

Veterinär-Anatomisches Institut
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. H. Geyer

**The elephant's hoof: Macroscopic and microscopic morphology of
defined locations under consideration of pathological changes**

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Andreas Benz

Tierarzt
von Schübelbach, SZ

genehmigt auf Antrag von

Prof. Dr. Hans Geyer, Referent
Prof. Dr. Klaus Eulenberger, Korreferent

Zürich 2005

Druck: RoNexus Services AG, Basel

“The feet of elephants, both in captivity and in work camps, are probably the single greatest source of medical problems, which confront veterinarians working with elephants”
(M.J. Schmidt, 1986)

*Meiner Familie und insbesondere meiner Mutter für Ihre immerwährende Unterstützung
jeglicher Art in grosser Dankbarkeit gewidmet*

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1 Summary

This study analyzes the normal macroscopic and microscopic morphology of elephant hooves under consideration of pathological changes. Biotin plasma concentrations are examined to establish values as a basis for possible treatment.

86 feet of 24 Asian (*Elephas maximus*) and 9 African (*Loxodonta africana*) elephants were macroscopically examined and 727 histological samples from defined locations of hooves of both species were studied.

The macroscopic anatomy shows differences between the feet of the two species and between wild and captive animals, but similarities to other ungulates. An important finding is the comparatively thin horn layer of the weight bearing surface in zoo elephants (about 10 mm). The horn wall grows between 5 – 8 mm/28 d, depending on feet and species. Minor differences between the feet and species are seen histologically, but with similarities to other ungulates. Poor horn quality in captive elephants' hooves (e.g. vacuoles in the stratum spinosum, decayed marrows of the horn tubules) and "loci of minor resistance" in captive and wild animals (e.g. micro cracks close to the papillae in the pad segment) are detected. Pathological changes are assessed and described microscopically. On the basis of initial biotin plasma specimens (n = 16), 500 ng biotin/l plasma is estimated to be the approximate borderline between animals with or without biotin supplementation.

Knowledge of morphology and horn growth rates is essential for diagnosis and therapy. Variations in foot appearance between species might reflect differing body builds, origins and habitats. The histological findings might explain some of the foot problems, but also give rise to questions about the quality and correctness of current husbandry techniques.

2 Zusammenfassung

Die Arbeit erfasst die normale makroskopische und mikroskopische Morphologie des Zehenendorgans vom Elefanten unter Berücksichtigung von pathologischen Veränderungen. Weiter wurden Biotin-Plasmakonzentrationen untersucht, um Werte als Basis für allfällige Behandlungen zu erhalten.

86 Füße von 24 asiatischen (*Elephas maximus*) und 9 afrikanischen (*Loxodonta africana*) Elefanten wurden makroskopisch erfasst und 727 Gewebeproben von definierten Stellen des Zehendenorgans histologisch untersucht.

Die makroskopische Anatomie zeigt einige Unterschiede zwischen den zwei Arten sowie zwischen Wildtieren und den in Gefangenschaft gehaltenen Elefanten. Dabei bestehen aber einige Parallelen zu anderen Huf- und Klautieren. Ein wichtiger Befund ist die vergleichsweise dünne Hornschicht an Sohle und Ballen der Zooelefanten (ca. 10mm). Die Hornwand wächst zwischen 5-8 mm / 28 Tage, mit geringeren Wachstumsraten an den Hinterfüßen im Vergleich zu den Vorderfüßen und bei den afrikanischen gegenüber den asiatischen Elefanten. Die histologischen Befunde zeigen an verschiedenen Lokalisationen und zwischen den beiden Spezies ebenfalls relativ geringe Unterschiede und vielfach ähnliche Strukturen wie bei anderen Huf- und Klautieren. Pathologische Veränderungen wurden auch mikroskopisch erfasst. Am Zehenendorgan werden schlechte Hornqualität mit Vakuolen im Stratum spinosum und erweiterten Markräumen von Hornröhrchen sowie Schwachstellen, wie Mikrorisse nahe der Papillen im Ballensegment, bei den in Gefangenschaft gehaltenen Elefanten und den Wildtieren beschrieben. Nach ersten Biotinplasmaproben wurde 500 ng Biotin pro Liter Plasma als Grenzwert zwischen Elefanten mit und ohne Biotinbehandlung angenommen.

Die Kenntnis der Morphologie und der Wachstumsraten am Zehenendorgan ist eine wichtige Grundlage zur Diagnostik und Therapie von Schäden. Die Morphologie der Füße ist auch geprägt von Unterschieden des Körperbaus und des Lebensraumes der beiden Elefantenarten. Die histologischen Befunde sind in der Lage, einige der Fussprobleme der Elefanten zu klären, werfen aber auch Fragen zu heutigen Haltungssystemen auf.

3 Introduction and aim of the study

As the elephant is the largest living terrestrial mammal, its feet are probably one of the most important parts of its body. They have to carry an enormous weight. Thus, it is very important that the hooves are of good horn quality that can withstand all the mechanical and environmental influences. Therefore, it is easy to appreciate that elephant husbandry demands a great deal from zoological institutions. Control and maintenance of the hooves' good condition is a major part of the work of an elephant keeper in a zoo.

As early as 1929, Zimmer had drawn the conclusion from old Asian traditions that foot diseases are grave and inexorable. Seilkopf (1959) also emphasized that many captive elephants died or were euthanised due to hoof disorders and Kuntze (1980) also accentuated the importance of the feet for the general health of elephants. However, Mikota et al. (1994) reported the infrequent appearance of foot problems of elephants in the literature, in contrary to their commonness in the captive population. This common occurrence in Asian (*Elephas maximus*) and African elephants (*Loxodonta africana*) had already been confirmed by Seilkopf (1959), Salzert (1972) and Kuntze (1980).

The most important and most extensive study about care and management of elephants in captivity was done by Mikota et al. (1994). They reported disorders of the musculoskeletal system as common (1323 of 5415 medical events). 586 cases of these 1323 events involved the feet. 50% of the study population was affected and occurred at 63 of 69 zoos. About 30% of the elephants examined had more than one episode. In spite of the poor description of foot problems in the medical records of the respective zoos, they found in their study that 158 events involved the nail (cracks, overgrowth, overwear), 42 events involved the pad (erosions, cracks, separations) and 14 events involved the cuticle. Other recorded signs were cracks in the sole, abscesses, non-specific lesions, injuries (e.g. foreign body) and wounds. During their study, seven elephants were even euthanized as a result of chronic pododermatitis.

Additionally, there are also numerous other case reports and therapy suggestions about foot problems in Asian and African elephants, which are well known in many zoos and circuses (Heck and Schlossarek, 1955; Ruthe and Seilkopf, 1962; Kuntze, 1980; Schaller, 1986; Schanberger, 1990; Rakes, 1996; Csuti et al., 2001; Flügger, 2002; Ollivet-Courtois et al., 2003; Rajankutty, 2004). According to the general view that African elephants do not experience as many diseases under captive conditions as the Asian subspecies, this might also be valid for foot disorders. There also exist a number of publications about beneficial foot care for elephants (e.g. (Wallach and Silberman, 1977; Roocroft and Atwell Zoll, 1994; Kock, 1997; Csuti et al., 2001).

Biotin is mentioned again and again by keepers and vets with regard to pathological alterations in the elephants' hooves as a possible solution for many problems. However, this is undoubtedly not a universal cure. Additionally, very different opinions exist as to whether biotin has any effect on elephants' hooves at all. One reason for these differences in opinion is certainly that there are no scientific studies about biotin that could prove its direct effect on elephant hoof horn quality.

All the above explanations show the importance of elephants' feet for medical care and husbandry in captivity. But to evaluate the pathological alterations in connection with the husbandry or the causes for these diseases, the normal anatomical occurrence has to be known. This is necessary to be able to perceive any pathological divergences. Indeed, there have been macroscopic (Watson, 1873; Virchow, 1910; Wettstein, 1920; Mariappa, 1955a; Mariappa, 1955b; Mariappa, 1955c; Mariappa, 1955d; Güßgen, 1988) and histological (Smith, 1890; Horstmann, 1966; Bragulla and Hirschberg, 2001; Lamps et al., 2001) research studies into parts of the leg and the feet of elephants, but a systematic macroscopic and microscopic investigation of elephants' hooves, which would make it possible to detect more details about the feet and the tendency for foot problems, is still lacking.

The goal of the present study is to analyse the normal macroscopic morphology and the normal microscopic structure of both African and Asian elephant species' hooves by means of different measurements at defined locations, with regard to pathological changes. The normal histological findings provide a basis for assessing histopathological changes and especially horn quality. Examinations of biotin concentration in the plasma should establish the values of biotin with and without biotin supplementation as a basis for controlled biotin treatment.

This study will hopefully be a starting point for further investigations in every field of this subject so that elephant husbandry can be improved with reference to foot problems.

4 Review of the literature

4.1 Definition of elephants' hooves

The word “hoof” has two dictionary definitions: 1st the foot of an ungulate mammal, 2nd the horny covering of the end of the foot in hoofed mammals. The different possible designations of the horn part of the elephants' foot (e.g. nail, hoof) can lead to different opinions among authors, as Seilkopf (1959) showed. **In this study, the “hoof” of the elephant, “nails” included, is meant as all structures that are surrounded by the horn capsule.** It represents the digital organ consisting of central supporting structures and the surrounded modified skin with subcutis, corium and epidermis (see chapter 4.5). The stratum corneum of the epidermis, which also means horny covering or horn capsule of the hoof, is also often named hoof capsule.

4.2 General facts about Asian and African elephants

The elephants, non-ruminant herbivores, belong to the family Elephantidae under the order Proboscidea. There are two living genera and species, *Elephas maximus*, of Southern Asia and *Loxodonta africana*, of Africa (Nowak, 1999).

4.2.1 Classification

4.2.1.1 Asian elephant

The Asian elephants are subdivided into three different subspecies: *Elephas maximus maximus* (common on the island of Sri Lanka), *Elephas maximus indicus* (common in mainland Asia) and *Elephas maximus sumatranus* (common on the island of Sumatra) (Shoshani and Eisenberg, 1982). The Asian elephant is classified as endangered by the International Union for Conservation of Nature (IUCN) and the United States Department of the Interior (USDI) and is on the list in Appendix 1 of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES); see Nowak, 1999.

4.2.1.2 African elephant

There are two kinds of *Loxodonta africana*: the larger, paler bush or savannah elephant (*Loxodonta africana africana*) and the smaller, darker forest elephant (*Loxodonta africana cyclotis*), found in the tropical rainforest zone of West and Central Africa (Nowak, 1999). The African elephant is classified as endangered by IUCN and the USDI. It is listed in Appendix 1 of CITES, except for the elephants of Botswana, Namibia and Zimbabwe, which are classified in Appendix 2 of the CITES (Nowak, 1999).

4.2.2 Natural habitat

4.2.2.1 Asian elephant

The Asian elephants are still indigenous to the following countries: India, Nepal, Bhutan, Bangladesh, Sri Lanka, Myanmar, China, Thailand, Cambodia, Vietnam, Malaysia, Sumatra and Borneo (Malaysia and Indonesia) (Shoshani and Eisenberg, 1982). Their habitat, which is adapted to their diet (especially leaves and grass), includes primarily the tropical forests and grasslands (Keele, 1998b). The Asian elephants occupy a diversity of habitat types such as moist and dry deciduous forest, scrub thicket, swampy grasslands, riparian forest, patches of evergreen forest and alluvial floodplains of large rivers (Nowak, 1999; Sukumar, 1999) with mud and water pools, as well as places for licking salt (Altevogt et al., 1987).

4.2.2.2 African elephant

The species occurs from south of the Sahara to the northern parts of Namibia, Botswana, Zimbabwe and South Africa. *Loxodonta* occupies different types of habitat, from the desert regions of western Niger and the Etosha Pans of Namibia to the grassy savannahs of East Africa westward into the forests of Central Africa (Keele, 1998a). Bushland and forested habitats with e.g. scrub, leaves, twigs and forbs, as well as savannahs with mainly grasslands are the main habitat for the different subspecies (Ullrey, et al., 1997). To find their requirements as fresh water, plentiful food and some shade, the elephant may have to make annual migrations of several hundred kilometers (Nowak, 1999).

4.2.3 Natural diet

4.2.3.1 Asian elephant

The Asian elephant is a browser and a grazer, depending on the habitat and season (Ullrey, et al., 1997). The diet consists of different grasses, leaves (such as e.g. *karyota/caryota urenus*, *jak/artocarpus heterophylla*, coconut palm/*cocos nucifera*), bark of trees, wood and soil, for its mineral content. 400 different plants are known as part of the Asian elephants' nutrition (Altevogt et al., 1987). As Sukumar (1999) describes, parts of diverse plant categories in the diet vary enormously from one region to the other and therefore elephants of different countries establish different feeding patterns.

Generally, elephants are continuous feeders, spending an average of 12 to 20 hours a day eating because they have poor food utilization (lower efficiency of the gastro-intestinal tract) and therefore need a large amount of food per day, but because the vegetation is rather rich in the environment of the Asian elephant, it requires less food than the African species (about 150 kg per day, Keele, 1998b). According to the longer mean retention time of the food in the Asian elephant, they tend to have slightly higher digestion rates of cellulose and hemicellulose (Van Soest, 1996).

4.2.3.2 African elephant

The African elephant is also a grazer and a browser. As with the Asian elephants, the African species are generalist feeders consuming a large number of different plants, but they have a wide variety regionally and seasonally. The plant species range from grasses, sedges, forbs, shrubs, trees (especially the bark) to fruits, bulbs, plant bases and roots including soil for its mineral content, comparable to the elephants in Asia (Ullrey, et al., 1997). The African elephant may consume 200 – 300 kg of food (Nowak, 1999).

4.2.4 Elephants in captivity

During the last century, elephant husbandry, as with zoos in general, has gone through a great deal of development and change.

In the European Asian elephant stud book of January 2003 (Belterman, 2003), 92 institutions are listed holding 298 Asian elephants (64 males/ 234 females). 154 females and 43 males of the African elephants are kept in 47 zoos across Europe, according to the European African elephant stud book of June 2002 (Terkel, 2002).

Keeping Asian elephants in captivity has a much longer history, especially in the Asian countries where they are still used as working elephants (Shoshani and Tassy, 1996). The Asian species is known to be less aggressive and to be easier to handle due to their docile character (Nowak, 1999). This could be the explanation for the higher number of Asian elephants in European zoos.

In this chapter, the species are not differentiated because in the literature and in zoos no essential distinction is made between them with reference to husbandry and diet. But the health status varies.

4.2.4.1 Husbandry

In most European countries, some months of the year imply cold temperatures and a cold climate. Therefore the husbandry of elephants in Europe involves an indoor (at least 15°C) and outdoor enclosure. The indoor enclosures have a floor heating system in general and their floor surfaces vary. There are asphalt and rubber floors, plaster stones and poured cements. For the outdoor enclosures (Mikota et al., 1994), the floor system differs as well, but many zoos have sand and some additionally even a natural substrate and/or artificial ground (e.g. concrete, asphalt, plaster stones, marl). A floor that is too rough can cause excessive wear of the pads and skin abrasions when the elephants lie down. The elephants should be allowed to get off these hard surfaces for as many hours each day as the climate permits (Mikota et al., 1994).

There are three systems for keeping elephants: free contact, protected contact and hands off management. Free contact is the traditional way of keeping elephants, with direct contact between keepers and animals. Protected contact (no direct contact between men and elephant) is frequently used for bulls, but is becoming more popular even for groups (female

and offspring). It is a much safer system for the keepers. The hands off management involves no contact with the elephants at all. They live on their own in a big enclosure (typically seen in safari parks or reserves).

Due to the matriarchal society of the animals in the wild, the basic social unit in a zoo is a group of adult females with their offspring, as closely related as possible (Rees, 2000). The bulls are kept alone and in general they are brought together with the group daily.

Enrichment can contain, according to Mikota et al. (1994), natural happenings (as e.g. an encounter of the group with the bull, a birth, the raising of the young generation, the daily washing and the training of frequent orders), behavioral (e.g. occupying the animals longer with feeding, presenting some chained up toys, going for a walk in the nearby area) and environmental enrichment (e.g. mud bath areas, water pools, digging or dusting facilities, scratching trees, trees for moving). The access to water and mud is of great significance especially for the Asian elephant (Altevogt et al., 1987; Ponnappan, 2000; Buckley, 2001). In their natural habitat, the elephants spend several hours a day in rivers or pools, which may help for good foot health.

4.2.4.2 *Diet*

The digestion abilities of elephants, whose digestive physiology resembles that of horses, is quite low due to fast ingesta passage rates (Clauss et al., 2003). In captivity, the diets of African and Asian elephants are broadly identical (Hatt and Liesegang, 2001), although there is a difference in evolutionary adaptation to the difference in browse content between the species (Asian elephants achieve higher digestibility and longer mean ingesta retention times than African elephants, Hackenberger, 1987).

As mentioned in chapters 4.3.1.2 and 4.3.2.2, elephants are grazers and browsers and they feed in the herd. In many cases, the diet is offered to each individual at its own place so that the proper food intake of each elephant can be monitored. Hay and browse (or straw) should be given ad libitum for nutrition and to occupy the animals. Grain supplements are very common as well. Grass should be a part of the nutrition programme during the summer time. Fruits, vegetables and pellets are added to the daily feed portions. Vitamin and mineral supplementation should be provided. Analyzing the feeding and especially the hay ration is recommended by different authors (Ullrey, et al., 1997; Nijboer and Casteleijn, 2001). Overfeeding and therefore overweight is a common problem in zoos, which can be solved with a reduction of the diet (less energy) and with an increase in the amount of browse (meaning the fibre content of the diet) to compensate for the otherwise reduced occupation levels (Hatt and Liesegang, 2001).

4.2.4.3 Health status

The health status of animals in captivity depends on various factors, but e.g. nutrition and husbandry are among the most important (Mikota et al., 1994; Buckley, 2001; Sadler, 2001; Clauss and Kiefer, 2003; Schmitt, 2003).

Both species are susceptible to several common diseases, but each one also tends to be predisposed to particular maladies. For example, the Asian elephants tend to have more foot problems, although they occur in the African elephant as well (see chapter 4.6 and 5.2). On the other hand, the African elephants are more susceptible to salmonellosis than the Asian species (Schmitt, 2003).

A multitude of different diseases (infectious as e.g. tuberculosis, salmonellosis, herpesvirus, poxvirus, tetanus, as well as parasitic as e.g. gastric myiasis, trypanosomiasis, elephant lice, ticks, mites and non-infectious as e.g. foot problems, rheumatoid arthritis, cardiovascular and reproductive disorders) are seen and known in elephants (Rüedi, 1995; Chandrasekharan, 2002; Schmitt, 2003). In particular, the herpes virus can cause fatal disease in elephants in zoos, especially affecting young animals (Schmitt, 2003).

4.3 Different features between the species concerning anatomical appearance

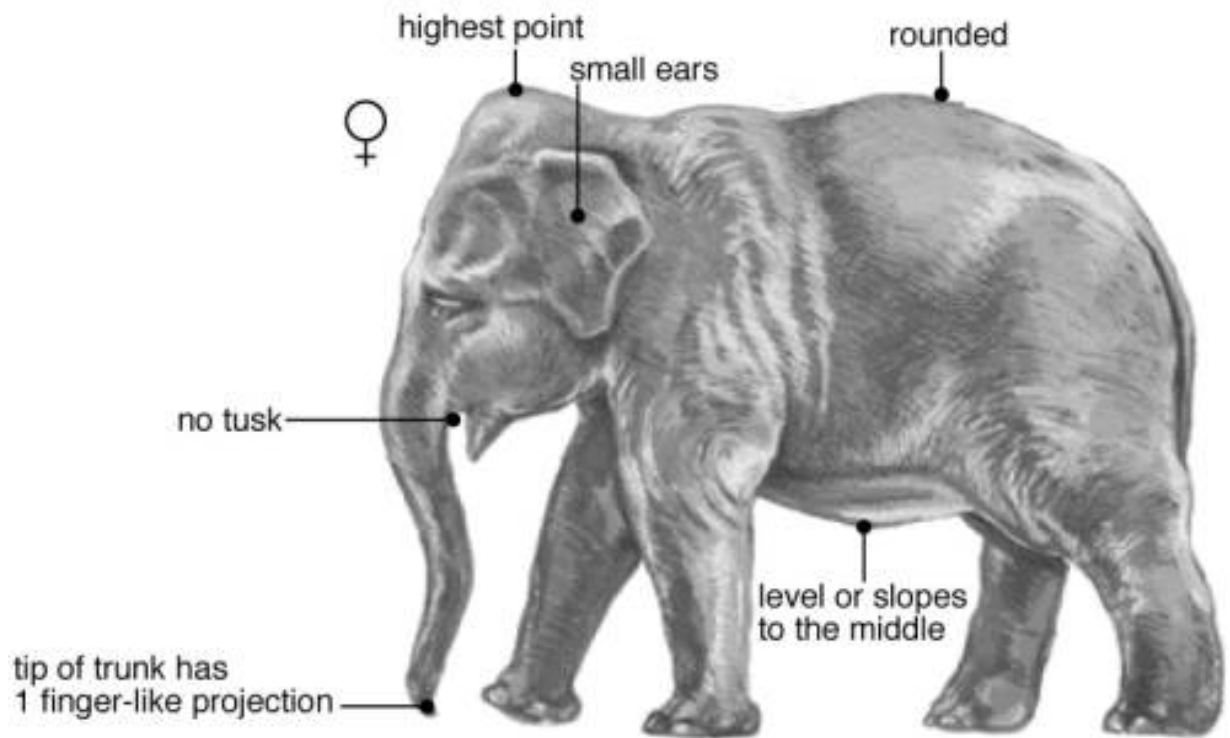
Even if the African and Asian elephant are of the same family, the two species differ from each other in many features of their anatomical appearance (see table 1, figure 1 and Nowak, 1999), but it has to be mentioned that although there are two different subspecies of the African elephant that diverge from each other in some attributes (e.g. *Loxodonta africana cyclotis* is smaller and darker in general than *Loxodonta africana africana*, see chapter 4.2.1.2, Nowak, 1999), they are not differentiated among themselves in table 1.

4.4 Anatomy of the elephants' foot

The elephant is classified as subungulate (Myers, 2000) and is digitigrade on the forefoot (as the hippopotamus and the tapir) and semiplantigrade on the hindfoot (Mikota et al., 1994), see figures 8 and 13. The elephant walks in an ambling way and the hind foot treads in the print of the fore foot. They cannot trot or gallop due to the almost vertical orientation of the bones of the limbs. Nevertheless, they can reach high speeds. The formation of the bones and their restricted agility mean that elephants are unable to cross even a seven foot furrow, but this bone formation supports their enormous body weight (Evans, 1910), see figure 2.

