17). The remainder of the hoof wall tubules of the stratum externum and stratum medium do not have an immunocytochemically identified BM. As a result of the appearance and the formation of the LDT, a plausible mechanism can be suggested from the sections, and it seems to occur as follows: the LDT are formed by the coalescence of two facing SEL on two adjacent PEL. The BM of the SEL appear to contact each other from the two adjacent PEL to become pinched off with the enclosed microvasculature to form a new LDT. The now "unattached and free" epithelial cells that composed the "contacted SEL" then course into the stratum internum and stratum medium of the hoof wall toward the newly formed tubule to surround the central medullary core and gradually become keratinized. The SEL lengths are usually shorter in this region between two PEL where the LDT appear to be formed (Fig. 13D, Fig. 14A and Fig. 14B). The extent of their passage into the stratum internum and stratum medium of the hoof wall seems to be related to the physical properties of the loads and stresses, etc. on the hoof wall.

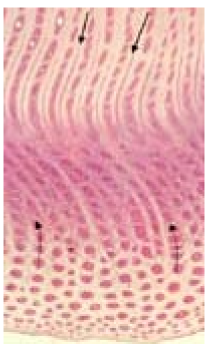

Figure 16. Photomicrographs showing LDT from inner hoof to surface at heels. Solid arrows indicate PEL, and the lines of the tubules are marked by dotted arrows. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 17. Photomicrograph of immunocytochemically stained section showing formation of LDT with basement membrane identified as laminin (arrows) enclosed by epithelial cells from SEL. - To view this image in full size go to the IVIS website at www.ivis.org . -

7. Bars and Its Contributions to the Sole

Another fascinating region of the hoof that undergoes growth and adaptive changes is the bars and the adjacent solar surfaces and heels. The bars are the part of the hoof wall that reflects on itself and extends toward the apex of the frog. We have always assumed that the microscopic structure of the bars is virtually identical to that of the hoof wall of the toe and quarters, because both will have PEL and SEL. However, while both the PEL and SEL are present around the perimeter of the hoof wall, there is a gradual change in their structure and appearance beginning at the angle of the hoof of the heels and continuing onto the bars. The epidermal laminae comprising the bars are more keratinized than those epidermal laminae of the hoof wall, and their shapes are different than those laminae at the toes and quarters. In addition to the PEL and SEL, there is another branch of keratinized laminae that we have called tertiary epidermal laminae (TEL) (Fig. 18). We have called them TEL rather than branched PEL to differentiate them from bifurcating the PEL present around the perimeter of the hoof. The bars seem to be important in that they aid in the support phase of the stride during movement, and, on a microscopic level, they appear to form tubular horn and contribute to the hoof wall and the sole. These TEL form branches from the PEL, with the SEL extending from the surface. The bars may be positioned uniquely, because they support the hoof at both the distal ends (solar surface) and at the proximal end (near the junction of the frog and bars and the caudal-most heels). At the proximal end, the hoof wall tubules are formed by the epithelial cells of the SEL coalescing as they extend toward the hoof wall surface at the heels (Fig. 16). At this site, the newly formed tubules appear to "bud off" and engulf the microvasculature of the corium as they align themselves to the hoof wall surface. At the more distal solar margin of the bars, the epithelial cells also form tubules by the apparent loss of distinct laminae comprising the SL as they form straight but parallel finger-like projections of epithelium. Between these two "epithelial fingers," cellular connections extend out to join with the opposite cellular mass at intermittent distances along the finger-like projections (Fig. 19). Such cross connections will create and form an epithelial tubule that extends onto the sole surface and a centrally localized vascular network. The epidermal cells from the "finger-like" projections extend forward from the bars and those tubules continuing dorsally in front of the bars themselves. The orientation of the solar tubules may be in part dependent on pressure during stance.

Figure 18. Photomicrograph of histological section from the bars showing primary (1), secondary (2), and tertiary (3) laminae. These tertiary laminae are only observed at the bars. - To view this image in full size go to the IVIS website at www.ivis.org . -

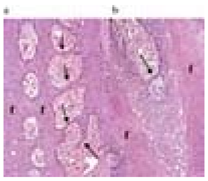

Figure 19. Photomicrograph showing finger-like projections (F) from the bars, where cross-linking of the epithelial cells (arrows) forms the tubules on the sole. - To view this image in full size go to the IVIS website at www.ivis.org . -

8. Summary of Adaptation

The information presented indicates that the structural composition of the foot is not constant from the late term fetus to the aged horse, but, instead, it varies considerably. Only in the fetus, where there is little exposure to the environmental influences, are the hoof wall structures similar in their structural composition. When a foal is born, the entire foot interacts with the environment and, thus, becomes exposed to the many loads and stresses applied to it as well as to the many diverse environmental conditions. The ability of the foot to respond to these external and perhaps internal forces is called adaptation. The cellular and structural make-up of the hoof at the toe, quarters, and heels and the cellular and structural make-up of the sole is exposed to different quantitative forces and external conditions, and, as a result, each region of the hoof and foot will respond slightly differently. These responses will be reflected in the changing morphological features of the three primary layers of the hoof wall, the stratum externum, the stratum medium, and the stratum internum, in terms of their relative thickness, structural organization, and cellular responses. With greater time of exposure to the environment, there is a greater opportunity for more adaptation and, thus, a wider range of variation between hooves under "normal conditions". This wide range of appearances of the hoof wall and the foot structures are commonly referred to as "conformation" of the horse. We define conformation of the foot as a "point in time" in which the structural appearance of the foot is a product of the environmental influences on the foot at the time that it is being examined. The structural appearance of the foot is continually being modified by the interactions of the foot with the environment and the environment's influences on the foot and hoof wall. The term "environmental influences" includes just about everything that the horse has come into contact with since birth, including the extent of movement, ground surfaces, trimming and shoeing procedures or the lack of these practices, nutrition, etc. As a result, the conformation of the hoof and the foot can change when a horse is moved to a different environment and/or to living conditions different from those to which the horse was first exposed. The hoof wall and foot will then undergo additional adaptive changes and gradually become modified to the new environmental conditions. Therefore, the hoof wall and foot will have different morphological features than they did at the horse's previous environment. An important concept for us as hoof care professionals is to begin to appreciate the significant influence that the environment has in determining the internal structure and the composition of the hoof wall and foot and to begin to apply these principles in the future.

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Footnotes

[a] Lancaster L, and Bowker R, Unpublished evidence, 2003.
[b] Troyer D, and Bowker R, Unpublished observations, 2003.

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