The foot is defined by the supporting structures and the surrounding, partly modified, skin, respectively horn capsule. The supporting structures contain the phalanges I to III, the metacarpal/-tarsal bones, the distal sesamoid bones, the several joints between the different

Asian elephant



African elephant

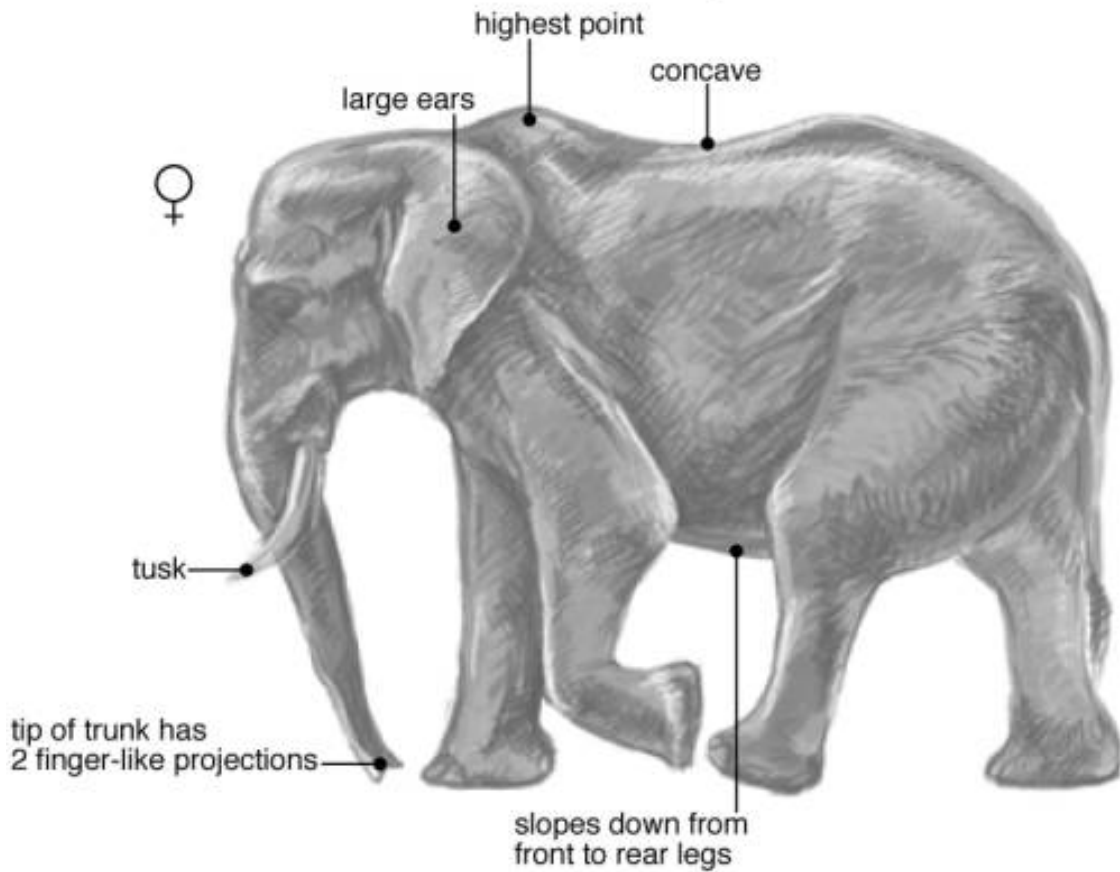


Figure 1: External appearance of an Asian and African female elephant

Table 1: List of the differences in anatomical appearance between the Asian and African elephant (International Elephant Foundation, 2003)

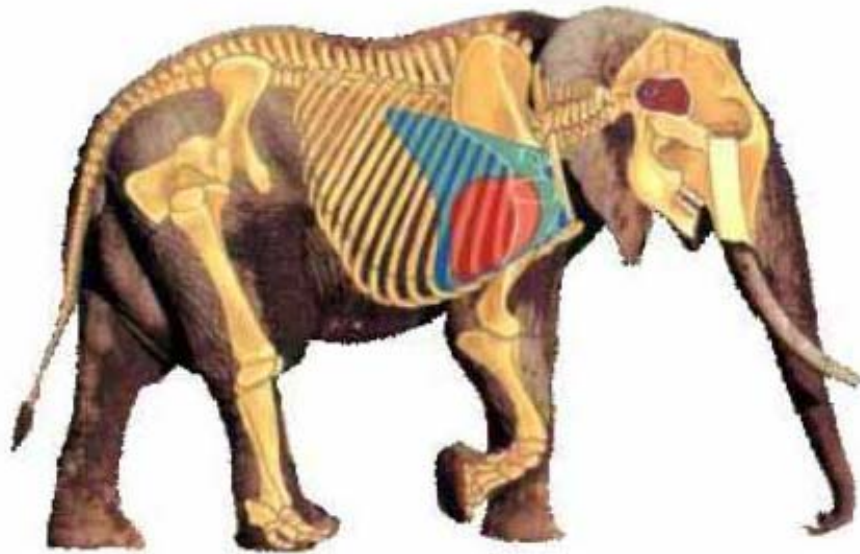
	Asian elephant	African elephant
Height	2 to 3.5 m	3 to 4 m
Weight	3 to 6 tons and the male is heavier	4 to 7 tons and the male is heavier
Tallest point	top of the head	top of the shoulders
Back	roundedly formed	concavely formed
Belly	level or slopes to the middle	slopes down from front to the hind legs
Head	two domes	one rounded dome
Ears	small and rectangular, don't reach over the neck	large, with a similar shape like the continent of Africa
Skin	lightly wrinkled with sparse hair over the entire body	deeply wrinkled with sparse hair over the entire body
Trunk	one finger-like projection of the tip	two finger-like projections of the tip
Tusks	only males have tusks, females have "tushes" ²	both sexes have tusks
Feet ¹	usually 5 toenails on each front and 4 on each hind foot	usually 4 toenails on each front foot and 3 on each hind foot

Notes: ¹ See chapter 4.5 and 6.1; ² "Tushes" seldom extend beyond the upper lip.

bones, the appropriate tendons and muscles, respectively, ligaments as well as the bursa and the digital cushion (everything for each toe except for the digital cushion) that enables the elephants to walk so noiselessly, even in forests (see figures 8 and 13).

In the **forefoot**, there are five metacarpal bones that permit very little abduction of the carpus and therefore their position and articulation is different from other ungulates (Mariappa, 1986). There is a prepollux reaching from the carpal bone I to the pad, medial of the midline and probably stabilizing the carpus over the digital cushion. The digits are in a certain spread, directed to the floor with their apices, but in an oblique incline, just the digit one (D-1) and D-5 stand steep (Virchow, 1910). This is also valid for the hind foot. The Asian elephant has two phalanges in D-1 and D-5, but three in D-2 to D-4 (see figure 2b). There are also

2a



2b

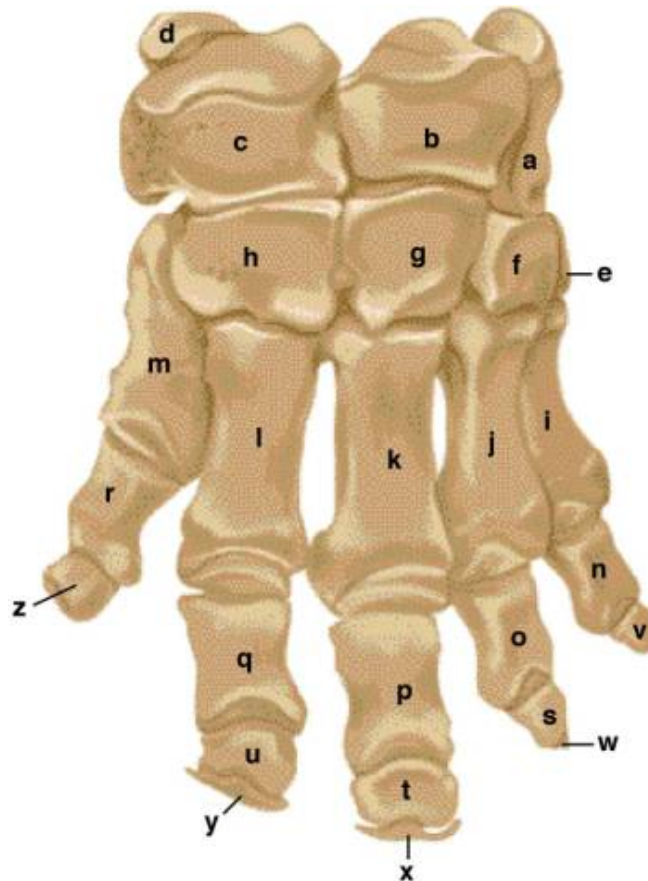


Figure 2: View of the skeleton of an elephant

a: diagram of the skeleton in an African elephant's body (Koehl, 2005)

b: the skeleton of an Asian elephant's right front foot based on the descriptions of Mariappa (1955 a, c) and Güßgen (1988)

a-Os carpi radiale, b-Os carpi intermedium, c-Os carpi ulnare, d-Os carpi accessorium s. Os pisiforme, e-Os carpale I, f-Os carpale II, g-Os carpale III, h-Os carpale IV, i – m: Ossa metacarpalia (Mc) I – V, i-Mc I, j-Mc II, k-Mc III, l-Mc IV, m-Mc V, n – r: Phalanx proximalis, n-digit I, o-digit II, p-digit III, q-digit IV, r-digit V, s – u: Phalanx media, s-digit II, t-digit III, u-digit IV, v – z: Phalanx distalis, v-digit I, w-digit II, x-digit III, y-digit IV, z-digit V

paired sesamoid bones palmar to the metacarpal-phalangeal joint in all digits except for D-1, which has a single sesamoid bone. The African elephant has the same structure, but D-1 has just one phalanx. D-3 is the largest digit. The phalanges are positioned similarly to those of the horse's foot. In the Asian species, the toenails protect the distal part of D-1 to D-5 but in the African elephants the usual four nails shield the D-2 to D-5 (see chapter 6.1.1 and figures 9a – b, 12a – p). Nevertheless, the number of nails (both in the front and hind feet, but also in both species and even in the subspecies of the African elephant) can vary individually (Ramsay and Henry, 2001).

The muscles in the fore foot are the extensor carpi quinti in the Asian elephant, the long digital extensor or communis digitorum, the palmaris longus and the long digital flexor (Ramsay and Henry, 2001). Ligaments between the bones are numerous.

The **hindfoot**, distally to the tarsus, resembles the forefoot. The rear foot also possesses five metatarsal bones and a prehallux, as the corresponding part of the prepollux in the fore foot and five digits (Ramsay and Henry, 2001). The D-1 of the Asian elephant has one phalanx without a sesamoid bone (Mariappa, 1986), but in the African elephant D-1 is represented by just one single sesamoid bone (Smuts and Bezuidenhout, 1994). The other digits are composed in the following way: D-2 has three digits in the Asian, but just two in the African elephant (Mariappa, 1986). Digits three and four are larger than the others in both species and also have three phalanges. On the other hand, D-5 has just two phalanges. D-2 to D-5 have paired sesamoid bones, plantar to the metatarsal-phalangeal articulation. The toenail corresponds to D-2 to D-5 in the Asian elephant and in general in the African subspecies *Loxodonta africana cyclotis*, but to D-2 to D-4 in the other African subspecies *Loxodonta africana africana*.

The muscles of the hind foot are the lateral digital extensor, the long digital extensor, the short digital extensor, the abductor of D-3, the deep digital flexor, the superficial digital flexor. The tendons of the lateral and long digital extensors and the ones of the deep digital flexor totally cover phalanx three of D-3 and D-4 and insert into the nail. (Ramsay and Henry, 2001). The musculature of both species are similar but Shindo and Mori (1956) describe the long digital extensor, the extensor of D-5 and the peroneus tertius in the Asian elephant that are fused to one large muscle belly. This belly divides into a medial and lateral portion.

4.5 Summary of opinions about the occurrence and causes of elephants' foot problems in zoos

4.5.1 General statements

Zimmer (1929) and Lahiri-Choudhury (2001) noted that even older Indian people had observed frequent foot problems with elephants in captivity. Rajankutty (2004) however

declares foot disorders to be common in both wild and captive elephants. Nevertheless, the Asian elephants seem to have more diseases of the foot than the African species.

According to the extensive study of Mikota et al. (1994), the elephants show the same types of problems as seen in domestic and non-domestic ungulates, such as penetrating injuries, sole cracks, nail cracks, overgrowth and infection. In their investigation, about 10% of all medical events found in 69 zoos were problems involving the feet and affected 50 % of the study population. The most common alterations in their study were cracks in the nail or cuticle, abscesses and non-specific lesions, injuries or wounds.

Kuntze (1980) classifies the foot problems into 4 categories, pododermatitis acuta aseptica diffusa (laminitis), pododermatitis acuta septica (metastatica) e.g. after a pox virus infection, pododermatitis chronica suppurativa et proliferativa and pododermatitis traumatica superficialis et profunda.

Two characteristic problems of elephants and other soft-footed species such as camels, tapirs and hippopotami are described by Fowler (1980): overgrowth of the sole as a result of inadequate wear and cracks developing on the surface of the sole. Such cracks act as a potential portal of entry for bacteria. The same also described von Houwald (2001) in rhinoceros. Additionally, Fowler (1993) stated that overgrown or split toenails, cuticle problems, pododermatitis, osteomyelitis, suppurative arthritis, fractures, dislocations and degenerative joint diseases are frequent diseases of elephants' feet. On the other hand, West (2001) declares nail infections as the most common foot problem with a great potential for subsequent serious sequels (e.g. osteomyelitis). He suggests that the front feet have more foot problems than the rear feet and these are caused by the increased weight load on the front feet.

Different causes are mentioned for the occurrence of foot problems. Lack of exercise, overgrowth of the nail and/or sole, improper enclosure surface, excessive moisture, insufficient foot grooming and insanitary enclosures, inherited poor foot structure, malnutrition and skeletal disorders (arthritis) are suggested as predisposing factors for foot problems by Fowler (2001). Recurrent foot infection and arthritis are mentioned by the same author as the main causes for euthanizing elephants. Mikota et al. (1994), Rüedi (1995), Schmitt (2003) and Rajankutty (2004) propagate the following as causes for foot disorders: wet and dirty conditions, hard concrete floors, lack of exercise and movement as well as specific diseases (e.g. foot and mouth disease, penetrating injuries, falling of heavy logs on the foot, prolonged work) as causes for foot disorders. West (2001) sees the etiology of foot diseases in inactivity, lack of trimming, overweight, preexisting diseases such as e.g. arthritis, hard, wet or dirty surfaces and old age.

Most foot diseases are treatable, but some can result in disability or death according to Schmitt (2003). He also states that in 50% of captive elephants foot problems occur at some

point in their lifetime. Therefore, he suggests taking radiographs in advance for reference and recognition of any changes in the case of a foot problem. The healing of most diseases lasts several weeks to months. As Wallach and Silberman (1977) reported: "It takes a considerable amount of time for keratinized sole to heal by second intention or granulation."

4.5.2 Short abstract on the most frequent foot diseases

The classification of these diseases as the most frequent ones was done on the basis of the volume of references found in the literature. A similar division can be observed in horses (Hermans, 1992) and in cattle (Ruthe et al., 1997; Pollit, 1999; Lischer et al., 2000), where the diseases are related (Hertsch et al., 1997; Geyer, 2005) to the different parts of the hoof.

4.5.2.1 Cracks

The cracks appear most commonly in the horn wall (see figure 23). There they are by definition separations of the horn wall proceeding in the direction of the horn tubules (Hermans, 1992) and can be superficial, but they can also reach the living part of the hoof (Fowler, 1980; Rajankutty, 2004). They can start from the coronary border or from the bearing border but, according to Fowler (1978), cracks generally begin at the weight bearing border and progress upwards. Size, width and depth can vary greatly. The weight bearing border or even the sole can be involved. Nevertheless, cracks in the sole can also appear as a singular event and then are partly associated with abscesses or fistules, but they have the same characteristics as in the nails. Bacteria, such as *Streptococcus* spp. and *Staphylococcus* spp., can be found in some infected cracks, but also fungi are common. The cracks are unstable and therefore enlarge under the great weight of the elephant and occur primarily in the hind feet (West, 2001). The healing process takes several months according to the horn growth rate. A recurring tendency exists.

Ruthe and Seilkopf (1962) identify several factors that they consider responsible for the occurrence of the cracks (e.g. bad horn quality and too much utilization of the nail) and Fowler (1978) declared cracks just a sign of general poor hoof health. West (2001) declares cracks to be associated with overgrowth of the nail, trauma to the nail or constant exposure to hard or wet substrates. Flügger (2002) listed the same reasons, but additionally overweight. Rajankutty (2004) sees inadequate wear or trimming combined with moist conditions as reasons for cracks. Nutrition was also named as a cause for the occurrence of cracks (Ruthe and Seilkopf, 1962; Fowler, 1993; Buckley, 2001).

4.5.2.2 Pododermatitis traumatica superficialis et profunda or abscess

Abscesses can arise from different causes and can reach different sizes and be situated in different locations (nail, sole, pad), see figure 23. West (2001) declared abscessation as a result of nail infections that occur after microtraumas to the nails allowing bacteria to invade and necrotize the deeper tissue.

Infections can be caused by foreign bodies as e.g. sharp pointed objects like nails, thorns, needles, stones according to Kuntze (1980), Mikota et al. (1994) and Rajankutty (2004). Foreign bodies are a frequent occurrence in the pad. According to elephant keepers this is due to the surface of the enclosure, especially in the outdoor enclosure (stones). A foreign body can penetrate the horn and even invade the “living” tissue. The bacteria involved are *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Proteus*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* (Mikota et al., 1994). Poor foot care is another possible cause (Rajankutty, 2004). In comparison, West (2001) explained nail infections as a symptom of a variety of husbandry problems. If nail infections are not treated in time, they are susceptible to serious sequelae.

4.5.2.3 *Pododermatitis acuta aseptica diffusa or laminitis*

The symptoms of laminitis are described as severe lameness, inability to stand, high fever, hotness around the foot pads on palpation (Kuntze, 1980; Rüedi, 1995).

According to Rüedi (1995) and Rajankutty (2004), prolonged work, chilling of exhausted animals or overfeeding are the main reasons for laminitis. Chronic laminitis can also be the result of keeping elephants on hard surfaces as well as of a feeding pattern with too much soluble carbohydrate leading to an acidosis and finally to laminitis (Clauss and Kiefer, 2003).

4.5.2.4 *Pododermatitis chronica suppurativa et proliferativa or foot rot*

According to Göltenboth and Klös (1995), the footpads of elephants are prone to foot rot (see figure 3), especially if the pad horn is overgrown with developing pockets within the horn. These compartments attract bacteria and end in the degeneration of the horn. Fowler (1980) stated that the wild hoofed animals are susceptible to infectious agents similar to those that affect the domestic hoofed ones. He called it “infectious pododermatitis”. Elderly Asian elephants are predisposed to this illness according to Seilkopf (1959).

Rajankutty (2004) describes this disease as necrotic changes with foul smelling discharge. In the worst case, it leads to a deterioration of the nails, sole and pad (Seilkopf, 1959). In the front foot, the injury is the vegetative type and in the rear foot the ulcerative type (different bacteria can be involved such as *Streptococcus agalactiae*, *Bacillus cereus*, *Corynebacterium* spp., *Dichelobacter nodosus*, *Clostridium tetani*, *Staphylococcus* spp. (Keet et al., 1997)). Different types of local and laminar inflammations of the foot, nails and pad are possible (Seilkopf, 1959; Rüedi, 1995). Exungulation can be a sequel (Seilkopf, 1959).

Seilkopf (1959), Kuntze (1972 and 1997) and Chandrasekharan et al. (2004) mentioned unhygienic management with elephants standing for long periods on unclean (wetness mixed with urine and dung) surfaces as the most common reason for foot rot. Further Seilkopf (1959) and Kuntze (1972 and 1997) listed insufficient foot care and lack of exercise, in comparison Mikota et al. (1994) refers to cracks and overgrown nails as preconditions for the foot rot.

This kind of problem was also seen in wild elephants, both in Asia (Alahakoon, 2003) and Africa (Keet et al., 1997). The causes for this occurrence in the wild were declared as “drought together with constant elephant browsing, [which] resulted in sharp, woody projections or stubble”. Contamination with faeces and urine of the surroundings of rare water sources increases the occurrence of the pododermatitis (Keet et al., 1997).

Osteomyelitis could be a complication of any pododermatitis if the infection is able to invade deep into the foot.

4.5.2.5 Overgrown horn

An excessive horn growth (see figure 4) underlies this kind of alteration. It is often mentioned as the general cause for other foot problems such as e.g. cracks, infection, ingrown nails and lameness (Fowler, 2001; von Houwald, 2001; Flügger, 2002). The nails extend further than the sole and become deformed with a roughened and layered appearance. The weight bearing surface develops an irregular shape and a layered appearance. Secondary infections and abscessations can arise from this.

Insufficient wear of the horn by lack of movement (Rüedi, 1995) or improper trimming (Rajankutty, 2004) and husbandry on moist floors (Mikota et al., 1994; Rüedi, 1995) are the most often named causes. Chronic laminitis also leads to nail overgrowth (Boosman et al., 1991), which also happens in elephants (Clauss and Kiefer, 2003).

4.5.2.6 Exungulation

Exungulation (see figure 5) means the partial or entire detaching of the nails after e.g. grave foot and joint diseases (Seilkopf, 1959), pox virus infections (Kuntze, 1980; Pilaski et al., 1995) and foot and mouth disease, but also after overloading (Rüedi, 1995).



Figure 3: Adult Asian elephant. Right front foot. Pododermatitis

Figure 4: Adult Asian elephant. Left front foot. Overgrown nails (figure from Dr. H. Schwammer, “Schönbrunner Tiergarten”/Vienna)



Figure 5: Adult Asian elephant. Left front foot. Exungulation of one nail (figure from Dr. W. Rietschel, “Zoologisch Botanischer Garten Wilhelma”/Stuttgart)

5 Material and methods

To realize this study, zoos were visited to inspect the husbandry conditions of the elephants. Macroscopic foot measurements were collected in order to build up an idea of the health status. Further, the feet of dead elephants were examined for the histological part of the thesis.

Feet of both species, *Elephas maximus spp.* and *Loxodonta africana* - subspecies are not differentiated, have been incorporated into the two main parts of the study. The difficulty lay in finding and defining sound feet, which is essential for the description of the normal macroscopic and microscopic anatomy. Logically, the feet of wild animals would represent the normal anatomical state, but it was impossible to organize enough material using only the feet of wild elephants. Thus, the feet of animals in captivity have been examined. However, it was impossible to organize sufficient feet being macroscopically in perfect condition for the macroscopic and microscopic assessments, as well. Therefore, feet with no or little macroscopically visible alterations have been used for the macroscopic examinations. The matter is different with the material for the investigations of the histologically normal state. Because it is difficult to collect feet of dead elephants, every foot that could be obtained was taken. So, the feet in general show partial macroscopic alterations, but the samples for the histological process were taken from macroscopically unchanged locations. Moreover some specimens of dead wild African elephants could be included in the study. From this material the general construction of the structures could be described. In this study it is assumed that the normality of the structures is defined as the unchanged, optimal state of the structures (with some exceptions; see below).

As explained above, it was not possible to obtain a systematic number of feet from each animal nor of the four different legs for either the macroscopic or the microscopic investigations. Even the age of the assessed animals differs greatly, so that the results of the different age groups are treated separately.

5.1 Macroscopic investigations

5.1.1 General macroscopic examinations and measurements (figure 7)

During the period from 2002 to 2003, 61 feet of 24 captive and semi-wild, respectively, Asian elephants (5 males/19 females) and 25 feet of 9 captive African elephants (1 male/8 females), living in 10 separate institutions were examined and macroscopically measured. See table 2 and 3.

Table 2: Institutions participating in the study and numbers of examined elephants

Asian elephants				African elephants			
Zoo	Animals	Male	Female	Zoo	Animals	Male	Female
Zurich Zoo, Switzerland	5	1	4	Tiergarten Schönbrunn Vienna, Austria	4		4
Circus Knie/Zoo Rapperswil, Switzerland	4		4	Basel Zoo, Switzerland	6	2	4
Stuttgart Wilhelma, Germany	1		1	Tierpark Berlin- Friedrichsfelde, Germany	3	1	2
Berlin Zoo, Germany	2		2				
Tierpark Berlin- Friedrichsfelde, Germany	1		1				
Diergaarde Blijdorp Rotterdam, The Netherlands	2		2				
Leipzig Zoo, Germany	1		1				
Pinnewala Elephant Orphanage, Sri Lanka	8	4	4				
Total	24	5	19	Total	13	3	10
Total of Asian and African elephants	37	8	29				

With one African male elephant at the Basel zoo, it was only possible to draw the outline of one front and one hind foot on a plastic sheet. The feet of the African elephants of Tierpark Berlin-Friedrichsfelde could only be macroscopically assessed; no measurements were possible.

The elephants in the Pinnewala Elephant Orphanage in Sri Lanka, which are declared to be semi-wild animals, were measured by Dr. W. Zenker, zoo veterinarian of the “Schönbrunner Tiergarten”, Vienna.

The ages of the Asian elephants range from 1 to 63 years old. For the African elephants, the range of ages is narrower (7 to 45 years old). The different age-groups were treated separately in the measurements.

The macroscopic measurements¹ that were taken are visible in figure 7. In addition, the husbandry has been surveyed with the help of a questionnaire. If necessary, pathological findings have been noted and described.

¹ The measurements of the horn wall always refer to one nail of the appropriate foot, generally the most centrally located and sound nail of the front and hind feet

Table 3: Numbers and locations of the examined and macroscopically measured feet

	Asian elephant					African elephant					Total Feet
	left front foot	right front foot	left hind foot	right hind foot	Total	left front foot	right front foot	left hind foot	right hind foot	Total	
Animals	20	12	12	17	61	9	3	5	8	25	86
Male	2	4	3	3	12	1	1	1	1	4	16
Female	18	8	9	14	49	8	2	4	7	21	70

During the period of the study, one examined animal (1.0) died and all four feet were brought to the Institute of Veterinary Anatomy, Vetsuisse Faculty of the University of Zurich/Switzerland for further macroscopic and microscopic investigations.

5.1.2 Determination of the horn growth rate in the horn wall

The growth rate in the horn wall was measured in 12 animals (8 Asian elephants and 4 African elephants), living in 4 different zoos. This was done by cutting a small notch close to the coronary border in one nail of one front and one hind foot, when possible (see figure 6). The distal movement of the cut was observed and read once a month as long as it was possible (before it disappeared, for various reasons) or for a certain time. Usually the notch was placed into the biggest nail of the foot, generally the third nail of the front hoof and the second of the hind hoof when they were free of pathological alterations. The growth rate was read by the keepers or the vets on location.

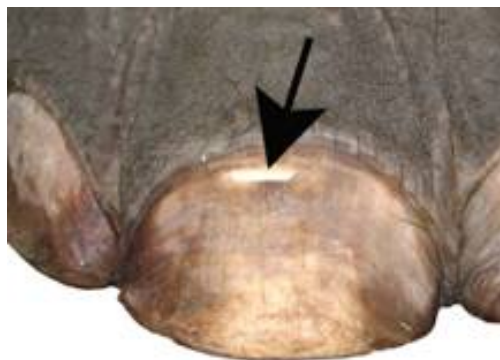
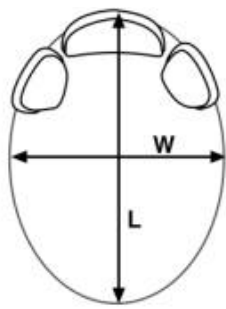


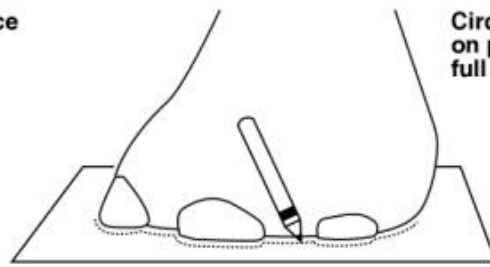
Figure 6: Notch for measuring the growth rate of the horn in the horn wall of the elephants' feet, Asian elephant

5.1.3 Exungulation of the horn capsule

Several singular horn capsules of different feet, consisting of horn wall with appropriate sole and a part of the pad, were exungulated for the macroscopic examination of the horn structure and its construction. For this, the hooves were placed for one hour in water heated

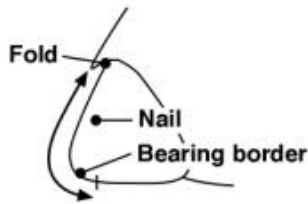


Weight bearing surface
L = length
W = width

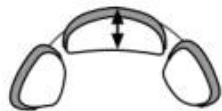


Circumscribing of the foot
on plastic foil (alive feet by
full load on the feet)

palmar/plantar

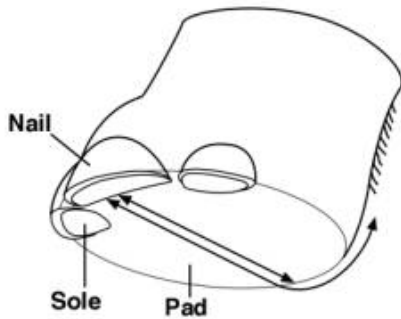


Fold to the middle of bearing border



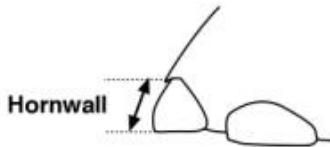
Length of the sole

palmar/plantar

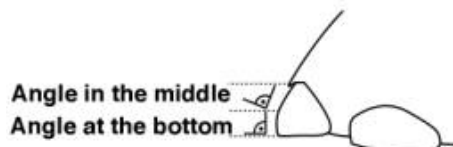
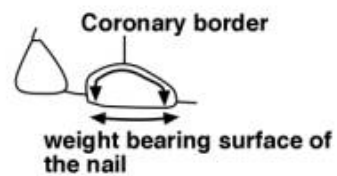


Length of the pad (from the end of the sole to the hair
and to the end of the weight bearing surface)

Length of the horn wall



Tangent of the horn wall



Angle of the horn wall

Figure 7: Macroscopic measurement locations for the examined elephant's feet

to 65°C and then in cold water for two hours. Finally, the horn capsules were removed with a forceps. The separation of the exungulation usually takes place within the germinative layer of the epidermis. Accordingly, there is a thin layer of remaining epidermal cells above the corium, consisting of papillae and leaflets (see figures 15a – b).

5.1.4 Comparison of wild and semi-wild elephants of both species

Several figures of the feet of wild, semi-wild and captive elephants still living in their countries of origin were provided by Dr. W. Zenker, zoo veterinarian of the “Schönbrunner Tiergarten” in Vienna (feet of 8 different semi-wild Asian elephants at the Pinnewala Orphanage in Sri Lanka, kept in almost wild conditions), Dr. M. Hofmeyer, principal scientist of the Veterinary Services and Dr I. Whyte, Program Manager for Large Herbivores, both of the Kruger National Park in South Africa (feet of 10 different wild African elephants from the Kruger National Park) as well as by Dr. P. Martelli, director of the Veterinary/Conservation/Research Department of the Singapore Zoological Gardens in Singapore (feet of 10 different Asian zoo elephants in Cambodia and Vietnam).

From these figures and visits to different elephant institutions in Sri Lanka (see chapter 5.1.5.1), examining the feet of 9 different elephants, it was possible to compare the macroscopic anatomical appearance of the feet of the elephants in European zoos with the feet of wild animals of both species and of semi-wild/captive relatives of the Asian species, living in institutions in their countries of actual origin.

5.1.5 Husbandry conditions of the participating institutions

In this section, the husbandry of different institutions will be listed in a table (see table 4). The data were collected with the help of a questionnaire and discussions with the respective staff. Therefore, the list does not correspond to that in chapter 5.1.1: some additional zoos answered the questionnaire. The information about elephants’ institutions in Sri Lanka is given after the following table.

5.1.5.1 Visits to elephant institutions in Sri Lanka

Several elephant institutions (Colombo National Zoo/Colombo, Millenium Elephant Orphanage/Randeniya, Pinnewala Elephant Orphanage/Kegalla, Elephant Transit Home at the Udawalawe National Park, Udawalawe National Park), guided by Dr. J. Deepanij, were visited in 2003. Additional information was collected in discussions with the responsible vets. The husbandry, the feet of different animals, the foot care and the occurrence of any foot problems were considered, although the husbandry conditions are not comparable to European zoos. Nevertheless, the statements of Sri Lankan vets, coming from a country where elephants live wild and with a long history in keeping elephants as a domesticated animals, should carry some weight.

Table 4: A list of European zoos questioned and their husbandry systems – especially in relation to elephants' feet²

Zoo	No. of elephants	Husbandry (incl. environment, enrichment, feeding ³)	Inside enclosure	Foot care ⁴	Special features
1	2.4 African elephants	Environment: inside and outside enclosure Enrichment: free-ranging, several feeding stations, natural substrates in the enclosure; exercise, walking, digging, daily washing, mud bath area, pool, training, moving tree stumps, dusting, feeding at different times/places Food: <u>branches and leaves</u> , apples, dried bread, <u>grass or hay</u> , pellets, automatic drinking bowl	Heated floor, asphalt in boxes, rubber floor, surface is sloped for good drainage, floor and wall heating	Individual daily visual checks and foot scrubs, every three months proper foot care Equipment: hoof knife, woodworking draws knife, hoof rasp	Mud bath is used quite often, but pool is not used very often
2	2.5 African elephants	Environment: inside and outside enclosure, chained during feeding and washing Enrichment: Several feeding stations, vast area of diverse terrain, partially natural substrate in the enclosure, elephant riding by keepers, digging, daily washing, mud bath area, pool, training, dusting, demonstrations, feeding at different places and of branches and bark Food: <u>hay or grass</u> , dried bread, <u>straw, branches and bark</u> , pellets, apples, bananas or pumpkin, wheat bran, carrots, additional: calcium-hydrogen-phosphate-powder daily to improve taste	Concrete	Daily visual checks, proper foot care every three months Equipment: hoof knife, hoof rasp	
3	1.5 Asian elephants	Environment: inside and outside enclosure, chained during night and feeding and washing (changed after rebuilding and after giving away elephants) Enrichment: exercise, walking, digging, daily washing, mud bath area, pool, training, moving tree stumps, dusting Food: <u>hay or grass</u> , dried bread, <u>wheat/barley</u> , apples, bananas, <u>branches and leaves</u> , pellets, vegetables, mangold or dried sugar-beet pulp, salt, vitamins and calcium	Asphalt and partly stallet, plaster stones (rebuilt in summer 2004)	Daily visual checks, proper foot care every three months Equipment: hoof knife, hoof rasp	Pools indoors and outdoors are used regularly;

² The author has not verified whether the answers provided to the husbandry questionnaires present a true and accurate figure.

³ Underlined are the foodstuffs that constitute the biggest portion of the whole feed

Table 4 (continued)

Zoo	No. of elephants	Husbandry (incl.environment, enrichment, feeding ⁵)	Inside enclosure	Foot care ⁶	Special features
4	0.11 Asian elephants	Environment: inside and outside enclosure, chained during night, possibility to walk through washes and/or streams, vast area of diverse terrain Enrichment: free-ranging, elephant riding, walking, daily washing, pool, training (several hours per day), moving tree stumps, dusting, demonstrations, feeding at different times/places Food: hay or grass, dried bread, wheat/barley, straw, apples, bananas, branches, corn	Isolated-concrete-bricks	Sand, grass, plaster stones, concrete, different natural substrates	The inside enclosure for the winter time contains few enrichments and is a small space, but a lot of training is done with the elephants
5	1.7 Asian elephants	Environment: inside and outside enclosure, chained during feeding, possibility to walk through washes and/or streams Enrichment: digging, daily washing, mud bath area, pool training, dusting, feeding at different times/places Food: <u>hay</u> , dried bread, <u>straw</u> , silage, apples, bananas, branches and leaves, concentrates, biotin-supplementation, pellets (with much Vit. E and other vitamin-mineral-supplements)	Concrete, floor is heated	Sand; enclosure is formed like a hill so that the elephants have to walk quite a lot to have an view over their enclosure	More feet problems on hard surfaces; since they have been given biotin, it seems that foot problems have been diminished; all elephants use the pool indoors and outdoors
6	0.4 Asian elephants	Environment: inside and outside enclosure Enrichment: free-ranging, elephant riding (by keepers), walking in zoo, daily washing, pool, training, moving tree stumps, dusting, feeding at different times/places Food: automatic drinking bowl; <u>hay</u> (and grass in summer time), <u>beet pulp</u> , mixed vegetables, carrots, dried bread, wheat/barley, compressed oats, bran, alfalfa, straw, apples, bananas, branches, concentrates, biotin (for about a year); feeding changed recently: less bread and concentrates and pellets in small amounts	Stallit (since summer 2003, previously latex flooring), floor is heated (also since summer 2003)	Sand, concrete, plaster stones	Change of the husbandry during the study (feeding and inside enclosure surface) and according to the staff an improvement in foot health

⁴ The list of foot care performed is a general one. It has to be adapted to every animal individually, especially the repetition of the foot care.

⁵ Underlined are the foodstuffs that constitute the biggest portion of the whole feed

⁶ The list of foot care performed is a general one. It has to be adapted to every animal individually, especially the repetition of the foot care.

Table 4 (continued)

Zoo	No. of elephants	Husbandry (incl.environment, enrichment, feeding ⁷)	Inside enclosure		Foot care ⁸	Special features
7	1.5 Asian elephants	Environment: inside and outside enclosure, chained during washing, possibility to walk through washes and/or streams, several feeding stations Enrichment: exercise, walking, digging, daily washing, mud bath area, pool, training, moving tree stumps, dusting, feeding at different times/places Food: automatic drinking bowl, hay (in summer time: grass mixed with hay), oat hay, dried bread, oat hulls, pellets, apples, bananas, carrots, lettuce, beet pulp, bran, <u>root vegetables</u> , <u>straw</u> , <u>branches</u> and leaves, vitamin-mineral mix; feeding changed in 2000: more straw and more movement	Concrete with crystal-sand and epoxid-resin mixed, floor is heated, surface is sloped for good drainage	Marl, chaff, loam, sand just for top surface from time to time	Daily visual checks, foot care every two months Equipment: hoof knife, hoof rasp, file	Weaving as a suspected cause of cracks in the outer nails
8 ⁹	2.4 Asian elephants	Environment: inside and outside enclosure, several feeding stations, chained 1 h per day during washing Enrichment: exercise, daily washing, mud bath area, pool, training, demonstrations, feeding at different times/places Food: automatic drinking bowl, hay or grass, dried bread, straw, apples, branches, one elephant with less food and vitamin E/Selen and biotin supplementation; feeding changed recently: more leaves and branches than bread	Asphalt, rubber mats, floor is heated (partially)	Concrete, sand, grass	Daily visual checks, once every second week with one elephant, foot care every three months, no foot care with the bull in the free-range enclosure Equipment: hoof knife, hoof rasp	More feet problems on hard surfaces in the opinion of the staff
9 ⁶	1.5 Asian elephants	Environment: inside and outside enclosure, pasture Enrichment: exercise, walking, digging, daily washing, pool, dusting Food: hay or grass, dried bread, apples, bananas, branches, oranges, carrots, cabbage, Ele-vite	Concrete (cow barn), rubber floor (bull barn)	Concrete, sand, grass	Daily visual checks, daily foot scrubs, and foot care as soon as problem is seen Equipment: hoof knife, hobby knife, hoof rasp	
10 ¹⁰	1.10 Asian elephants	Environment: inside and outside enclosure, chained during night (some elephants) Enrichment: elephant riding (some elephants), walking, daily washing, mud bath area, pool, training, moving tree stumps Food: hay or grass, straw, <u>branches</u> , fed by public	Asphalt, rubber mats, floor is heated (in some stables)	Sand	Daily visual checks and cleaning, foot care every two months Equipment: hoof knife, hoof rasp	

⁷ Underlined are the foodstuffs that constitute the biggest portion of the whole feed

⁸ The list of foot care performed is a general one. It has to be adapted to every animal individually, especially the repetition of the foot care.

⁹ This zoo was not visited on location, but the information was received via the questionnaire and some samples of its elephants were also sent for examination.

¹⁰ These zoos were not visited on location, but the information was received via the questionnaire.

There are four different elephant populations: wild, semi-wild (e.g. Pinnewala Elephant Orphanage), domesticated (privately owned elephants, mainly temple and working animals) and zoo elephants. The environment consists mainly of natural substrates or partially hard floors, such as e.g. concrete, for the captive elephants. The wild and semi-wild elephants live in bigger groups with different, related generations in natural (for the wild) and almost natural (for the semi-wild) environments. The captive animals are mostly kept in smaller groups or alone. The domesticated animals are in a free contact system and therefore well trained by their keepers ("mahouts"). Each mahout takes care of one elephant almost twenty-four hours per day, including most importantly bathing the elephant (scrubbing, rubbing and brushing the whole body with a coconut shell) and being responsible for a hygienic environment (removing urine and faeces where the elephant stands and walks). Therefore, elephants chained to one place sometimes stand on wood to keep their feet dry and to avoid contact with soil contaminated with urine and faeces. The feed of captive and semi-wild Sri Lankan elephants consists of different leaves and barks, grass, special elephant-pellets (only for thin and sick animals), mineral nutrients (Ca for cracks), vitamins (especially vitamin C for healing of wounds).

Foot care is only done if necessary and mainly by the mahouts during the daily bathing routine. The horn wall never gets trimmed but is rubbed with the coconut shell. Treatment, if still necessary, is done relatively early to prevent bigger problems developing.

Some special features have to be mentioned: Firstly the climate in Sri Lanka, which is much more humid and moist than in Europe, certainly influences the skin and horn quality. Secondly there is a widely held opinion among elephant specialists in Sri Lanka that the type of floor the animals have to stand on is not as important as taking care of the elephants by bathing them, giving them the right food and removing urine and faeces. This is confirmed by the fact that elephants stand chained to the same place for hours per day without developing foot problems.

5.1.6 Statistical analysis

For the data gathered through the different anatomical measurements, the descriptive statistic was applied and mean values with ranges were given.

Statistical analysis was done for the growth rate of the horn in the nail of captive Asian and African elephants with the help of the computer programme StatView 5.0¹¹. For a comparison among the different species, the unpaired T-Test and the Mann-Whitney-Test were applied. For the test of differences among the same species, the paired T-Test and the Wilcoxon

¹¹ SAS Institut, 8602 Wangen, Switzerland

signed RankTest, as well as the analysis of variance according to ANOVA were applied. The significant differences are set with $p = 0.05$.

5.2 Microscopic investigations

5.2.1 Histological material

24 feet (17 of the Asian and 7 of the African species) of 10 captive and 4 wild animals (6 males / 1 female / 7 of unknown sex and exact age) were collected from 6 different institutions (see table 5 and 6).

Table 5: Numbers and origin of examined and histologically processed feet of dead African and Asian elephants

Asian elephants					African elephants				
Zoo	Animals	Male	Female	Unknown	Zoo	Animals	Male	Female	Unknown
Zurich Zoo, Switzerland	2	2			Institute of Zoo and Wildlife Research, Berlin (IZW), Germany ¹	3			3
Institute of Zoo and Wildlife Research, Berlin (IZW), Germany ¹	2			2	Museum für Naturkunde Berlin, Germany	2			2
Basel Zoo, Switzerland	1		1		Kruger Nationalpark, South Africa	2	2		
Diergaarde Blijdorp Rotterdam, The Netherlands	2	2							
Total	7	4	1	2	Total	7	2		5
Total of Asian and African elephants	14	6	1	7					

Note: ¹ The feet are from different European Zoos the IZW collected for a project of their own.

Unfortunately, neither the sex, nor age or origin of the feet of the Institute for Zoo and Wildlife Research, Berlin and of the „Naturkundemuseum“ Berlin is known (they are referred to as “Unknown” in the table 5). Only the denomination of African or Asian species was given. According to the size of the feet, it was possible to determine that they are the feet of adult animals. Additionally, with the help of different anatomical structures, the orientation of right and left and the determination of front and rear foot, could be worked out. The feet from the „Naturkundemuseum“ Berlin originated from African elephants living in the wild.

The samples of African elephants from the Kruger Nationalpark, taken in South Africa by Dr. G. Weissengruber from the Department for Pathobiology and Anatomy of the University of Veterinary Medicine in Vienna, were mixed specimens of 2 different animals that were juvenile and from the wild.

The ages of the animals that are known (and these are just the Asian elephants) range from almost 1 to 27 years old. The different age-groups were treated separately in the examinations.

Table 6: Numbers and locations of examined feet of dead African and Asian elephants

	Asian elephant					African elephant				Total Feet
	left front foot	right front foot	left hind foot	right hind foot	Total	left front foot	right front foot	right hind foot1	Total	
Animals	6	3	3	5	17	3	1	3	7	24
Male	4	3	3	4	14			2	2	16
Female				1	1					1
Unknown	2				2	3	1	1	5	7

Note: ¹ The number of the right hind foot of an African elephant consist of a mixture sample of two young wild male elephants of the Kruger National Park and of an animal from the Institute for Zoo and Wildlife Research.

The feet of the dead elephants were generally cut approximately at the height of the carpal or tarsal joint, respectively. The majority of the material was stored frozen at -20°C , except for the feet from Basel Zoo, which were conserved in formalin, and those from the „Naturkundemuseum“ Berlin, which were dried meaning only the skin remained and all the other tissues were gone. The samples from the Kruger Nationalpark were conserved in formalin.

In 2 different zoos and 1 circus (Leipzig zoo, Basel zoo and Kinderzoo Rapperswil/Circus Knie), it was possible to collect some horn material at different locations (coronary border, horn wall, weight-bearing border, sole or pad) during the normal foot care of 4 female animals. These samples consisted of macroscopically normal-looking hooves and of one nail showing foot rot. The horn samples from the Leipzig zoo were organized by Prof. Dr. K. Eulenberger, zoo veterinarian of the Leipzig zoo. The ages of the animals were 35, 42 and 50 years old for the Asian elephants and 32 years old for the African elephant at the time of collection.

Additionally, 41 histological slides of the feet of two dead Asian elephants (one young male and one new-born female, four feet of each animal) and 13 histological horn samples from two living animals (two female elephants of the same zoological garden) already processed by Dr. F. von Houwald were examined.

5.2.2 Processing of the histological material

5.2.2.1 Macroscopic foot measurements

The feet were measured as for the macroscopic investigations (see chapter 5.1.1)¹². Additionally, measurements of the thickness of the pad epidermis (average over the whole length of the foot) and of the digital cushion (height and width¹³) were collected. Whenever possible and logical, these data were included in the results of the macroscopic measurements. For gathering these further measurements, the feet were cut in half using a band saw. This was not allowed with the feet from the „Naturkundemuseum“ Berlin and so it was not possible to measure all parameters of them.

5.2.2.2 Sampling sites from the hooves of the feet

9 and 12 blocks for the longitudinal section as well as 9 and 10 blocks for the transverse section were cut out with a band saw at defined locations of a 1 cm thick longitudinal slice through a centrally situated nail that appeared to be sound macroscopically.

These blocks had a size of around 1.5 x 1.5 x 1 cm and included the epidermis, the corium and a part of the subcutis (to differing degrees). The actual sampling sites were at 18 locations.

Longitudinal sections (see figure 8a):

1-coronary border/periople, 2-central part of the horn wall, 3-weight bearing border/sole, 4-apical, 5-central and 6-palmar/plantar part of the pad and 7-middle, 8-palmar/plantar part and 9-apical part of the digital cushion.

Transverse sections (see figure 8b):

1-skin/periople/fold, 2-periople, 3-hornwall, 4 and 5-weight bearing border, 6-apical part of the sole, 7-apical, 8-central and 9-palmar/plantar part of the pad.

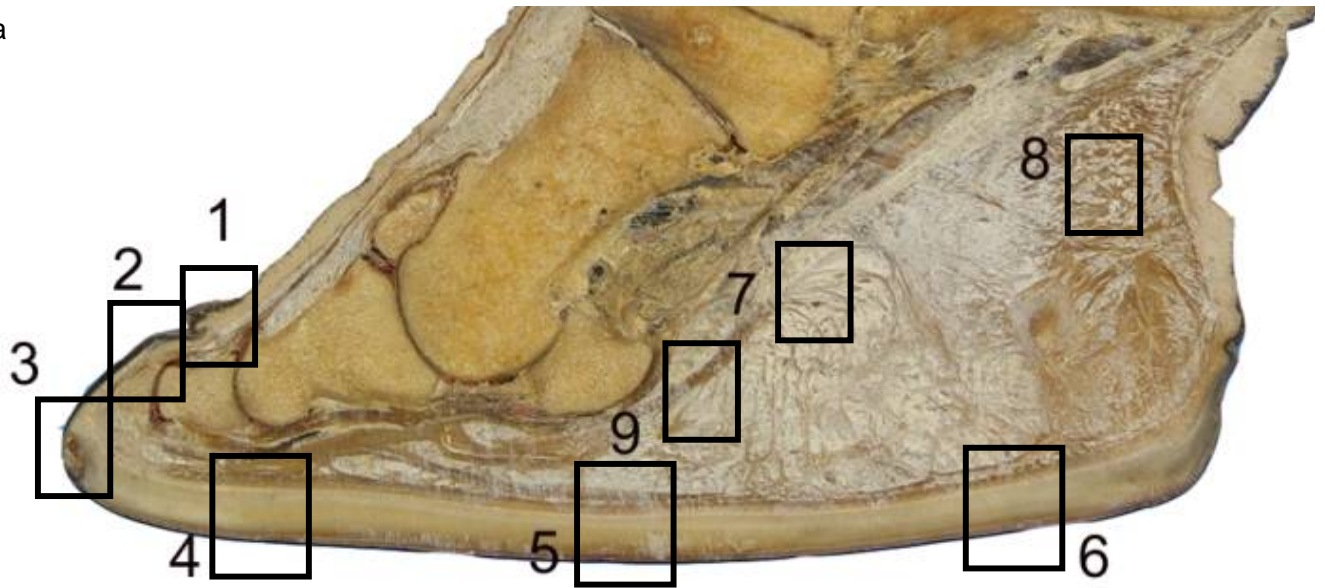
12 blocks for the longitudinal sections were cut out when additional specimens at the central part of the horn wall, at the transition between weight bearing border / sole and at the sole were taken. This was done with the bigger nails and feet. 10 blocks were taken from the transverse sections when further samplings at the transition of the weight bearing border/sole were cut.

The blocks of the feet from the „Naturkundemuseum“ Berlin were sawn out by using a diamond saw, oscillating saw, borer and jigsaw.

¹² The measurements of the horn wall always refer to one nail of the appropriate foot, generally the most centrally located and sound nail of the front and hind feet

¹³ The greatest dimensions in horizontal (width) and perpendicular (height) direction was measured.

8a



8b



Figure 8: Sampling sites for the histological section of the hoof, (for the designation of the numbers see the text; the longitudinal slices in the figures are from a right hind foot of an African male elephant and were provided by the “Naturhistorisches Museum” in Basel)
a: longitudinal sections
b: transverse sections

5.2.2.3 Handling of the specimen

The blocks were put in a mixture of 2.5% glutaraldehyde and 1.3% formaldehyde in 0.1 mol Na/K-P buffer, pH 7.2 for at least 2 days and then sectioned lengthwise and across with a thickness of 10 µm in the cryostat (Leica CM 3050, D knife) at -20°C. The fat samples were sectioned lengthwise with a thickness of 20 µm at -32°C. These latter samples were then directly mounted on a microscope slide (Super®Frost). The blocks of the feet from the „Naturkundemuseum“ Berlin had to be treated differently. They were put into water for at least three days and were cut using a carbide-knife.

The sections of horn samples were stained floating by using haematoxylin and eosin (HE) and Alcian Blue periodic acid-Schiff (AB-PAS) stain. The sections of fat samples were stained with HE and Oil red, instead of AB-PAS. The stained sections were mounted in glycerol gelatin. To cover the slides with glass, the sections of the feet from the „Naturkundemuseum“ Berlin had to be weighted down with lead when mounted in glycerol gelatin. This was because the section was too stiff and air was able to enter between the cover glass and the section.

HE stains the nuclei dark blue and the cytoplasm of the horn cells within the periople and pad light violet. The horn cells of the other segments stain pink. Cells that are not cornified stain red. Cell membranes with the intercellular glue do not show up well and the marrow of the tubules stains bright pink.

AB-PAS stains the nuclei dark blue and the cytoplasm of the horn cells within the periople and the pad segment light blue and in the coronary horn bright. The horn cells of the other segments appear light red in colour. This staining can lead to a PAS positive reaction, which means that the respective structure is discoloured red. So, the cell membranes, the intercellular membrane coating material and the basal membrane are Pas-positive and stain red.

All slides were examined according to the same scheme: subcutis first, then the corium, the epidermis and the different cell layers and types of cells. Finally, every deviation from the normal appearance was described and noted.

In total, 650 prepared specimens were examined histologically. This included some specially cut slides (sometimes some blocks were sectioned again in the middle or every 10th cut was taken to allow the development of the histological structures to be examined).

5.2.2.4 Handling of the horn samples

The horn samples were frozen at –20°C or preserved in 4% formaline. At the laboratory of the Institute of Veterinary-Anatomy, Vetsuisse Faculty of the University of Zurich, the samples were cut into pieces and then treated in the same way as the normal blocks of histological material. The horn samples from living animals resulted in 27 histological samples.

5.2.2.5 Histopathological examinations of macroscopically pathological alterations

In comparison to the normal structures, a selection of macroscopic pathological alterations that occurred in the microscopically examined feet of some Asian elephants (especially cracks in the horn wall and sole and “horn rings”, but also some alterations in the nail that could not be defined exactly) were histologically processed at similar locations and treated in the same way as the normal blocks of histological material. They resulted in 77 additional histopathological slides.

5.2.3 Statistical analysis

Descriptive statistics were applied for the measurements of the histological and pathological description and of the thickness of the horn at different locations in the hooves of Asian and African elephants and mean values with ranges were given.

5.3 Examinations of biotin concentration in the blood plasma of elephants both with and without biotin supplementation

Blood samples of 4 Asian elephants with biotin supplementation and of 3 Asian elephants without biotin supplementation, living in two different zoos, were collected for the determination of the biotin-concentration in the blood plasma. Both zoos were also involved in the rest of the study, which means that some of their elephants were also used for macroscopic data measurements, for measuring the horn growth rate and to inspect some macroscopic alterations. One zoo gave some of their stored feet out of its freezer for the histological examinations.

Zoo A decided to give biotin to 2 of their Asian elephants due to long-lasting severe foot problems over a period of about 13 months. One of the elephants was affected severely, especially by horizontal cracks in the nail, fistules in the sole and bad horn quality. For 8 months, 75 mg D-biotin with 12 mg zinc ("Biotin-Agraria" for horses by Agraria Pharma GmbH¹⁴) then, for 100 days, 100 mg D-biotin ("Rovimix[®]H-2" by Roche Vitamine GmbH¹⁵) and, for two months, the same biotin in the same dosage as at the beginning were given as supplements to both elephants daily. Blood samples of these two elephants were apparently taken prior to every change of the supplements with biotin. It should be noted that it was not possible to get a clear statement from the responsible person as to whether biotin was given to the elephants at the time of the blood collection or how much time elapsed between biotin feeding and blood collection. Equally, it was not clear whether the elephants received the biotin every day during the 13 months or if there were any breaks in between.

Additionally, blood was collected from the other two Asian elephants of the group that were not fed with biotin in order to compare the concentrations of biotin in their blood. After the last blood collection, the staff decided to stop the supplements.

Zoo B gives biotin regularly to each of their animals (35 – 50 mg of the "2% biotin" of Pre-Mervo per day per elephant¹⁶). This is due to a long history of foot problems some years ago. They provided blood samples from two elephants of their group, one before and one during the time of biotin treatment. Unfortunately, one unsupplemented plasma sample was too

¹⁴ Agraria Pharma GmbH, Kesseldorfer Str. 116, D-01159 Dresden

¹⁵ Roche Vitamine GmbH, Emil-Barrell-Strasse 3, Postfach 1145, D-79639 Grenzach-Wyhlen

¹⁶ Pre-Mervo, Posthus 40248, NL-3504 AJ Utrecht

small for a useful analysis. Of one elephant, two different supplemented samples were made available. Biotin is given about eight o'clock in the morning, just once a day and the blood collection is performed about 5 to 10 minutes later.

The plasma samples were examined for the blood concentration of biotin by DSM Nutritional Products, R&D Human Nutrition and Health Analytics (VFHA), registered as Roche Vitamins Ltd¹⁷ with the method of Frigg and Brubacher (1976).

Statistical analysis was done for the biotin determination in the blood plasma samples of 6 different Asian elephants, supplemented and non-supplemented, with the help of the computer programme StatView 5.0¹⁸. Here the Mann-Whitney-Test and the analysis of variance according to ANOVA were applied and the significant differences are set with $p = 0.05$.

¹⁷ DSM Nutritional Products, Sample Registration, Bldg. 205/6, R&D Human Nutrition and Health – Analytics (VFHA), Wurmisweg 576, CH-4303 Kaiseraugst

¹⁸ SAS Institut, 8602 Wangen, Switzerland

6 Results

6.1 Macroscopic examinations of the feet of captive Asian and African elephants

The measurements given in this chapter are taken from different Asian (*Elephas maximus* spp.) and African (*Loxodonta africana*) zoo elephants and also from the histologically examined feet of both species. The subspecies could not be differentiated. The results are divided into females, males and young animals. (see chapter 5.1.1 and 5.2.1). As already mentioned, these animals vary in age and in the size of their feet (see chapter 5). Additionally, some feet or nails showed slight pathological changes, which influenced the dimensions in part. Therefore, the measurements only constitute guidelines. They are calculated according to the arithmetic mean using the descriptive statistic method and ranges are given.

There are a some divergences between the Asian and the African elephant concerning the anatomical appearance and measurements of the feet. These differences will be pointed out in the appropriate chapter. Where the anatomy is congruent, it will not be itemized separately.

If the age and sex of the elephants are unknown, the dimensions of their feet are declared separately. This is only the case with the histologically examined feet. So, the data for “female” and “young” of the African elephants were exclusively collected from zoo elephants. There are no measurements for male African elephants, except for the circumference of a footprint.

6.1.1 The foot

The foot or digital organ (see figure 9) is composed of the internal supporting structures surrounded by modified skin. The supporting structures consist of bones, tendons and ligaments. The digital phalanges and the metacarpal/-tarsal bones together with the sesamoid bones are the bones of it. In the central digits (II, III and IV), three phalanges are present whereas in the lateral/medial digits (I and V) only two digital phalanges can be found. For more details concerning bones and tendons see chapter 4.5. The modified skin itself consists of various layers, which are explained in chapters 6.1.2 and 6.1.3. The nomenclature of all anatomical descriptions refers to the *Nomina Anatomica Veterinaria* (1994).

For a complete figure of the foot anatomy, the whole leg and its position to the rest of the body has also to be taken into account. Due to the enormous size of an elephant, the legs have to carry a massive weight and are correspondingly very straight and perpendicular to the trunk. They appear like columns (see figures 1 and 2). They are positioned well

underneath the body and so the rear view appears pear-shaped in form (see figure 10). The hind feet turn partially outwards, especially in a standing position. This is not the case, or only slightly so, for the front feet.

The feet of the elephants are specialized in weight bearing and therefore, within the foot, there is an enormous digital cushion that enables the foot to absorb the whole mass of the animal (see figures 8a – b and 13).

The circumference of the front feet of Asian elephants (122 – 125 cm for the female and 136 - 146 cm for the male) is generally larger than in the rear feet (around 113 cm for the female and about 127 cm for the male). In comparison, the circumference of front and hind feet do not differ very much for the African elephants (for the front foot, 124 cm for the female and about 149 cm for the male; for the rear foot, 123.5 cm for the female and about 142.5 cm for the male), see table 7 and figure 7. The different sizes of the fore and hind feet corresponds to the differences in the weight bearing surfaces too (see figure 7). Males generally have larger feet than females and African elephants slightly larger ones than Asian elephants (see table 7). At the same time, the shape of the feet in the front differs from those behind (see figures 12d, h, l, p). The front foot is round and broad and the rear foot elongated. Additionally, the rear foot of the African elephant displays a narrower shape because the nails stand much closer to each other so that an interdigital gap is sometimes missed. Equally the centrally situated nail (usually the middle of three) of the African hind foot often stands exactly on the foremost point of the foot with the smaller nails being positioned sideways (see figures 12m – o).

The differences in the numbers of nails (generally 5 nails in the fore foot and 4 in the hind foot of the Asian elephant, on contrast to 4 nails in the fore foot and 3 in the hind foot of the African elephant) were mentioned in chapter 4.5 and also observed in this study. The nails touching the ground when standing are arranged in a semicircular way on the dorsal side of the foot and actually show individual size and shape. With every animal with its feet in a normal state, the laterally and medially located toenails are much smaller than those dorsally and centrally located (see figures 12a – p). Between the nails, an interdigital space appears, which usually becomes larger along its latitude. Nevertheless, it can also display individual size and shape, and in some cases it disappears entirely (see figures 12a – p).

The weight bearing surface is composed of the weight bearing border of the nail, the sole and the pad (see figures 9 a – b, 13 and 14b). This palmar/plantar part of the foot is not curved, on the contrary it appears to be quite flat.

Table 7: Measurements of the weight bearing surface and circumferences of the feet examined (in mm), ranges according to the descriptive statistic method are given 1st part = arithmetic mean, in parentheses = minimum – maximum of the measurements, n = total number of elephants analysed; F = female, M = male, Y = young animals

Asian elephant		
Foot	weight bearing surface (width x length)	circumferences ³
left front foot		
F:	337x374, (290-390x330-440), n = 17	1220, (1115-1300), n = 8
M:	400x430, n = 1	1460, n = 1
Y ¹ :	205x250, (160-250x220-280), n = 2	940, n = 1
right front foot		
F:	355.5x385, (320-390x350-410), n = 7	1250, (1120-1300), n = 6
M:	386.5x436.5, (320-490x370-510), n = 3	1360, n = 1
Y ¹ :	235x260, (200-270x240-280), n = 2	955, n = 1
left hind foot		
F:	266x397, (234-355x305-430), n = 8	1130 (975-1200), n = 5
M:	292.5x435 (285-300x420-450), n = 2	1270, n = 1
Y ¹ :	177.5x267.5, (160-195x230-305), n = 2	865, n = 1
right hind foot		
F:	169x387.5, (220-370x310-425), n = 14	1135, (1030-1200), n = 9
M:	300x475, (250-350x370-580), n = 2	1275, n = 1
Y ² :	190x310, (1x m, 3y), n = 1	880, n = 1

African elephant		
Foot ⁴	weight bearing surface (width x length)	circumferences ⁵
left front foot		
F:	333.5x375, (300-350x350-395), n = 6	1240, (1105-1330), n = 4
Y:	270x330, (260-280x320-340), n = 3	
right front foot		
F:	250x380, n = 1	
Y:	275x325, (275x 320-330), n = 2	
left hind foot		
F:	255x445, (250-260x440-450), n = 2	
Y:	266.5x388.5 (200-380x370-405), n = 3	
right hind foot		
F:	254x435 (220-270x410-465), n = 5	1235, (1195-1270), n = 4
Y:	211.5x380 (195-220x360-400), n = 3	

Notes: ¹ Y = 1 male (3 years old) and 1 female (1 years old) for the weight bearing surface and a 2 years old male for the circumferences; ² Y = a 3 years old male for the weight bearing surface and a 2 years old male for the circumferences; ³ Measurements of the males are from the histological examined feet; ⁴ The young elephants are 7, 8 and 9 years old, both sexes; ⁵ The circumferences for the male are taken from one footprint of a zoo bull: front foot about 149 cm and hind foot about 142 cm.

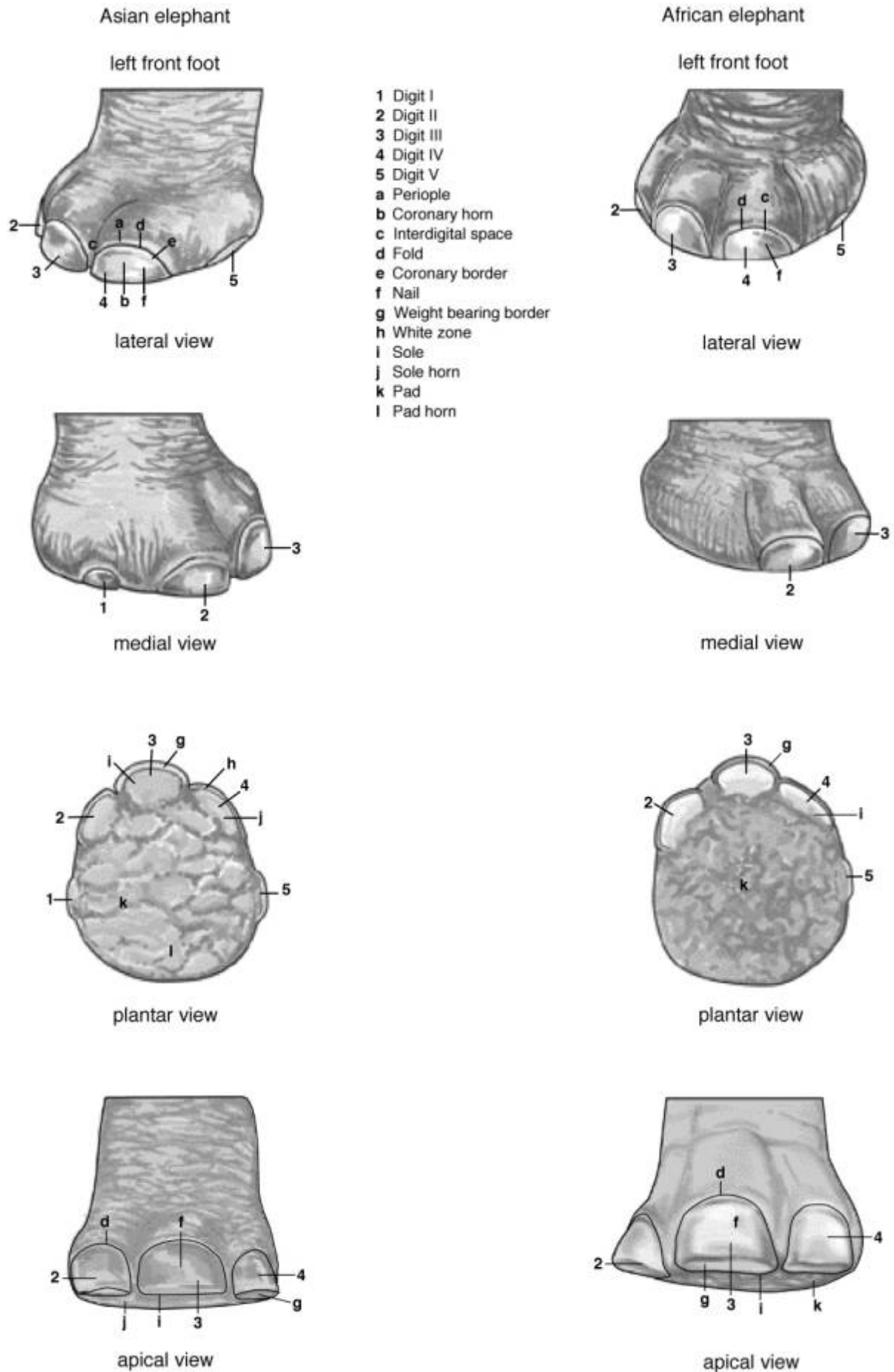


Figure 9a: Anatomical designation of the front foot of the elephants

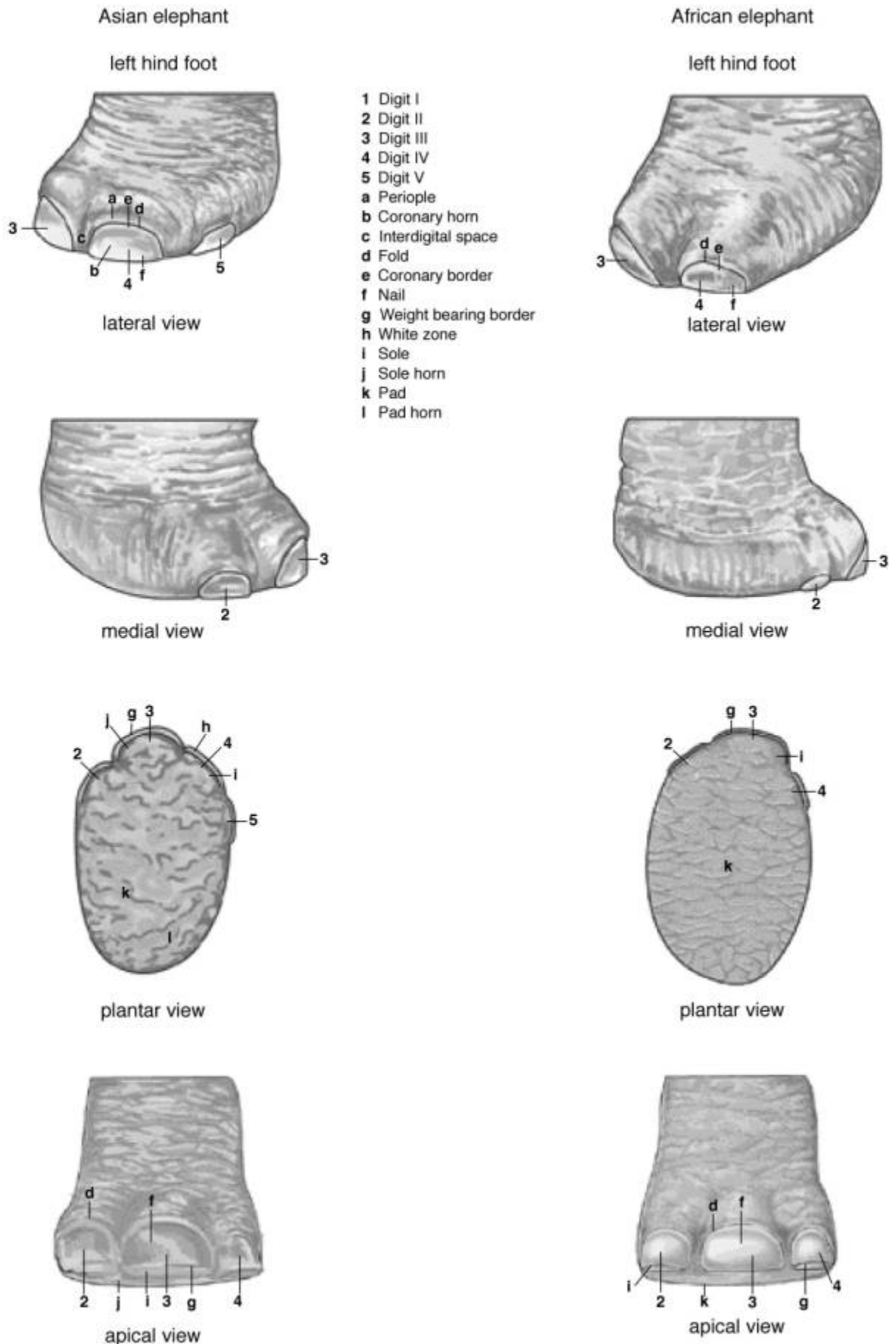


Figure 9b: Anatomical designation of the hind foot of the elephants



Figure 10: Hind view of an elephant, African elephant

Because of the significant differences in the circumference and weight bearing surface between alive and dead feet that have been frozen, only the measurements of the zoo elephants are considered in table 7, except for the data regarding the circumferences of the feet from Asian males. These last measurements derive from the histologically examined feet.

6.1.2 The hoof

The horn walls or nails are formed in a plate-like, semi-circular way and describe a half-moon shape (see figures 12 and 16). The nails situated centrally are bigger than the ones located on the side, which can only reach a size of around half that of the central nails. The horn wall is curved in the horizontal and longitudinal direction (see figures 12a – p). The angle this produces has about the same mean value for the Asian elephant ($72 - 79^\circ$) in every foot, except for instances where the horn walls were too curved so that it was necessary to measure two angles (see table 8). The angle in the African nails is slightly greater. The lengths of horn walls in the front feet are larger than in the hind feet. Males have longer horn walls than females. The African elephants' nails are shorter in size than the Asian ones. However, the tangent of the horn walls in table 8 is about the same for all feet, although generally bigger in males and Asian elephants. As a special feature, the horn wall also has a fold that is visible macroscopically and reaches around the half-moon shaped border of the nail (see figures 14a – b). Most of the nails are pigmented or at least show some pigmentation. On the palmar/plantar side of the horn wall a white zone appears, partly ribbed through the different microscopic structures (see chapter 6.3.3.2 and figures 9, 11, 15b).

A sole belongs to each nail and adjoins the weight bearing border (see chapter 6.1.2.3). Its size for each nail varies to the same degree as the horn walls. It is sometimes hardly distinguishable from the pad and therefore (together with the nail) merges completely with the structures of the pad so that no part of the hoof is independently mobile in any way. The

sole is largely unpigmented, especially on the front feet, and is flat on the surface. The shape describes a semi-circular narrow strip with the greatest width in the centre. As mentioned before, the length of the sole is difficult to identify. Sometimes there is a furrow between sole and pad (see figure 11). Therefore, this segment can only be measured with accuracy in the longitudinal cut of a dead foot, because the sole is better distinguishable from the pad due to the missing subcutis (see figure 18 and chapter 6.3.4). The length of the sole varies between 20 mm and 65 mm (see table 8).



Figure 11: Part of the weight bearing surface of a hind foot, Asian elephant
The arrow shows a trench between the sole and the pad

The pad representing the contact surface to the base adjoins the sole directly, but also together with the sole and weight bearing border the pad connects the nails in the interdigital space (see figures 12d, e, j, 18 and chapter 6.1.3.1, 6.1.3.2). The pad is an independent structure, which is not linked to any single horn wall as the sole was. It generally has the same height as the sole and has an even level over the whole foot. The length of the pad is dissimilar between the front and hind feet, as well as for African and Asian elephants. The measurements are 316.5 to 371.5 mm for the front of the adult Asian elephants and 341.5 to 360 mm for the female African elephant, but 350.5 to 396.5 mm for the hind foot of the adult Asian and 382.5 to 408 mm for the female African species (see table 8).

The exterior of the pad shows macroscopically visible furrows on the surface, which means that ditches plunge into the pad horn (see figures 12d, h, l, p). Those ditches can reach rather deep or can be almost invisible depending on the individual and on the time that has elapsed since the most recent foot care. With the Asian elephant, the furrows are much more present and deeper in general. In comparison, they are hardly found on the pad surface of the African species. As a rule, the African elephant generally has a much smoother pad. In some hind feet, the sole and pad are divided by an easily visible groove, as mentioned in the section regarding the sole (see figure 11). Sometimes the pad horn is quite thin, especially after foot care. Contrary to the sole, the pad horn is mostly pigmented and feels much softer

than the sole horn. Nevertheless, it is difficult to distinguish the horn types from the exterior. Fissures on the surface of macroscopically sound feet can be considered as a normal finding.

It has to be emphasized that the description of the pad in the previous section reproduces only the macroscopic appearance of the examined captive elephants of both species. This is important because there are some differences in the pads and other structures between captive and wild animals of both species. This is dealt with further in the chapter considering additional macroscopic distinctions between the captive and wild animals (see chapter 6.1.4).

6.1.2.1 Subcutis

The subcutis, consisting of elastic-fibrous connective tissue, fat, nerves and vessels, occurs just behind the periople, part of the coronary and the pad corium (see chapter 6.3.1 – 6.3.5). A well-developed subcutis (about 2 cm thick) is found between the coronary border and the joint capsule of the second and third phalanx. Especially underneath the pad cutis, the subcutis grows to a huge size. It fills about half of the interior space of the digital organ and is named the digital cushion or foot cushion (*Pulvinus digitalis*). This cushion extends from the palmar/plantar part of the sole to the palmar/plantar end of the foot. There it reaches the greatest dimensions and has the shape of a right-angled triangle. The size varies between the female and male, but is about the same between Asian and African elephants (see table 8 and chapter 6.3.5 and 6.4.6). This subcutis is bordered above by the bones of the phalanges. These bones are therefore well protected by the digital cushion. Towards apical, the size of the digital cushion and height decreases markedly (the distance from the appropriate corium perpendicular to the joint of the phalanges I and II above ranges from 2.5 to 3.5 cm). Surrounded by connective tissue, the digital cushion consists of highly elastic tissue and a lot of fat cells. The fat tissue appears much whiter in younger animals and sometimes even reddish. It is covered by thick cords of connective tissue (*retinacula*) that attach the corium to the periost and give the impression of different compartments (see figures 8a – b and 13).

6.1.2.2 Corium

The corium, with its *stratum papillare* and *stratum reticulare*, represents the “living part” of the hoof because it contains a lot of vessels and nerves and is consequently very sensitive to any kind of pain-impulse. It also nourishes the non-vascularized epidermis. This happens through a special arrangement of the *stratum papillare* adjacent to the epidermis. This layer creates corial papillae in every segment (see chapter 6.1.3), except for the wall segment where it forms leaflets. In addition, both structures (papillae and leaflets) increase the surface area and the tight connection between corium and epidermis. In the distal part of the coronary segment, as well as in the wall and the sole segment, the corium borders directly

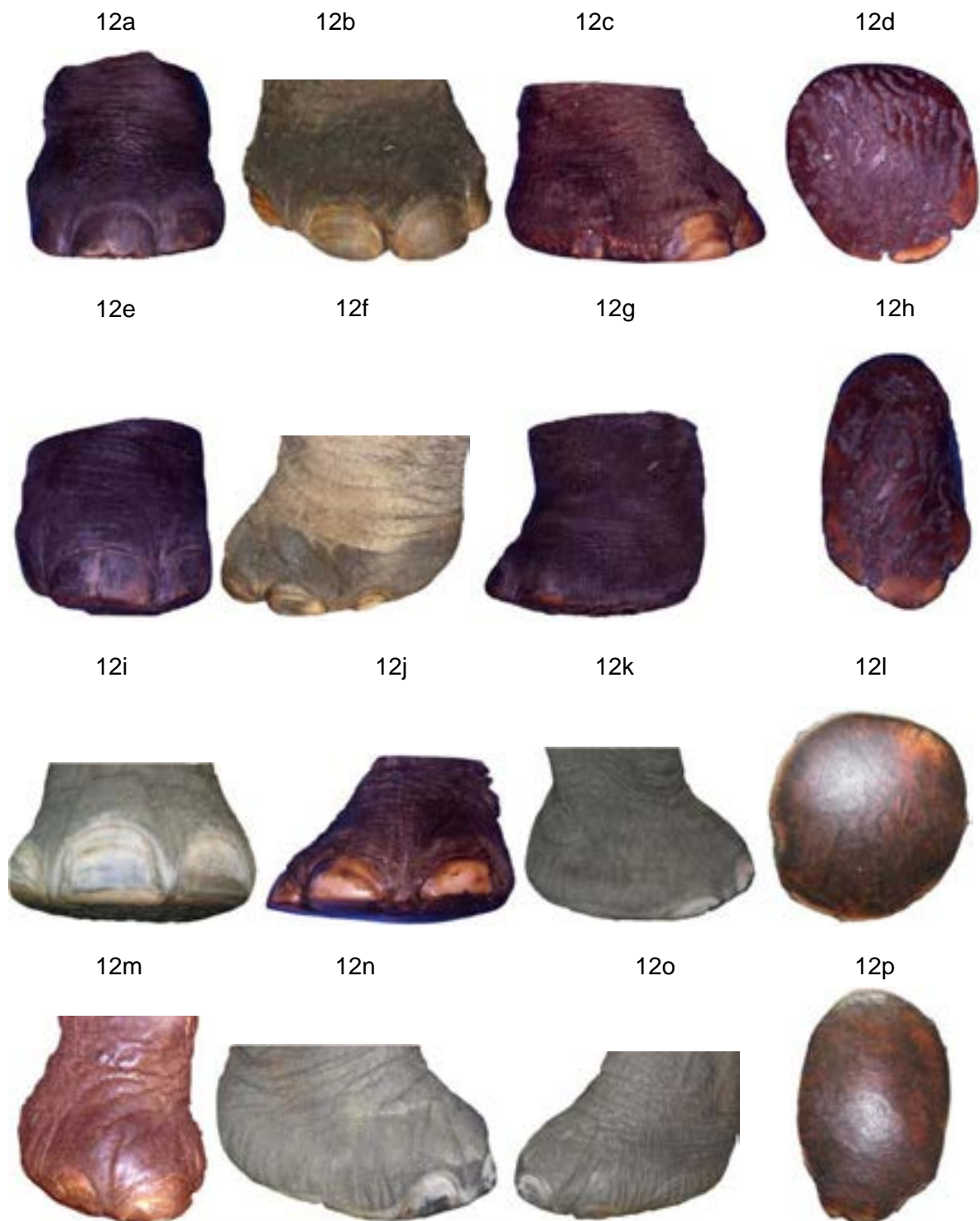


Figure 12: Different views of the front and hind leg of different captive Asian and African elephants
a – d: front feet of Asian elephants (a: apical, b: lateral, c: medial, d: palmar)
e – h: hind feet of Asian elephants (e: apical, f: lateral, g: medial, h: plantar)
i – l: front feet of African elephants (i: apical, j: lateral, k: medial, l: palmar)
m – p: hind feet of African elephants (m: apical, n: lateral, o: medial, p: plantar)

Table 8a: Measurements from the hooves of Asian elephants examined (in mm)¹⁹, ranges according to the descriptive statistic method are given
1st part = arithmetic mean, in parentheses = minimum – maximum of the measurements, n = total number of elephants analysed;
F = female, M = male, Y = young animals, U = sex unknown, for the histological examined feet (see chapter 5.2.1)

Asian elephant				
Foot ¹	angle of the nail ²	length of the horn wall	tangent of the horn wall ³	
left front foot				
F:	72° (64-79), n = 6; 71°/66.5° (59-83/59-72), n = 3	76 (56-95), n = 17	C: 204.5 (170-230), n = 13 / W: 155 (130-190), n = 15	
M:	77°/65°, n = 1	85 (80-90), n = 2	C: 225 / W: 180, n = 1	
Y:	72° (70-74), n = 2; 79°/69° (71-87/62-76), n = 2	46.5 (40-55), n = 5	C: 135 (120-155) / W: 105 (90-120), n = 5	
U:	88/67.5° (86-90/66-69), n = 2	80 (75-85), n = 2	C: 212.5 (185-240) / W: 135 (90-180), n = 2	
right front foot				
F:	72° (62-88), n = 4; 73°/69.5° (59-83/69-71), n = 3	78 (70-95), n = 7	C: 224.5 (195-270) / W: 172 (145-220), n = 7	
M:	86°/69°, n = 1	91.5 (85-100), n = 3	C: 280, n = 1 / W: 196.5 (190-210), n = 3	
Y:	75° (71-79), n = 2; 88°/72°, n = 1	46.5 (40-55), n = 3	C: 130 (120-150) / W: 101.5 (90-115), n = 3	
left hind foot				
F:	76° (70-84), n = 5	66.5 (60-75), n = 8	C: 203.5 (180-240), n = 6 / W: 151.5 (140-170), n = 7	
M:	72°, n = 1	82.5 (75-90), n = 3	C: 225, n = 1 / W: 165 (160-170), n = 2	
Y:	78.5° (78-79), n = 2; 87°/80°, n = 1	42 (25-60), n = 5	C: 138 (105-160) / W: 108 (95-120), n = 5	
right hind foot				
F:	79° (68-87), n = 6; 84.5°/72.5° (81-87/70-75), n = 3	64.5 (50-80), n = 15	C: 191.5 (155-225), n = 14 / W: 155 (120-200), n = 15	
M:	79°, n = 1	70 (60-80), n = 3	C: 200, n = 1 / W: 176.5 (140-230), n = 3	
Y:	77.5° (74-80), n = 3; 80°/66°, n = 1	41.5 (35-45), n = 4	C: 141.5 (130-150) / W: 110 (100-120), n = 4	

Asian elephant				
Foot ¹	fold to the middle of the bearing border along the horn wall	length of the sole ⁴	length of the pad ⁵	thickness of the digital cushion (height x length) ⁶
left front foot				
F:	88.5 (75-120), n = 16	20-25, n = 1	316.5 (250-370), n = 16	?x350, n = 1
M:	95 (90-100), n = 2	22.5 (20-28), n = 3	370, n = 1	100.5x183.5, (87-110x170-190), n = 3
Y:	57.5 (50-75), n = 5	45 (20-70), n = 2	221.5 (175-260), n = 4	115x260, (90-140x250-270), n = 2
U:	90 (85-95), n = 2		360, n = 1	
right front foot				
F:	87 (55-110), n = 7		325 (270-375), n = 7	?x260, n = 1
M:	99.5 (85-110), n = 4	30, n = 1	371.5 (320-480), n = 4	99x177.5, (88-110x175-180), n = 2
Y:	59.5 (45-75), n = 4	21 (17-25), n = 2	224 (175-260), n = 4	
left hind foot				
F:	79.5 (70-95), n = 8	32, n = 1	350.5 (310-400), n = 8	?x370, n = 1
M:	94 (90-100), n = 3	21.5 (18-25), n = 2	391.5 (380-400), n = 3	90x235, (80-100x200-270), n = 2
Y:	55 (50-60), n = 3		261 (225-280), n = 4	
right hind foot				
F:	80 (63-105), n = 15	20-55, n = 1	351 (310-390), n = 15	135x240, n = 1
M:	81.5 (70-90), n = 3	25, n = 1	396.5 (340-450), n = 3	170x395, n = 1
Y:	46.5 (35-55), n = 5	26 (25-28), n = 3	255 (220-270), n = 4	93.5x183.5, (90-100x220-245), n = 3

¹⁹ The measurements of the horn wall always refer to one nail of the appropriate foot, generally the most centrally located and sound nail from the front and hind feet (see chapter 5.1.1, 5.2.2.1 and figure 7)

Table 8b: Measurements of the examined hooves of the African elephant (in mm); description to the table see table 8a

African elephant				
Foot ⁷	angle of the nail ²	length of the horn wall	tangent of the horn wall ³	fold to the middle of the bearing border along the horn wall
left front foot				
F:	83.5°/64°, (76-89/59-75), n = 5	66, (55-75), n = 6	C: 171.5 (155-190) / W: 112.5 (100-120), n = 6	85, (75-95), n = 6
Y:	92.5°/76.5° (89->100/71-79), n = 3	58.5, (55-60), n = 3	C: 163 (150-180 / W: 116.5 (100-150), n = 3	70, (65-75), n = 3
U:	73°, n = 1	81.5, (80-85), n = 3	C: 206.5 (200-220) / W: 140 (130-150), n = 3	105, (95-120), n = 3
right front foot				
F:	80°/63°, n = 1	70, n = 1	C: 185 / W: 110, n = 1	95, n = 1
Y:	82° (76-88), n = 2	60, n = 2	C: 155 (150-160) / W: 95 (85-105), n = 2	77.5, (75-80), n = 2
U:	68°, n = 1	55, n = 1	C: 175 / W: 145, n = 1	65, n = 1
left hind foot				
F:	75°, n = 1, >100°/81°, n = 1	57.5, (55-60), n = 2	C: 170 (160-180) / W: 120 (100-140), n = 2	70, (65-75), n = 2
Y:	79.5° (79-80), n = 3	43.5, (40-50), n = 3	C: 150 / W: 106.5 (100-120), n = 3	56.5, (45-65), n = 3
right hind foot				
F:	84.5°/70.5°, (79-89/67-76), n = 3	55, (25-65), n = 5	C: 171 (155-185) / W: 124 (110-160), n = 5	75, (65-85), n = 5
Y:	78.5° (76-80), n = 3	46.5, (30-60), n = 3	C: 153.5 (145-165) / W: 103.5 (95-120), n = 3	60, (50-65?), n = 3
U:	82/75°, n = 1	65, n = 1		70, n = 1

African elephant			
Foot ⁷	length of the sole ⁴	length of the pad ⁵	thickness of the digital cushion (height x length) ⁶
left front foot			
F:		341.5 (280-370), n = 6	
Y:		276.5 (260-305), n = 3	
U:	36.5, (30-40), n = 3	378.5 (350-410), n = 3	120x320, n = 1
right front foot			
F:		360, n = 1	
Y:		265 (250-280), n = 2	
U:	50, n = 1	310, n = 1	140x235, n = 1
left hind foot			
F:		382.5 (365-400), n = 2	
Y:	30, (20-40), n = 3	355 (325-380), n = 3	
right hind foot			
F:		408 (380-435), n = 5	
Y:		348.5 (325-380), n = 3	
U:	65, n = 1	345, n = 1	120x310, n = 1

Notes: ¹ U = sex and age unknown of histological examined feet, but they are definitely from adults; the young elephants are 1, 2 and 3 years old and of both sexes; ² The angle means: from the bottom to the nail with an average measure or when the nail is too curved than there are two measurements: before the slash means the angle at the bottom and after the slash means the angle in about the middle of the nail; ³ This measurement means: C = length of the nail along the coronary border, W = length of the nail on the weight bearing surface; ⁴ The length of the sole comes from the histological examined feet because the sole is better to differentiate from the pad by the subcutis; ⁵ See chapter 5.1.1 and 5.2.1; ⁶ The thickness of the digital cushion comes from the histological examined feet as the feet have to be cut longitudinally for measuring, but in some cases the measurements of the height of the males are not possible because the foot was cut too shortly; ⁷ U = sex and age unknown of histological examined feet, but they are definitely from adults; the young elephants are 7, 8 and 9 years old, both sexes.

onto the periost. The stratum reticulare (see figures 13 and 14) is bordered by the subcutis and builds a “web-like” construction of connective tissue (about 0.8 to 1.5 cm thick).

Both papillae and leaflets are macroscopically visible in longitudinal sections (see figures 14 and 15).

6.1.2.3 Epidermis

The epidermis is the outermost part of the common integument and covers the corium (see figures 8, 13 and 14). It actually forms the hoof capsule of the foot (see figures 15c – e). It is composed of a soft and a cornified part. The soft part consists of the stratum germinativum (built from the stratum basale and stratum spinosum), which are living cells lying next to the dermis. The stratum basale represents a single layer that is connected to the corium by a basal membrane. In some parts of the epidermal layer, there is a stratum granulosum between the stratum germinativum and corneum. This layer is only found where soft horn is produced (periople and pad horn).

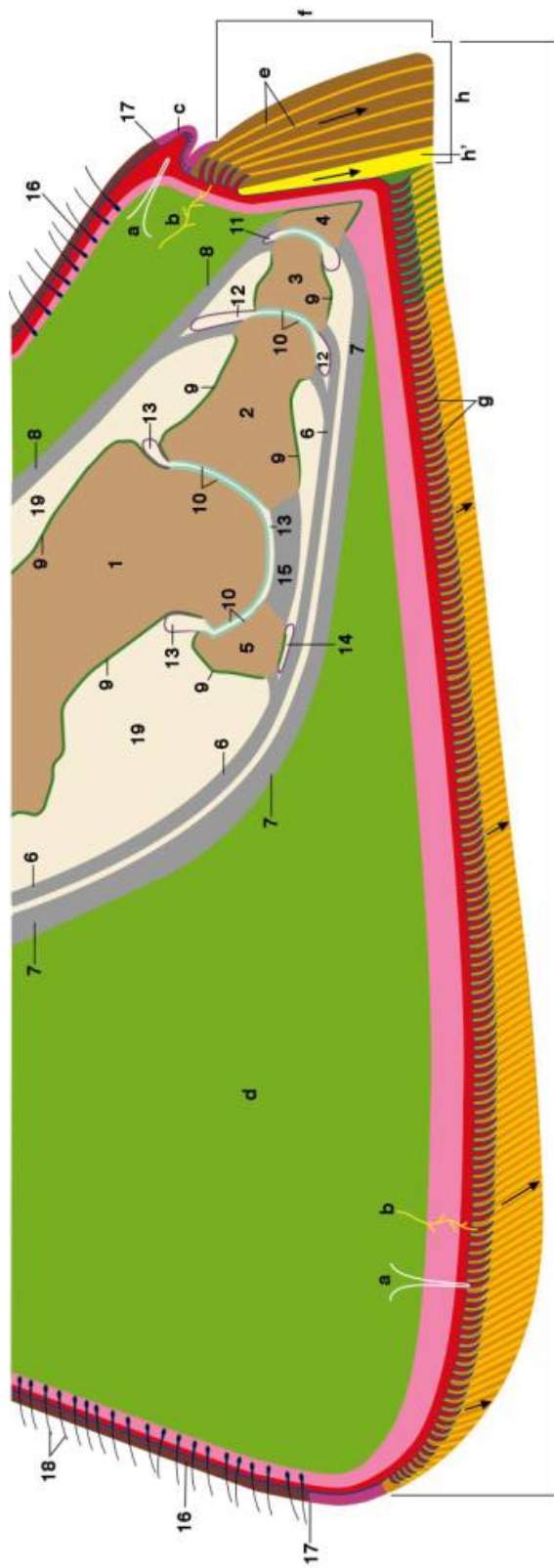
The horn part of the epidermis is made up of the stratum corneum, which is macroscopically visible. It consists of tubular horn, meaning horn tubules, and intertubular horn, which are found in the periople, coronary, sole and pad segment, and of horn leaflets, which is seen in the wall segment. The horn leaflets are bright in colour. The horn tubules are also macroscopically recognisable (see figures 14a – b).

The stratum corneum of the hoof is divided into different horn parts, being the perioplic, coronary, sole and pad horn, and horn leaflets with terminal horn (see chapter 6.1.3 and figures 14, 15). The nail or horn wall is composed of the perioplic and coronary horn as well as the horn leaflets with the terminal horn. The transition from the horn wall to the hairy skin is called the coronary border and the distal end of the nail is named the weight bearing border (see figure 9).

6.1.3 The hoof segments

The hoof is divided into five different segments (see figures 14 and 15). The segments are the periople, coronary, wall, sole and pad segments. Each segment consists of corium and epidermis and contains a subcutis as well if cushioning should be permitted. The name of the segment is related to the place of its production and not to its position. This is of most importance for the location where several horn layers from different origins overlap.

The segments can best be differentiated, visualized and measured macroscopically in specimens after the removal of the horn capsule (the details of the measurements refer to the adult animals), see figures 15a – e.














- | | | | | | |
|---|-------------------|---|----------------|---|----------|
|  | Subcutis |  | Epidermis |  | Pad horn |
|  | Corium |  | Peritopic horn | | |
|  | Str. papillare |  | Coronary horn | | |
|  | Corium |  | Horn leaflets | | |
|  | Str. reticulare | | Sole horn | | |
|  | Epidermis | | | | |
| | Str. germinativum | | | | |
-
- | | | | |
|----|--------------------------------------|----|--|
| 1 | Metacarpal/ metatarsal bone | 11 | Distal interphalangeal articulation |
| 2 | Phalanx I | 12 | Proximal interphalangeal articulation |
| 3 | Phalanx II | 13 | Metacarpal, metatarsal palangeal joint |
| 4 | Phalanx III | 14 | Bursa |
| 5 | Sesamoid bone | 15 | Ligament |
| 6 | Tendon of deep digital flexor | 16 | Stratum corneum of hairy skin |
| 7 | Tendon of superficial digital flexor | 17 | Border between hairy skin and peritopic horn |
| 8 | Tendon of digital extensor | 18 | Hair |
| 9 | Periost | 19 | Connective tissue, vessels, ligaments |
| 10 | Articular cartilage | | |
-
- | | |
|----|----------------------------|
| a | Blood vessels |
| b | Nerves |
| c | Fold |
| d | Digital cushion of the pad |
| e | Horn tubules |
| f | Horn wall |
| g | Papillae of the corium |
| h | Weight bearing border |
| h' | White zone |
| i | Weight bearing surface |

Figure 13: General nomenclature of the inner structures of an elephants' foot

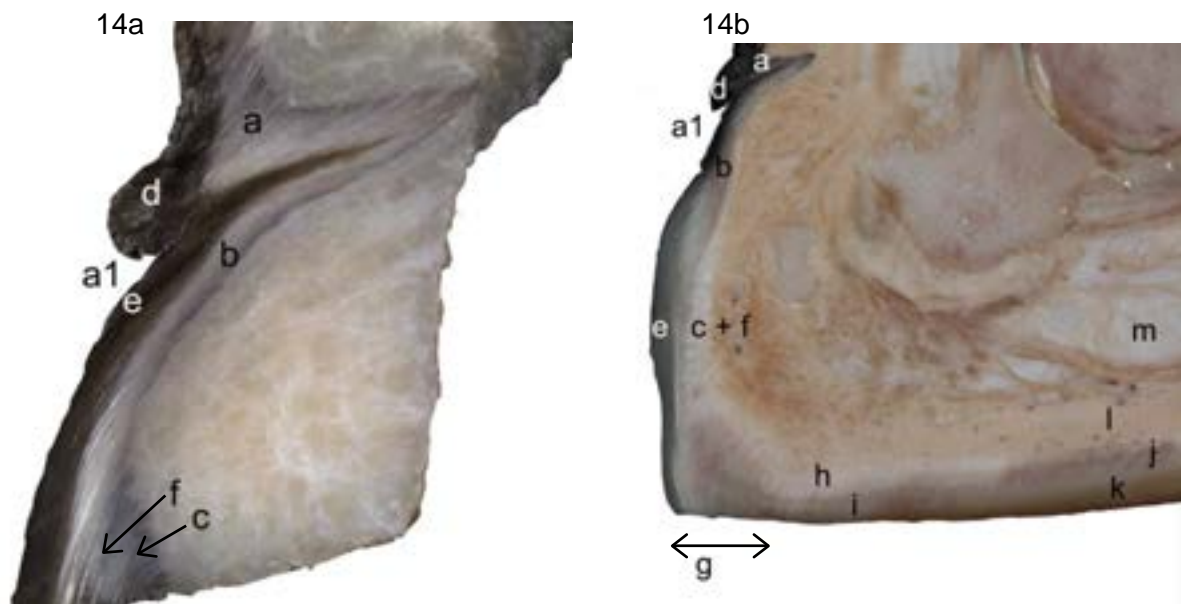


Figure 14: Enlargement of different segments of the elephant's hoof
a: section through the middle of the horn wall with coronary border and beginning of the horn wall (large enlargement), Asian elephant
b: peripheral section through the horn wall, sole and pad (small enlargement), Asian elephant
a-papillae of the periopic segment, a1-fold, b-papillae of the coronary segment, c-corial leaflets, d-stratum corneum of the periopic segment (with horn tubules and intertubular horn), e-stratum corneum of the coronary segment (with horn tubules and intertubular horn), f-horn leaflets, g-weight bearing border, h-papillae of the sole segment, i-stratum corneum of the sole segment (with horn tubules and intertubular horn), j-papillae of the pad segment, k-stratum corneum of the pad segment (with horn tubules and intertubular horn), l-stratum reticulare of the pad segment, m-subcutis of the pad segment

6.1.3.1 The periopic segment

The periopic segment, the proximal part of the hoof integument, is next to the hairy skin and on the coronary segment. It is also responsible for the building of the fold that forms a ridge in the periopic corium ending in a depression in the coronary corium and it lies on the surface of the horn wall. Therefore, only the periople limits the fold. The fold is only found around the nails (see figures 14a – b).

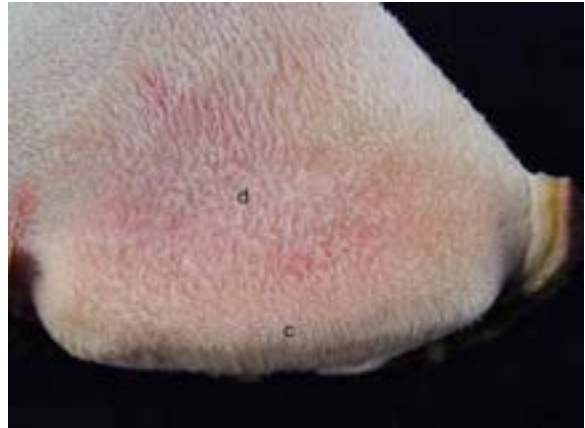
The horn of this segment does not reach the weight bearing border along the horn wall. It is only seen on the proximal part of the horn wall. Most of it looks rough (see figure 16). Besides, the periopic horn can not only be seen in the area of the horn wall, but even as a transition (broadness of about 1 to 5.5 cm) from the pad segment to the hairy skin around the foot (i.e. in the interdigital region and on the palmar/plantar side of the foot, see figures 12j and 17). Sometimes this area has some macroscopically visible fissures.

The periopic horn is pigmented. It appears brighter than the other cornified horny layers. The distance from the fold to the proximal interdigital joint (between proximal and medium phalanges) ranges from 2 to 3.5 cm.

15a



15b



15c



15d



15e

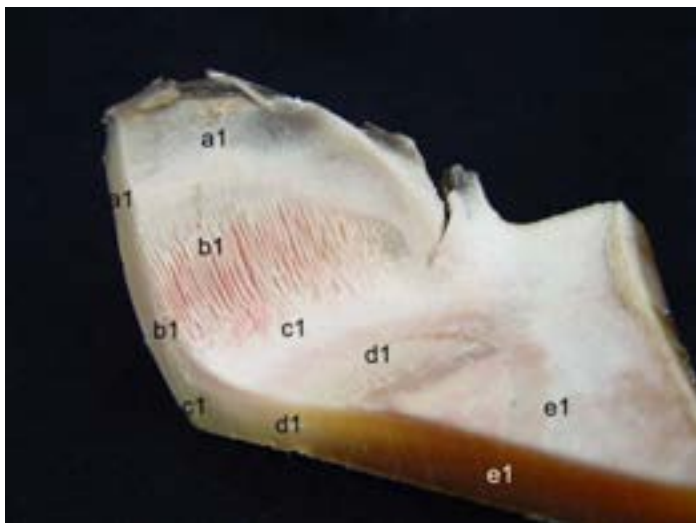


Figure 15: View after artificial exungulation, different Asian elephants

- a:** corial part of the horn wall
 - b:** corial part of the weight bearing border, sole and pad
 - c:** horn part of the horn wall
 - d:** horn part of horn wall, weight bearing border and sole
 - e:** half of the horn part of the horn wall, weight bearing border and sole
- a-cornary segment, b-wall segment, c-corial part of the white zone, d-sole segment, a1-cornary horn, b1-horn leaflets, c1-horn part of the white zone, d1-sole horn, e1-pad horn, f1-periopic horn

6.1.3.2 *The coronary segment*

The coronary segment is located distal to the periopic segment and also borders the wall segment. The shape of the segment is a semi-circular stripe always of approximately the same length (2.0 – 2.5 cm and see figure 15a). This streak borders distally at the lateral and medial side the sole, pad and interdigital region.

The corial papillae are ordered underneath in the same size as the coronary segment. Thus, the coronary horn, similar to the periople, is pushed distally and new horn material is continuously added to the inside surface of the existing horn. Therefore, the width of the horn increases within the coronary segment from proximal to distal (see figures 13 and 14a). The coronary horn is the hardest and strongest part of the horn wall. It is also pigmented.

6.1.3.3 *The wall segment*

The wall segment is adjoined to the coronary segment and the sole segment. In this segment, horn leaflets are connected with soft leaflets, which are lying between the horn leaflets. The horn leaflets are built by ridges at the transition between the coronary and wall segment. These horn leaflets grow from proximal to distal. The soft leaflets consist of corium and a germinative layer of the epidermis, which covers the corium. The soft leaflets are wider than the horn leaflets. Distally above the bearing border, the soft leaflets divide into papillae whose covering epidermis forms the terminal horn, which is found between the horn leaflets. Concerning the size of the soft and horn leaflets, the longest leaflets are in the middle of the half-moon (proximo-distally directed and about 3.5 – 3.8 cm long). They become shorter towards the sides (see figures 15a and c). The wall segment begins about 2.0 – 3.1 cm underneath the coronary border for the front feet of adult Asian elephants and 1.0 - 2.5 cm for the front feet of African elephants, but only about 2.0 – 2.5 cm underneath for the rear feet of the adult Asian species and 1.6 cm for the hind feet of the adult African elephants (see table 9). The distal width of the segment amounts to about 9.5 cm for the Asian and 11.5 cm for the African elephant.

On the palmar/plantar undersurface of the hoof, the breadth of the weight bearing border ranges from 1.0 to 1.5 cm. The part of the wall segment at the weight bearing border is called the white zone. It connects the coronary horn with the sole horn and is created by the transition from corial leaflets to terminal papillae (see figures 14b and 28h), where the horn builds the white zone together with the horn leaflets and the cap horn (see chapter 6.3.3.2). This white zone shows in parts some bars perpendicular to the horn wall.

The keratinized horn part of the wall segment is only small and, as already mentioned, at the distal part of the wall segment, horn tubules are formed, again by the terminal papillae. The wall segment is not actually pigmented, but the terminal horn is.

Table 9: Measurements of the distance from the coronary border to the beginning of the wall segment of examined elephants' hooves (in mm), see figure 13 and 15a, ranges according to the descriptive statistic method are given
^{1st} part = arithmetic mean, in parentheses = minimum – maximum of the measurements, n = total number of elephants analysed; F = female, M = male, Y = young animals, U = sex unknown, for the histological examined feet (see chapter 5.2.1)

Distance from coronary border to the beginning of the wall segment ¹		
Foot ²	Asian elephant	African elephant
left front foot		
M:	31, n = 1	
Y:	22, (19-25), n = 3	
U:	26, (25-27), n = 2	24.5, (19-30), n = 3
right front foot		
M:	31, n = 1	
Y:	21, (20-22), n = 2	
U:		10, n = 1
left hind foot		
M:	25, n = 1	
Y:	12 (11-13), n = 2	
right hind foot		
F:	20, n = 1	
M:	25, n = 1	
Y:	17, (15-19), n = 3	
U:		16, n = 1

Notes: ¹ The distance from the coronary border to the begin of the wall segment is measured in the middle of the segment; ² U = sex and age unknown of histological examined feet, but they are definitely from adults; the young elephants are 1, 2 and 3 years old, just males

6.1.3.4 The sole segment

The shape of the sole segment that joins onto the wall segment on the inside and borders the pad segment has already been described in chapter 6.1.2. There is no special transition line between the sole and the pad horn, except that some animals have a furrow built into the sole and also except for the fact that the pad segment differs in height to the sole (see figure 18 and chapter 6.1.2). The only sure way of differentiation is through the characteristic lack of subcutis in the sole in comparison to the subcutis present in the pad (see figures 13 and 18), which means that the corium borders directly onto the periost of the third phalanx. This can only be seen in longitudinal cuts of the foot. The area of the sole segment reaches about 1.5 x 9.0 cm for the Asian elephant and about 3.0 x 9.5 cm for the African species.

The bending of the almost perpendicular corium of the wall segment causes the corial papillae of the sole to arise. They appear longer than those of the pad segment. The sole horn is unpigmented and, therefore, the sole and pad can be distinguished from each other in part by the pigmentation of the stratum corneum, and also by the type of horn. The sole horn generally is smoother and stronger than the pad horn. The horn tubules of the sole section are also more perpendicular than those of the pad section. Interestingly, the

thickness of the sole horn and of the pad horn was quite thin in all examined feet (see figures 13 and 18). But, the sole horn contributes only a little to weight bearing function.

6.1.3.5 *The pad segment*

The segments adjacent to the pad segment have already been described in the previous chapters. The same is valid for the length of the pad and the description of the undersurface of this segment (see chapter 6.1.2). The construction of the subcutis is the most significant feature in the pad. The subcutis reaches its greatest dimension here and assumes special functions (see table 8 and chapter 6.1.2.1).

The corial papillae generally are shorter than those of the sole dermis. The order of the papillae is much more irregular than in the other segments and they can be observed lying in almost every direction. The horn tubules that join the corial papillae do not occur in a perpendicular form either; they are generally inclined to one side and sometimes also even undulating.

The pad epidermis also has its own characteristics. The thickness of the epidermis varies quite significantly within the individual animal, but also between the different ages, sexes and species of elephant and even between feet (see table 10). Consequently, the stratum corneum also exhibits varying thicknesses of horn. Sometimes, the horn is extremely thin (about 10 mm) in relation to the size and weight of the animal (see figures 13 and 18). The stratum germinativum does not run in a straight line, but in a corrugated way (see figures 8 and 18). There is pigmentation in the horn cells, which appears even darker than the other horn materials. It is much softer than the horn in the coronary or sole segment and has similarities to the periople segment.

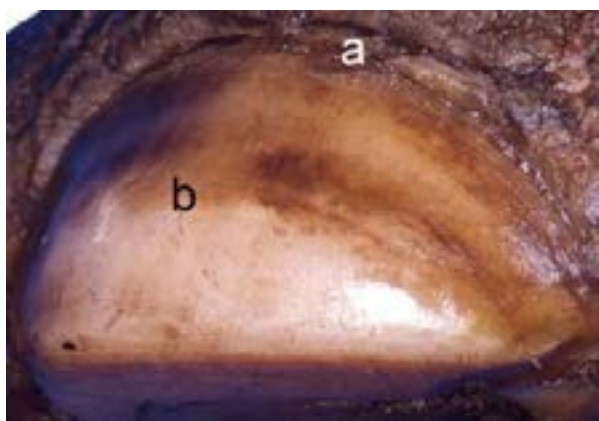


Figure 16: Periopic part of the horn wall of the elephant's hoof, African elephant
a-periopic horn, b-coronary horn



Figure 17: Periopic segment as a transition from the hairy skin to the pad segment of the elephants hoof, Asian elephant
a-hairy skin, b-periopic horn, c-pad horn, d-horn wall of a nail, e-sole horn

Table 10: Measurements of the thickness of the epidermis²⁰ of the pad of the examined elephants' hooves (in mm), ranges according to the descriptive statistic method are given

1st part = arithmetic mean, in parentheses = minimum – maximum of the measurements, n = total number of elephants analysed; F = female, M = male, Y = young animals, U = sex unknown, for the histological examined feet (see chapter 5.2.1)

Thickness of the epidermis of the pad ¹		
Foot ²	Asian elephant	African elephant
left front foot		
M:	13-18, n = 1	
Y:	6.5-8.5, (6-7 till 8-10), n = 3	
U:	11.5-15, (8-15 till 10-20), n = 2	16.5 -22.5, (9-23 till 20-26), n = 3
right front foot		
M:	7-18, n = 1	
Y:	8-10, n = 2	
U:		10-15, n = 1
left hind foot		
M:	10, n = 1	
Y:	7-9.5, (6-8 till 8-11), n = 2	
right hind foot		
F:	9-11, n = 1	
M:	11-13, n = 1	
Y:	6.5-10, (6-7 till 8-10), n = 3	
U:		5-10, n = 1

Notes: ¹ Average over the whole length of feet ²; U = sex and age unknown of histological examined feet, but they are definitely from adults; the young elephants are 1, 2 and 3 years old, just males

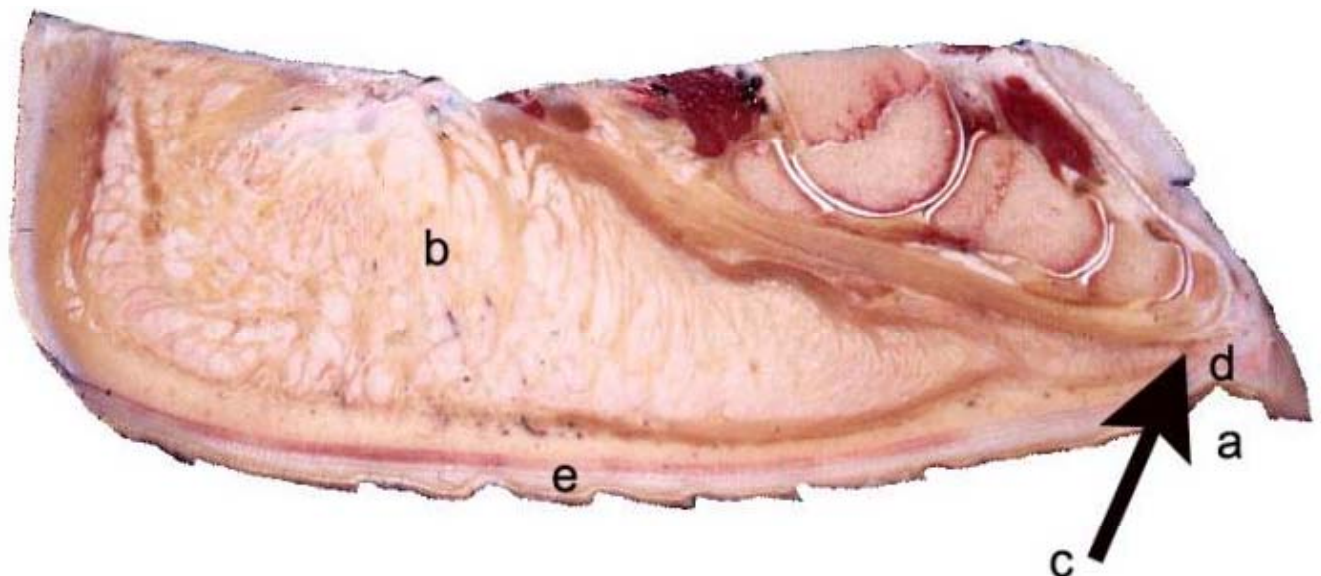


Figure 18: Differentiation of the sole and the pad segment in the elephant's foot, longitudinal cut of an Asian elephant's foot
a-trench between sole/pad and differentiation of the height between sole/pad respectively, b-digital cushion and subcutis of the pad segment respectively, c-beginning of the subcutis of the pad segment, d-sole epidermis, e-pad epidermis

²⁰ The thickness of the epidermis of the pad comes from the histologically examined feet, as the feet have to be cut longitudinally to take this measurement. At the same time, the thickness is not constant over the whole length of the foot and therefore a range is given.

6.1.4 Macroscopic differences between the captive and wild elephants

By evaluating figures and examining semi-wild and wild animals, obvious differences between captive and wild African elephants can be seen. In particular, the wild animals have more furrows on the undersurface of their pads (see figure 19). These trenches also give the impression that the pad horn is much thicker in comparison to those of the zoo elephants, which show a thinner horn layer especially after foot care. The furrows sometimes pass into the sole. In zoo elephants, the sole is flat and only a few furrows can be seen in the pad horn (see figures 12 l and p).

The same observations on horn thickness are valid for Asian elephant, which generally has much stronger horn in the wild than in captivity. In the wild, the pad horn has built up circle-shaped horn pieces about 5 cm in diameter, which provide much more resistance to the environment (see figure 20). On the contrary, the captive Asian elephants show furrows like the wild African elephants, but less pronounced (see figures 12 d and h). So, this illustrates the big difference in the macroscopic anatomy of the weight bearing surface between captive and wild relatives of both species (see chapter 6.1.2). Summarized, it can be said that the pad surface of the African elephants is furrowed and the Asian elephants have circle-shaped horn pieces in the undersurface of the hoof. Additionally, the captive elephants have a much thinner horn layer in the weight bearing surface in comparison to their wild relatives.

None of the wild animals' feet showed cracks, fissures, holes or other pathological alterations and they looked much sounder than those of the captive elephants (see figures 3, 4, 21 and 23). According to assertions by various vets working in the wild in Sri Lanka and South Africa, wild elephants' foot problems are usually related to traumas (penetration by shot, traps or sharp objects on the ground, but also burn wounds) and resulting infections. In the feet of both wild and captive elephants, grooves in the horn wall can be detected. On the other hand, no overgrowth of the periople nor overgrown nails can be recognised in the wild, but in the semi wild Asian elephants hyperkeratosis of the periople is seen.

The macroscopically estimated horn quality of wild elephants looks good in all visible segments, which gives the impression that the horn is much more resistant, even if there are some fissures to be seen on the surface of the pad horn. But it does not look so smooth and thin.

6.1.5 Growth rate of the horn in the nail of different elephants

As a guideline value for the growth rate in the horn wall of the Asian and African elephant, the horn growth rate over 28 days was calculated using measurements from 6 Asian and 3 African elephants. Measurements were taken over a long period for each elephant. From this, the horn growth rate is calculated using an average period of 28 days for each animal (see table 11) and on the basis of the distance the notch grew down during the respective period.

19



20



21



Figure 19: Front feet of a wild African elephant from the Kruger National Park in South Africa (figure from Dr. M. Hofmeyer, Kruger National Park, South Africa)

Figure 20: Front foot of a semi-wild Asian elephant from the Pinnewala Elephant Orphanage in Kegalla, Sri Lanka (figure from Dr. W. Zenker, "Schönbrunner Tiergarten" in Vienna)

Figure 21: Front feet of a semi-wild Asian elephant from the Pinnewala Elephant Orphanage in Kegalla, Sri Lanka

With the African elephants, the growth rate was measured annually in two consecutive years (2003 and 2004).

According to Wilcoxon signed RankTest, the horn growth rate from all measured data of both species in the nails of the left front foot is significantly faster than in the right hind foot ($p = 0.0497$). With the analysis of variance according to ANOVA, the differences in the growth rates of the nails between Asian and African elephants is significant ($p = 0.0246$). This rate is 7.0 mm/28 d for the Asian elephant and 5.4 mm/28 d for the African elephant (see table 11 and 22a). With the Mann-Whitney-Test, the difference of the growth rate in the front foot nails of the two species is significant ($p = 0.0389$), with a growth rate for the Asian elephant of 7.6 mm/28 d and for the African elephant of 5.9 mm/28 d (see table 11 and 22b). A significance ($p = 0.0530$) can also be identified for the same comparison of the growth rate according to the unpaired t-test. The Mann-Whitney-Test suggests a tendency to faster growth of the Asian elephant's horn wall in the hind foot nails ($p = 0.1824$), with a growth rate for the Asian elephant from 6.4 mm/28 d and for the African elephant from 4.7 mm/28 d (see table 11 and 22b).

Table 11: Growth rate of the horn in the horn wall of 6 Asian and 3 African elephants, calculated over 28 days (in mm)²¹; see also figures 22a and 22b

Asian elephant	left fore nail	right hind nail	Period of measurement
1	7.8 mm/28 d	5.7 mm/28 d	June till November '04
2	8.5 mm/28 d	6.8 mm/28 d	June till November '04
3	8.5 mm/28 d	6.8 mm/28 d	June till November '04
4	7.9 mm/28 d	6.3 mm/28 d	June till November '04
5	5.7 mm/28 d	4.5 mm/28 d	August till December '04
6	6.9 mm/28 d	8.4 mm/28 d	August till December '04
\bar{x}	7.6 mm/28 d	6.4 mm/28 d	
\bar{x}	7.0 mm/ 28 d		

African elephant	left fore nail	right hind nail	Period of measurement
1	1 st : 6.8 mm/28 d		1 st : August till October 2003
2	1 st : 6.1 mm/28 d 2 nd : 4.3 mm/28 d	1 st : 4.8 mm/28 d 2 nd : 4.8 mm/28 d	1 st : August till December 2003 2 nd : October till November '04 for fore nail, June till November '04 for hind nail
3	1 st : 6.1 mm/28 d 2 nd : 5.2 mm/28 d	1 st : 5.0 mm/28 d 2 nd : 4.1 mm/28 d	1 st : August till December 2003 2 nd : June till November '04 for fore nail, July till November '04 for hind nail
\bar{x}^{18}	5.9 mm/28 d	4.7 mm/28 d	
\bar{x}^{22}	5.4 mm/ 28 d		

One African elephant measured growth rates that diverged from others of the same species ($\bar{x} = 3.6$ mm/28 d for the front nail and $\bar{x} = 2.5$ mm/28 d for the hind nail). The person responsible for the measurements admitted that they were not correct and could not be relied upon. Therefore, this elephant is not considered for the mean.

²¹ Elephants 1 to 4 of the Asian species are from the same zoo, as are the elephants 5 and 6. All 4 elephants of the African species are from the same zoo. Two elephants from one zoo have not been included in the calculations because the measurements taken are not considered reliable or secure due to a misunderstanding (the results have been 9.6 mm/28 d and 4.8 mm/28 d, respectively for a centrally situated nail of the right front foot of the two animals, the period of measurement was November '03 to April '04).

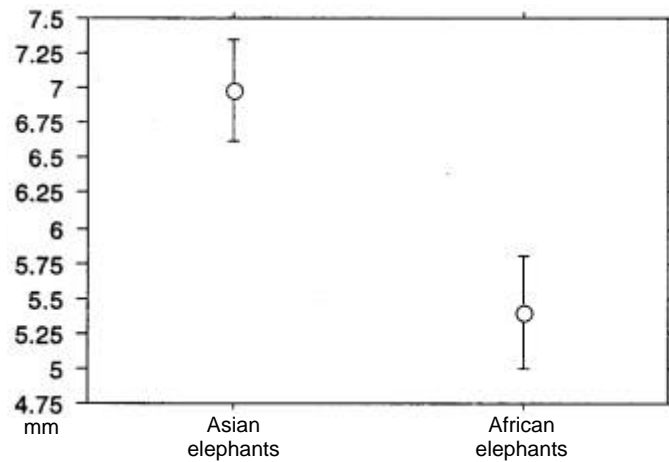


Figure 22a: The horn growth rate of all Asian (n = 6) and all African (n =3, partly measured twice) elephants, including all data from the measured front and hind feet, Interaction Line Plot (means and standard deviations); see also table 11

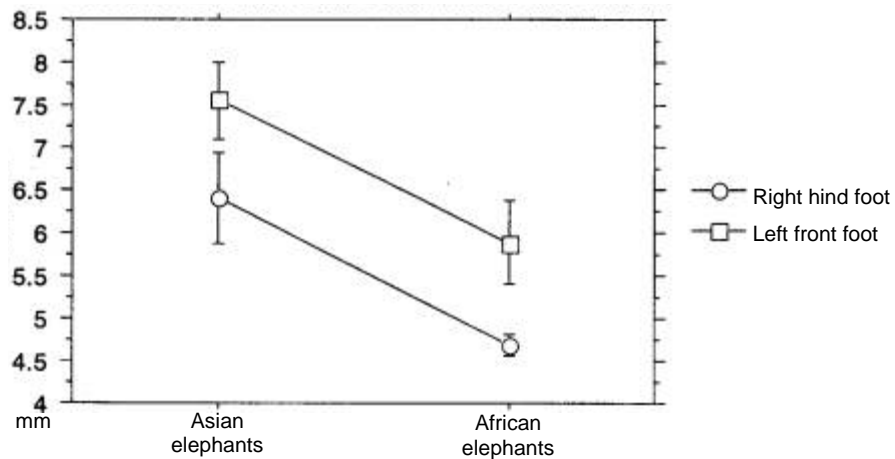


Figure 22b: The horn growth rate of all Asian (n = 6) and all African (n =3, partly measured twice) elephants divided into left front and right hind foot, including all data measured, Interaction Line Plot (means and standard deviations; see also table 11

In summary, it can be said that the growth rates for Asian elephants are faster than African elephants, and the nails of the front foot grow faster than those of the hind foot. Additionally, the faster horn growth rate of the nails of the front foot of the Asian elephant than that of the African elephants is statistically proven. For the differences between the nails of the hind foot for both species, a tendency can be identified. As a guideline, it can be said that the horn wall grows 5 – 8 mm/28 d, depending on feet and species.

²² For the elephants 2 and 3, the average of both measurements were taken for calculating \bar{x} .

6.2 Pathological findings in different husbandry systems

The husbandry systems of the zoos mentioned in table 4 of chapter 5.1.1 are compared with the occurrence of foot problems in each zoo (see table 12). The information is taken from the questionnaires, but also from visits to most of the zoos. The notes that were collected in Sri Lanka are included as well.

Every deviation from the normal anatomical state is considered a pathological alteration, even if it does not cause pain or a handicap for the animal. There are some findings that have been seen repeatedly in different zoos and one circus. In some institutions, the foot-status have been raised more than once and despite this the pathological degree has not improved noticeably.

Cracks in the horn wall arise in all institutions and nail abscesses occur in all zoos keeping Asian elephants (see figure 23). 50% of all zoos diagnose sole abscesses in the feet of their elephants and even 50% of the Asian elephant holding zoos detect overgrown nails (see figure 4).

In a compilation of all detected pathological findings for all feet of Asian and African elephants examined (this means the visited elephants living in the different institutions and the feet used for histological processing), of 31 Asian elephants only around one-fifth are without any alterations. The percentage is not much higher in the feet of African elephants. The left hind feet show the highest number of sound hooves in both species. However, the African elephant has fewer obvious changes. The cracks in the horn wall of the right front foot affected every second elephant. This was the most common change. Cracks in the sole are not so common as an alteration in the Asian elephant and are only rarely present in the African elephant (see figure 23). It should be noted that the so-called "cracks in the sole" mean in the "common sense" cracks in the pad. Overgrowth of the periople (see figure 23) is quite common, especially in the Asian elephants (13 out of 31), but only the adults are affected.

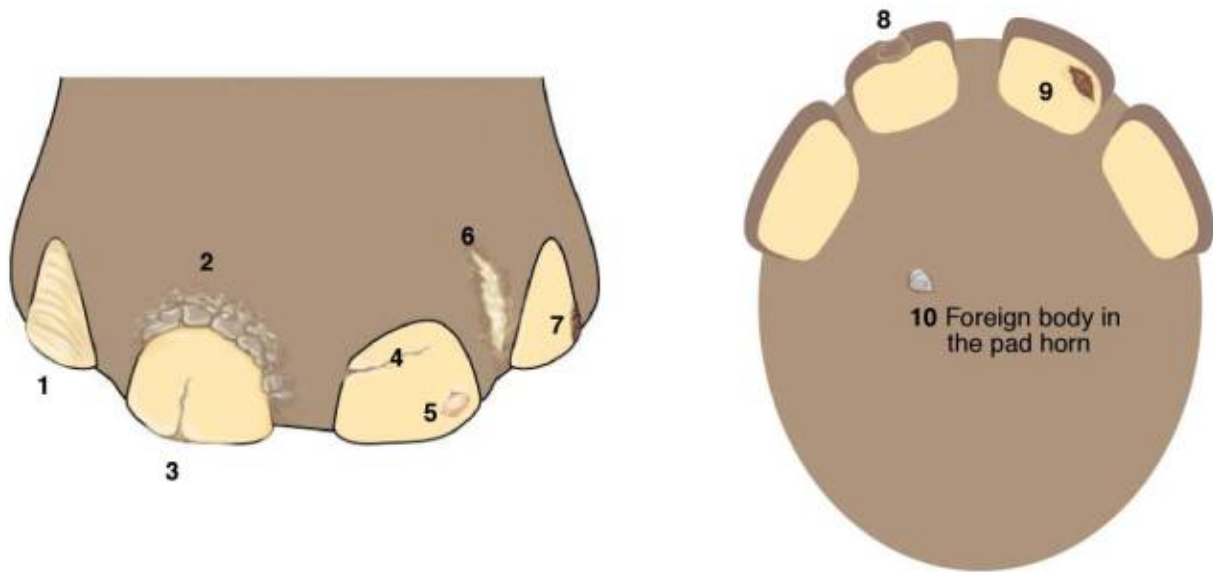
It is impossible to conclude from these data whether the front or the hind feet show more changes. For some alterations, the percentage of occurrence in the front feet is higher than in the rear feet for the Asian elephants, but this is not valid for the African species. Cracks and grooves in the horn wall (see figure 23) are common features in the African hooves, but additionally overgrowth of the periople (see figure 23) and overgrown nails are quite common in Asian elephants.

In the appendix, there is an examination form used for a practical survey of the different foot problems in different elephants.

Table 12: Pathological alterations in comparison to different husbandry systems of the zoos questioned and visited (the table refers to the table 2 and 4, in chapter 5.1.1; see also figures 3 – 5 and 23)

Zoo	No. of elephants ¹	Occurrence of foot problems
1	2.4 African elephants	Nail and sole abscesses, cracks in nails, grooves in the nails, exungulation of nail, fistule
2	2.5 African elephants	Cracks in nails and soles and pad, horizontal fissure, grooves in the nails
3	1.5 Asian elephants	Same problems in the same elephant recur; cracks in nails and soles, overgrown nails, nail abscesses, hyperkeratose in the fold, grooves in the nail
4	0.11 Asian elephants	Nail abscesses, holes in nails and soles, cracks in nails, horizontal fissures, foreign body, grooves in the nails
5	1.7 Asian elephants	Nail abscesses, cracks in nails, overgrowth in general, exungulation; more problems in winter time, when the floor is too dry (cracks) and often the same elephant has the same problem, grooves in nails
6	0.4 Asian elephants	Cracks in nails, nail and sole abscesses, fistule (once), overgrown feet, horizontal fissures with swelling of the corium, grooves in the nails, exungulation, holes in nails and soles, interdigital lesions, double horny layer, panaritium
7	1.5 Asian elephants	Same problems in the different elephants recur; panaritium, holes in nails and soles, cracks in nails and soles, nail (once) and sole abscesses, fistule, overgrown nails, foreign body, exungulation, grooves in the nails, double horny layer
8	2.4 Asian elephants	Same problems in the same elephant recur; cracks in nails, cracks in soles, holes in nails and soles, nail and sole abscesses, inflammation of low lying structure, overgrown nails, nail infections, foot rot
9	1.5 Asian elephants	Same problems in the same elephant recur; holes in nails and soles, nail and sole abscesses, cracks in nails and soles and pads, fistule, osteomyelitis of third and sometimes second phalanx
10	1.10 Asian elephants	Same problems in the same elephant recur; nail abscesses, cracks in nails, diseases of the coronary glands
11	Sri Lanka	Foot problems not common except for the privately owned elephants (because of a lack of knowledge and of walking long distances on hot streets): main problems are cracks, fungal infections around the coronary border and pododermatitis

Notes: ¹ The number before the dot means the male animals and the number behind the dot the female animals



1 Horn rings



2 Overgrowth of periople



3 Crack in the horn wall



4 Horizontal crack in the horn wall



5 Abscess in the horn wall



6 Fissure in the interdigital skin



7 Fistule in the horn wall



8 Crack in the horn wall to the sole



9 Fistule in the sole

Figure 23: Scheme and pictures of most frequent pathological alterations in the elephant's foot

6.3 Microscopic anatomy of the normal structure of an elephant's hoof

Firstly, it should be mentioned that it is not always viable to cut the horn samples from the different hooves at the very same position in the particular segments and therefore the measurements given should be understood as guidelines in the way of the descriptive statistics. Thus, the comparison between the different hooves at the same location can be challenging at times.

Some further opening remarks should be made: In a healthy foot, the stratum basale is separated from the corium by a basement membrane that can be seen in the AB-PAS staining. A positive reaction of the AB-PAS staining in the marrows of the horn tubules is also a normal appearance. The horn layer is generally composed of the horn tubules built by the basal epidermis layer of the papillae and the intertubular horn built by the basal cells in the area between the papillae. The horn tubules, for their part, consist of the cortex built by the basal layer next to the apex of the papillae and of the marrow built by the basal cells of the apex of the papillae.

A small number of vacuoles in the cells of the stratum spinosum or granulosum, as found in the perioplic, coronary, terminal, sole and pad segment can be regarded as normal, but many of them are pathological (see chapter 6.4).

The differences between the two elephant-species (if any) are mentioned where necessary.

6.3.1 The perioplic segment

The periople borders the skin, the coronary and the interdigital space, as well as in the palmar/plantar region the pad segment (see chapter 6.1.3.1, 6.3.2 and 6.3.5).

The perioplic segment has an average width of about 3.2 mm and an average length of 6.3 mm. It creates a fold by turning over and lying opposite itself (see figure 24). The papillae and the horn layer then reach over the fold and adjoin the coronary segment after the fold.

6.3.1.1 Subcutis

The subcutis of the periople borders the other low lying structures in the hoof. The connective tissue presents itself as a tight formation (see figure 24). The fibres-course is crossed. A lot of adipose tissue lies in the subcutis.

6.3.1.2 Corium

The stratum reticulare and papillare have a less denser connective tissue than the subcutis, especially the stratum papillare. The latter builds papillae. Their basis (width approximately 290 μm) lies vertical and oblique on the stratum reticulare. The length of the papillae ranges from 1.5 – 3.6 mm and they end in an apex. Some of them reach into the stratum corneum at their top end. Towards the fold, the papillae bend over at the distal end. The papillae of the African elephant hoof appear longer and narrower than in the Asian elephant hoof.

Some proximal papillae (this means that not all papillae are like this and there are some normal looking papillae even in the proximal part) are bigger than the others found distally and the neighbouring ones and are subdivided by cords belonging to the epidermis and consisting of stratum germinativum. As a consequence, smaller papillae arise from the proximal papillae and build a group formation (see figures 25 and 26) where the central papilla is bigger than the ones surrounding it. The horn tubules continue this group formation

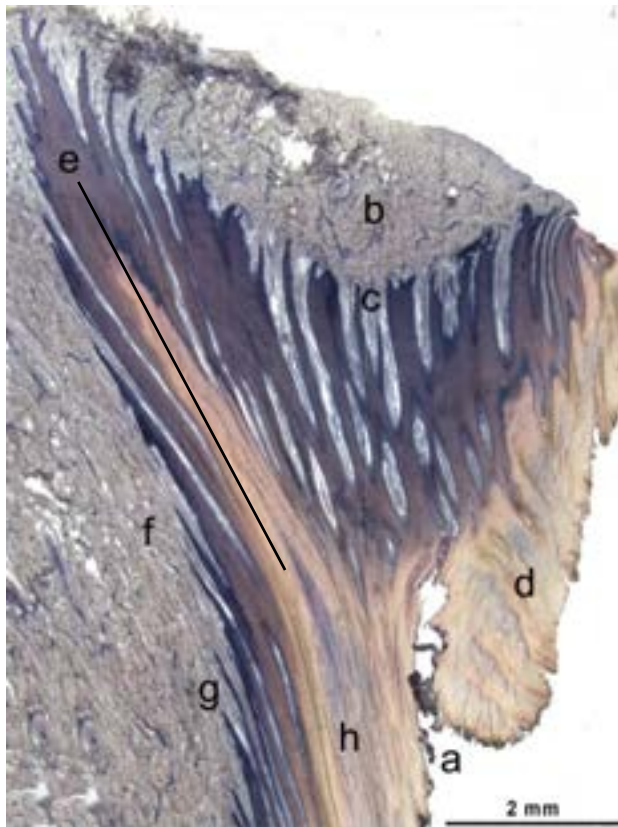


Figure 24: Longitudinal section through the periople and proximal part of the horn wall, Asian elephant, HE staining a-fold, b – d: periopic segment, b-stratum reticulare, c-stratum papillare, d-stratum corneum, e-transition from the periopic to the coronary segment, f – h: coronary segment, f-stratum reticulare, g-stratum papillare, h-stratum corneum

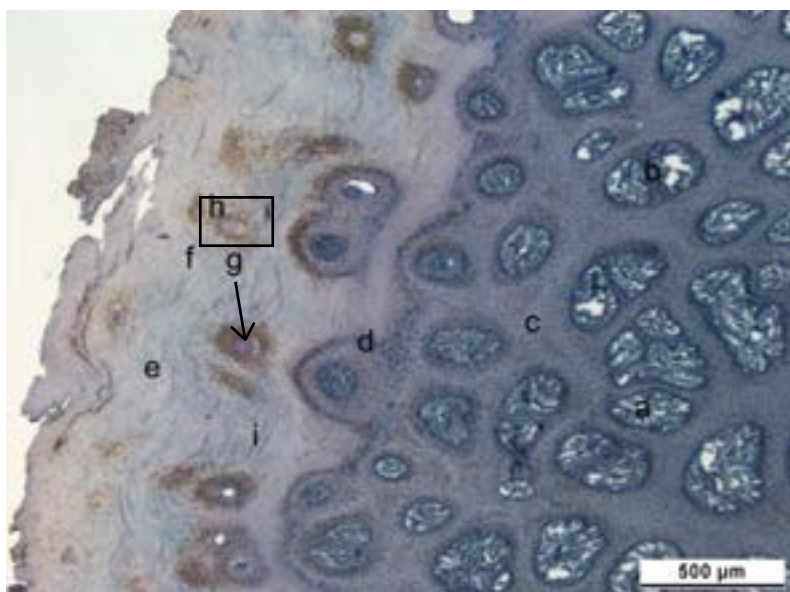


Figure 25: Transverse section through the periopic segment, Asian elephant, AB-PAS staining a-stratum papillare, b-papillae as a result of a papilla subdivided by cords belonging to the epidermis, c-stratum spinosum, d-stratum granulosum, e-intertubular horn, f-horn tubule, g-marrow of the horn tubule, h-cortical cells of the horn tubule, i-micro cracks in the intertubular horn

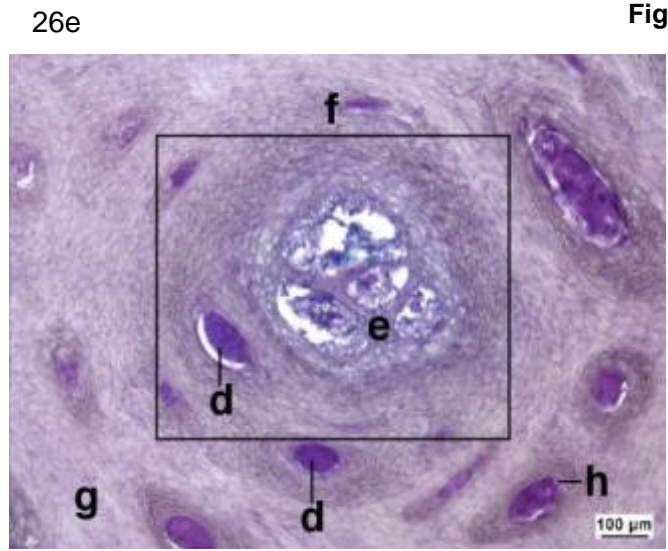
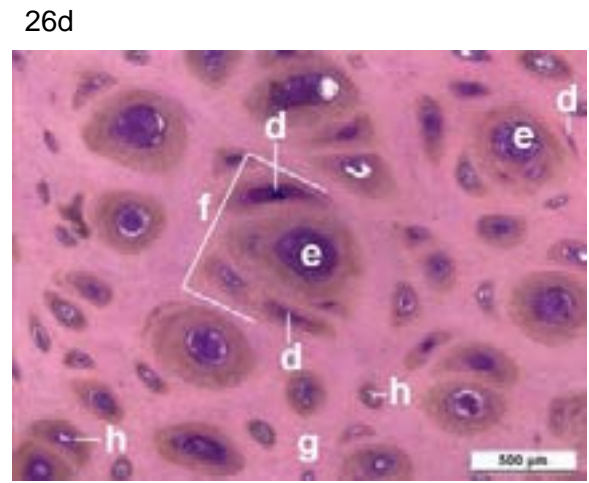
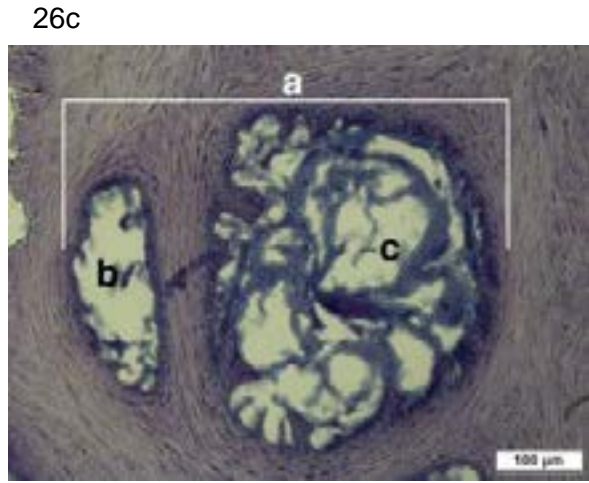
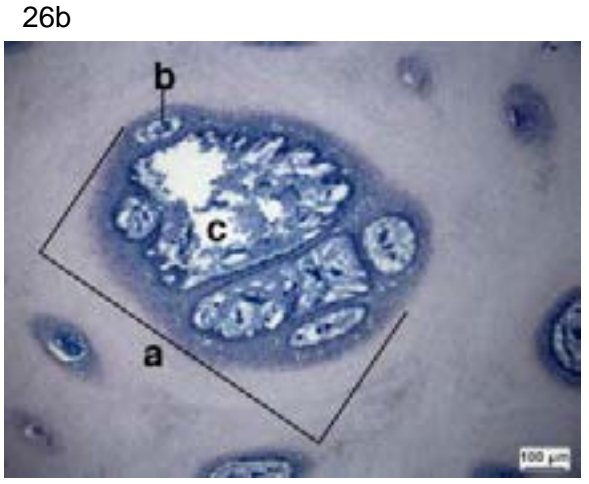
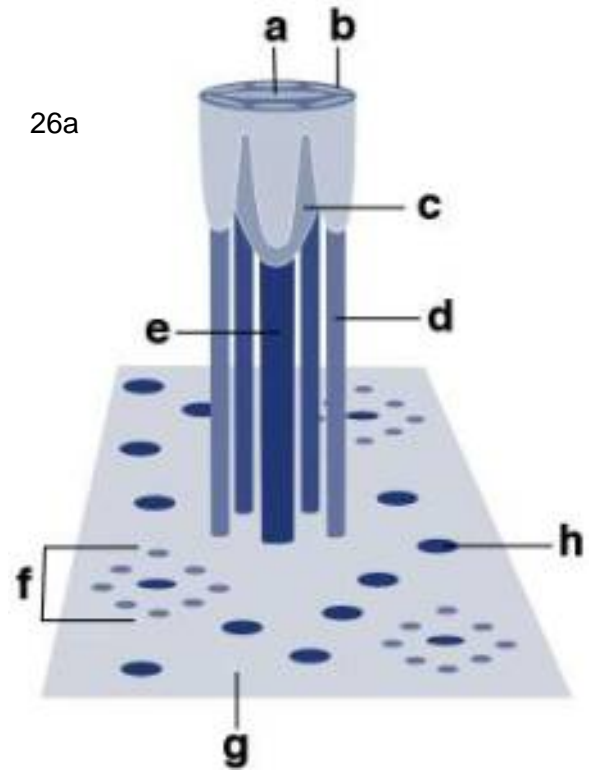


Figure 26: Special formation of some papillae and horn tubules in the periople, coronary, sole and pad segment (b – e: transverse sections of Asian elephants, AB-PAS staining)
a: scheme of the special formation
b: proximal part of the sole segment
c: proximal part of the pad segment
d: distal part of the sole horn
e: distal part of the sole horn
a-proximal papilla subdivided by cords, **b-**small papilla, **c-**big papilla, **d-**horn tubule of the group formation, **e-**big horn tubule in the centre, **f-**group formation, **g-**intertubular horn, **h-**normal horn tubule of the stratum corneum

in the same way. But as with the papillae, there are also normal horn tubules standing not in group formation. The same construction is found in the coronary, sole and pad segment.

6.3.1.3 Epidermis

The epidermis of the periople consists of the stratum germinativum, the stratum granulosum and the stratum corneum. The germinative layer ranges from 1.2 – 2.0 mm. The stratum basale is stained darker than the cells of the stratum spinosum and the basal cells present themselves as flat cells. Above the stratum spinosum, the stratum granulosum occurs as a transition to the stratum corneum. This stratum granulosum (see figure 25) consists of several cell layers between the papillae, but is just layered double or threefold over the apex of the papillae. The last layer follows the stratum corneum (1.0 – 1.9 mm). It is made up of horn tubules and blue-stained intertubular horn (see figure 25). The horn tubules are partly cut lengthwise and partly crosswise in the longitudinal and transverse section. The course is not perpendicular to the nail surface. The width of a tubule is around 140 – 260 μm . The tubules with the largest diameter and the widest marrow appear in the region of the fold. The cortical cells are delimited by the intertubular horn cells, which are stained a bluish colour. The marrow consists of cells taking over from the apex of the papillae and of decaying cortical cell material. In the longitudinal section, the cortical and intertubular horn cells appear lengthwise elliptically, which means that the tubules are flat and oval with the longer diameter parallel to the surface, but the cortical cells are polygonal in transverse section. Therefore they have a spindle-shaped form (type 2, Bolliger and Geyer, 1992). Additionally they are pigmented.

The perioplic horn reaches a length of about 9.0 mm (there was an exception in one African elephant's hoof, where the length was 16.5 mm) and a depth of 0.5 – 1.5 mm along the coronary horn, measured from underneath the fold. In sections from underneath the fold, no tubules were found. It is normal to find some micro transverse fissures in the stratum corneum located in the intertubular horn.

6.3.2 The coronary segment

The coronary segment lies between the perioplic and the wall segment (see chapter 6.1.3.2, 6.3.1 and 6.3.3). The demarcation between the coronary segment and the periople is simple due to the existence of a stratum granulosum, the bluish staining of the intertubular horn and the multitude of cracks in the perioplic horn.

According to the microscopic measurements, the average length of this segment ranges from 1.5 – 3.0 cm. In the proximal part, the average width is 2.4 mm (including the epidermis and the corium) and in the part where the building of the papillae ends a width of 4.4 mm is ordinarily measured.

The transition between the coronary segment and the adjoining segments occurs without any indication (see figure 24).

6.3.2.1 *Subcutis*

The subcutis is only present in the proximal region of the coronary segment, but there it is distinct. Otherwise, its characteristics are similar to the subcutis of the perioplic segment (see chapter 6.3.1.1).

6.3.2.2 *Corium*

As with the perioplic segment, the corium consists of a stratum papillare and a stratum reticulare. The stratum reticulare is packed with a dense connective tissue. The course of the fibres of this tissue are also crossed, like the subcutis. The connective tissue of the stratum papillare is looser. The papillae are orientated distally, but the basis lies perpendicular to the stratum reticulare. Therefore the papillae are inclined, but slope down towards the leaflet region (see figure 27a).

The papillae are broader (in the transverse section generally with an oval to lengthwise elliptical shape, 0.5 – 1.0 mm, but the shape and the size can vary), and also longer (up to 4.0 mm proximally and 1.9 – 2.5 mm distally, i.e. near the leaflets) than those in the periople. The papillae do not reach too far distally and are graduated, which means they are ordered one below the other from proximal to distal. All have approximately the same length. This gives an appearance of regularity (see figure 27a). On the height of the fold, the papillae do not just spread out vertically, but also in a palmar/plantar direction (see figure 27b) and at the height of the beginning of the wall segment, the building part of the papillae ends (see figure 28a). The papillae are surrounded by the stratum basale and spinosum of the epidermis. Their apex reaches into the stratum corneum.

6.3.2.3 *Epidermis*

The differences of the coronary epidermis to the epidermis of the periople are the lack of a stratum granulosum and the smaller size of the stratum spinosum (about a third of the one in the periople, approximately 0.5 mm).

The stratum basale also consists of dark cells. The first layers of the stratum spinosum appear bluish but the remaining ones are rather pink (HE, AB-PAS). The stratum corneum, which is less stained than that in the periople, is composed of horn tubules and of intertubular horn (see figure 27a – f). The horn tubules for their part contain the cortex and the marrow, which is made up of decaying material (PAS positive). The horn tubules are not grouped in an orderly way. Instead the cortical cells can be distinguished more or less from the intertubular horn because the cortex consists of flat horn cells (type 1, Bolliger and Geyer, 1992) and the intertubular horn of polygonal shaped cells, but these cells are not regular nor larger than the cortical cells (see figure 27c). Both cell types contain a lot of pigment. The coronary horn, which reaches an average depth of about 3.6 mm (including some horn leaflets) in the middle of the horn wall (see picture 19d and chapter 7.6), is pushed distally towards the weight bearing border, continuing the incline of the

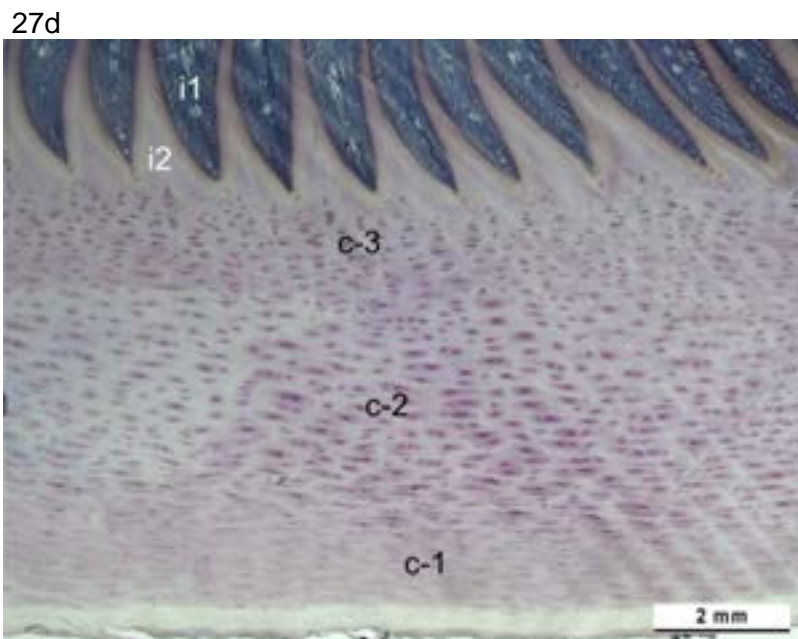
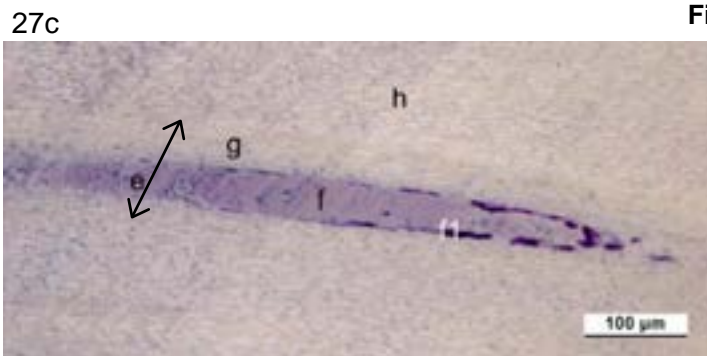
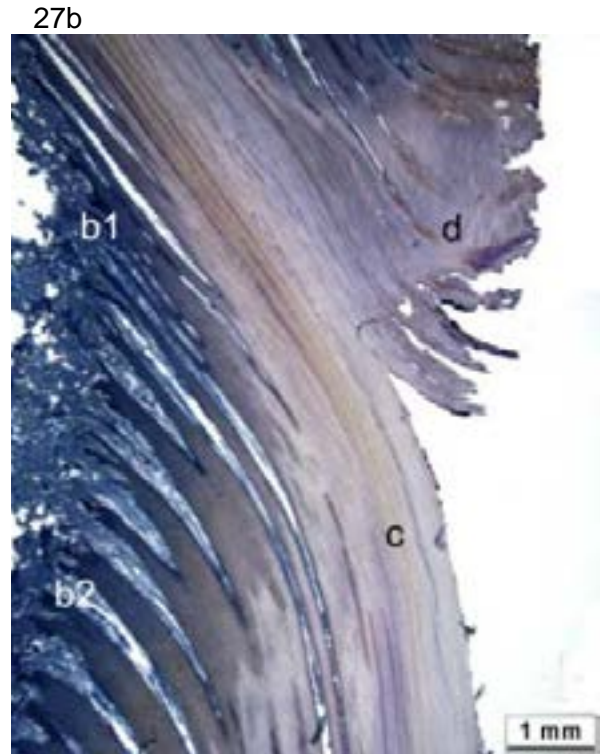
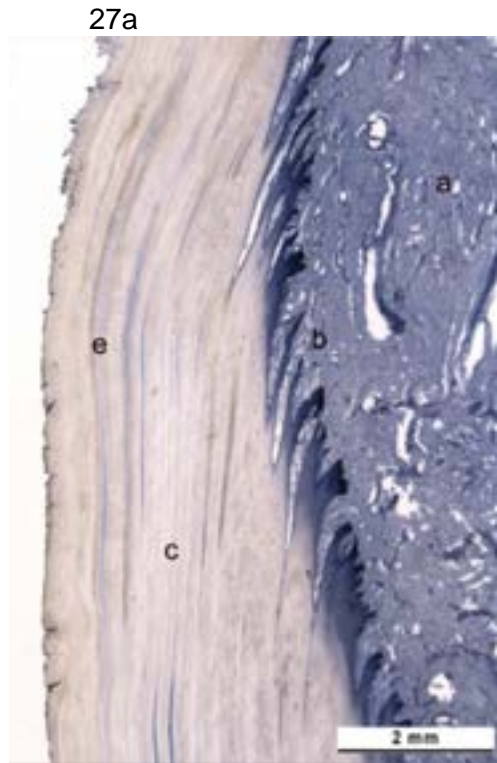
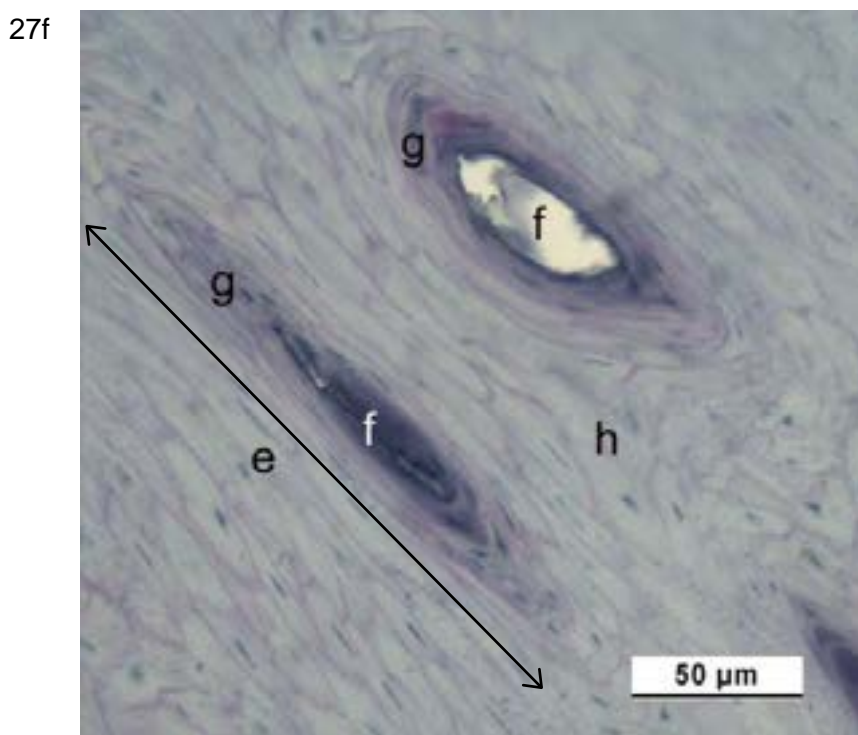


Figure 27: Histological sections of the coronary segment

- a:** longitudinal section of the proximal part of the horn wall, Asian elephant, HE staining
- b:** longitudinal section of the horn wall in the area of the fold for showing the expansion of the papillae to plantar, Asian elephant, AB-PAS staining
- c:** longitudinal section of a coronary horn tubule, African elephant, AB-PAS staining
- d:** transverse section of the horn wall, Asian elephant, AB-PAS staining
- e:** transverse section of a horn tubule of the middle zone, Asian elephant, AB-PAS staining
- f:** transverse section of a horn tubule of the inner zone, Asian elephant, AB-PAS staining

a-stratum reticulare, b- stratum papillare, b1-papillae running distal, b2-papillae running plantar, c: stratum corneum of the coronary segment, c1-outer zone, c2-middle zone, c3-inner zone, d-stratum corneum of the periopic segment, e – g:- horn tubule, e-horn tubule, f-marrow of the horn tubule, f1-PAS positive cortical cells of the horn tubule, g-cortex of the horn tubule, h-intertubular horn, i: leaflets, i1-corial leaflet, i2-horn leaflet



papillae, and is consequently responsible for the shape of the nail. The stratum corneum can be subdivided into three zones with the help of a transverse section of the horn tubules: the outer, the middle and the inner zone (see figure 27d). The distinctive features are the size and the shape of the horn tubules. The middle zone is the broadest of all, but its size varies depending on the height of the horn wall and the width of the coronary segment.

The outer zone is composed of small horn tubules that are flat and narrow. They can hardly be seen sometimes (stained weakly); also the cell borders of the cortical cells are difficult to detect. There is also a low density of horn tubules. This zone is created in the proximal coronary segment. The marrow (25x75 µm) consists mostly of well maintained material. The cortex is rather thin with 3 to 4 layers of cells.

The middle zone is made up of large horn tubules with partly dilated medullary space (see figure 27e), varying greatly in size between individuals. All the African elephants showed

large horn tubules in this zone. The shape of these structures is oval rather than round. In contrast to the outer zone, the density of the horn tubules is rather high. The middle part of the coronary segment produces the horn for this zone. Sometimes, 2 to 3 smaller tubules are arranged around a bigger one giving the impression of a group formation (see figure 26 and chapter 6.3.1.2). The content of the marrow (40x120 µm) is composed largely of disintegrating material that can be partially loosened, but the cortex consists of about 7 – 8 cell-layers. Therefore the proportion of cortex to marrow is small.

The inner zone, which is not much smaller in size than the middle zone, is made up of small, flat horn tubules, as in the outer zone, but larger than there (see figure 27f). They appear round or oval in diameter, are easy to distinguish and their density is lower than in the middle zone. In between, the tubules can also be larger and rather more oval than usual. Their place of creation is located in the distal part of the coronary segment. As in the outer zone, the marrow (25x60 µm) is mostly composed of well maintained material, but this can be partially loosened. 4 to 5 rows of cortical cells form the layer surrounding the marrow.

The coronary horn increases its depth from the proximal to the distal part, but at the fully distal part the width narrows again. Generally, it has to be said that the length of the papillae and the thickness of the coronary horn vary from animal to animal, and even between the front or hind hoof or the proximal or distal parts of the nail itself.

At the nail part of the weight bearing surface, the coronary horn is partly abraded and often only the terminal horn is left. It begins further upwards (around the middle of the nail), but then the outer or the outer and middle zones are worn away.

6.3.3 The wall segment

The wall segment adjoins the coronary and the sole segment (see chapter 6.1.3.3, 6.3.2 and 6.3.4). The wall and the coronary segment are easily differentiated by the varying structures of the segments. However, the changeover to the sole segment can be missed because the terminal papillae continue into the papillae of the sole segment. One indication of the transition to the sole segment is the absence of the row-arrangement of the papillae and horn tubules of the sole as seen in the terminal papillae and terminal horn tubules (see below). In addition, it can be said that the corium of the wall segment sits perpendicular to the horizontal and that of the sole segment is parallel (see figure 14b and 28h).

The wall segment begins at a distance of about 2.5 cm (1.9 – 3.1 cm) from the coronary border for the front hooves and 1.9 cm (1.5 – 2.5 cm) for the hind hooves (see also chapter 6.1.3), when measured microscopically.

The components of the wall segment are composed of corial and epidermal leaflets that convert to the terminal papillae and horn distally. Due to this arrangement of the corium and epidermis, it becomes clear that there is a large surface constituting a strong connection

between these two structures, which helps to fix the hoof epidermis firmly to its underlying tissues.

One big difference to the periople and coronary segments is the absence of a subcutis. Therefore the corium is directly linked to the periosteum of the digital bones. Another special feature is the absence of any pigment in the leaflet part of the wall segment, but this returns in the terminal horn. There, the hoof of the African elephant is much more pigmented than the Asian elephant.

Micro cracks and vacuoles in the terminal horn were found in all examined samples of captive elephants of both species and of the wild African elephants.

6.3.3.1 *Corium*

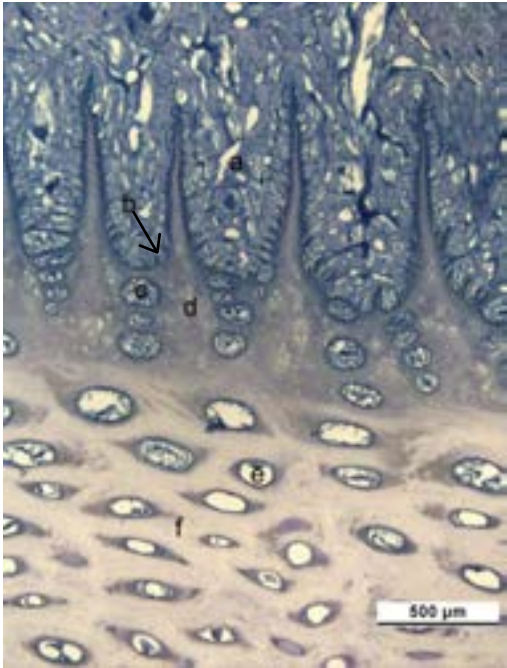
The usual construction of the corium consists of a stratum reticulare and corial leaflets in the stratum papillare. The former is made up of connective tissue, which contains fibres with a crossed course. The corial leaflets are composed of connective tissue even looser than the stratum reticulare, but their fibres have no crossed course.

The corial leaflets seem to join distally the papillae from the coronary segment(see figure 28a) and have a regular formation. Their position is more or less perpendicular to the surface of the horn wall, which can sometimes change to distal along their length. This means that the ridge of the leaflets is sometimes inclined. The corial leaflets are subdivided into primary

Figure 28: Histological sections of the wall segment

- a:** transverse section with the development of the leaflets out of the papillae, Asian elephant, AB-PAS staining
 - b:** transverse section through about the middle of the horn wall, Asian elephant, HE staining
 - c:** transverse section of the secondary corial leaflets, which are subdivided into tertiary leaflets, Asian elephant, HE staining
 - d:** longitudinal section through the middle of the horn wall, Asian elephant, HE staining
 - e:** transverse section through the distal part of the horn wall, Asian elephant, AB-PAS staining
 - f:** transverse section through the distal part of the horn wall for the cap horn, Asian elephant, AB-PAS staining
 - g:** transverse section through the distal part of the horn wall for the special arrangement of the terminal horn, Asian elephant, AB-PAS staining
 - h:** longitudinal section through the distal part of the horn wall and the beginning of the sole, Asian elephant, AB-PAS staining
- a-primary corial leaflet, b-secondary corial leaflet, b1-secondary epidermal leaflet, b2-subdivided secondary epidermal leaflet, c- stratum papillare, d-stratum spinosum of the epidermal leaflets (in figure 28a with vacuoles), e – f: coronary horn, e-horn tubule, f-intertubular horn, g-cap horn, h1-terminal papillae, h2-terminal horn tubules, i-horn leaflet, j-transitional and connecting horn cells, k-tertiary corial leaflets, l-special arrangement of the terminal horn, m-micro cracks in the horn leaflets and in the intertubular horn of the terminal horn, n-weight bearing border, o-white zone, p – q: sole segment, p- stratum papillare of the sole segment, q-horn tubule of the sole segment, r-transition from the white zone to the sole segment

28a



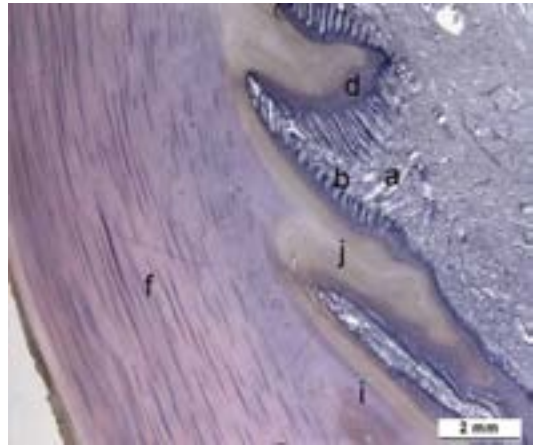
28b



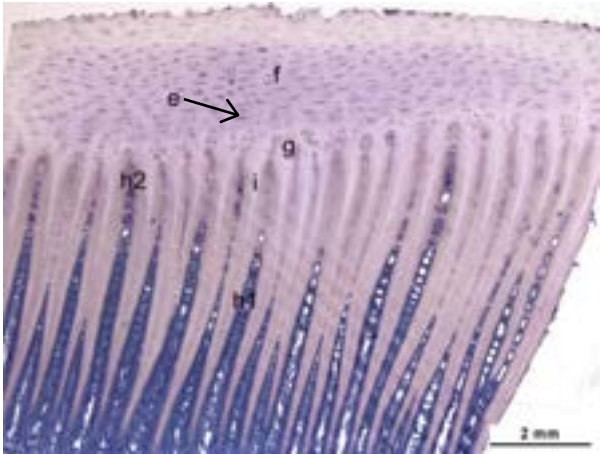
28c



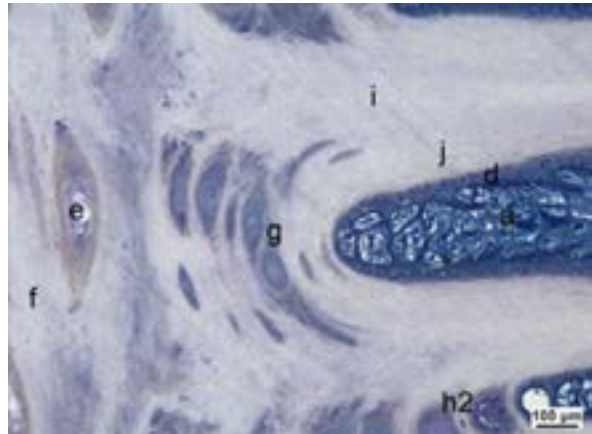
28d



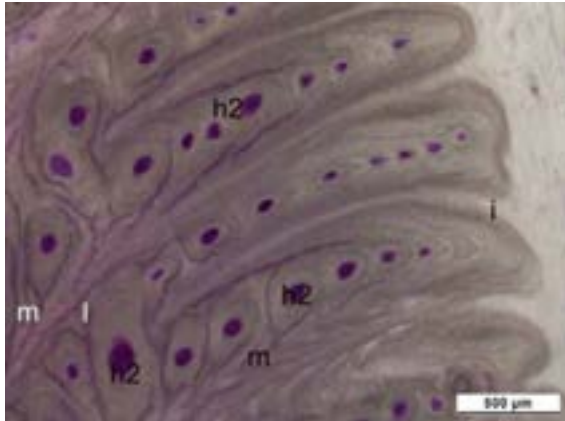
28e



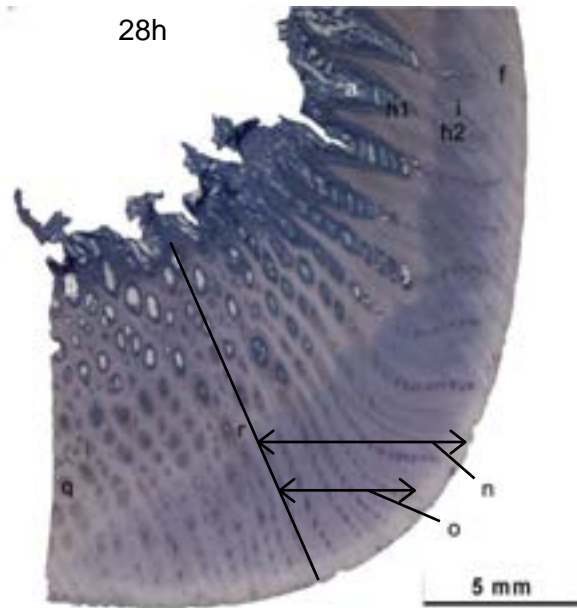
28f



28g



28h



and secondary leaflets, both of which are built proximally (see figure 28a, b and d). The secondary corial leaflets are bent towards apical and are larger at the palmar/plantar part than apical, as well as being larger at the proximal part, compared to the distal part. Their connective tissue is much looser than that of the primary leaflets. Altogether, they are broad and the stratum spinosum in between relatively thin. Some secondary corial leaflets are even subdivided into tertiary corial leaflets (see figure 28c), which makes an enormous surface connection between the corium and the epidermis. The primary and secondary leaflets of the wall segments in the African elephant are larger than those of the hooves of the Asian species. The corial leaflets are completely covered by a germinative layer of the epidermis and both together are named soft leaflets. Between the corial leaflets, horn leaflets can also be seen (see chapter 6.3.3.2 and figure 28b, d).

In the distal part of the wall segment, leaflets transform into papillae (terminal papillae) beginning at the apex of the leaflets and continuing to the base (see figure 28e). These papillae, which are round in shape, are similarly built to the others of the horn wall, just a bit larger and longer. They border the sole segment. The building of this terminal horn “segment” begins at a height of about 4.5 cm underneath the coronary border for the front hoof and about 3.5 cm for the hind hoof.

6.3.3.2 Epidermis

At the end of the coronary segment, the stratum germinativum begins to build horn leaflets of the wall segment instead of the horn tubules of the coronary horn. This happens at a distance of about 1.5 – 3.0 cm from the coronary band, depending on the size and length of the perspective nail and of the coronary segment (see figure 28b). The length of these leaflets, which consist of polygonal cells in the transverse section, increases from the

proximal to the distal part. Proximal, the length is 1.4 mm and distal, 2.9 mm (distal means at the weight bearing surface) for the Asian elephant and 3.5 mm in the proximal part and 10.8 mm in the distal part for the African elephant, respectively. In the area of the weight bearing surface, the size of the horn leaflets decreases again. Sometimes the horn leaflets are inclined towards the base of their structure, especially distally (see figure 28b). But they also mark the beginning of the sole segment at their distal end and their length actually represents the length of the white zone (see below).

In the proximal part of the wall segment, the epidermal leaflets start small, but soon get bigger (4.5 mm in length and 940 μm in width). They decrease distally; the rate at which this takes place differs for each individual elephant. In the lower section of the segment, there is the largest extension, after that the leaflets shorten and terminal horn is formed (see below). The width of the leaflets is relatively broad (approximately 875 μm) proximally, but decreases towards the distal end. The epidermal leaflets are perpendicular to the tangent of the horn wall (the horn wall is half-rounded). At the apex, there is a rim that can be bent and diverges from the perpendicular position distally. In the Asian elephant, the horn leaflets of the wall segment are only thin at their proximal beginning.

The stratum germinativum of the soft leaflets, which have fewer layers of cells at their apex than they do cross-ways, builds transitional and connecting horn cells (Bolliger, 1991; Fürst, 1992), which also keratinize and are connected with the horn leaflets (see figure 28b). In the elephant, this zone is rather broad and becomes broader towards the distal end. It even displaces the horn leaflets at the base of their structure. The stratum basale is composed of cuboidal and high-cuboidal cells. The stratum spiosum reaches a width of about 55 μm .

Another special feature in the wall segment is the presence of the cap horn, starting at a height of approximately 3.0 cm below the coronary border. It is made up of superposed cells and sometimes even tubules that are arranged sickle-shaped between the rim of the epidermal leaflets and the inner zone of the coronary horn and are smaller than those of the terminal horn (see figure 28f). In the transverse sections, these cells are generally lengthwise elliptical, but sometimes also polygonal. The thickness of the cap horn area in the distal wall segment reaches 0.8 cm. It is more distinct in African elephants than in Asian elephants, but it also differs individually.

The terminal papillae emerge from the leaflets in the distal part of the wall segment. They are covered by the epidermal stratum germinativum. This germinative layer forms horn tubules, the terminal horn, that are composed of marrow (150 μm) and cortex (100 μm). The terminal horn begins more proximal in the Asian elephant than in the African. The medullary cells contain decaying material (PAS positive) and in the cortex lengthwise elliptical cells can be seen in the transverse section. Even intertubular horn can be found between the tubules that are linearly arranged (see figures 28g and h), which can change towards the distal position.

There, the terminal horn rows are inclined at the apex. In some hooves, a special arrangement of the terminal horn tubules can be found, which gives the impression that two terminal horn rows in the apical part arise from one terminal horn row of the more palmar/plantar section (see figures 28g and h). The single terminal horn rows can develop differently, which means that terminal papillae can be beside terminal horn tubules in the same depth from the undersurface. Between the terminal horn, the horn leaflets can still be found. They grow together with the cap and the sole horn downwards to the weight bearing surface. The size of the white zone consisting of terminal and cap horn and horn leaflets ranges between 7.5 and 10 mm. The terminal horn is much softer and less steady than the horn leaflets and therefore it is rubbed off quicker than the horn leaflets. This explains the macroscopic comb-like look of the white zone. The proximo-distal length of the horn in the white zone ranges from 1.0 to 1.6 cm.

6.3.4 The sole segment

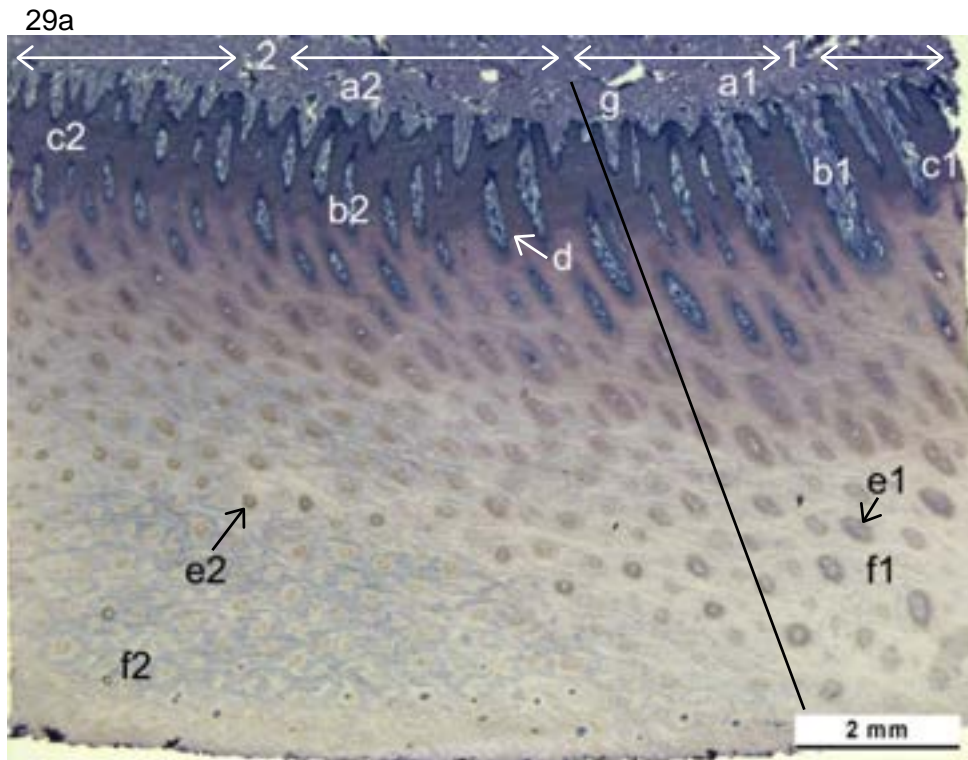
The sole segment is adjoined by the wall segment (exactly from the terminal horn) and the pad segment (see chapter 6.1.3.4 and 6.3.3 and 6.3.5). The major differences to the pad segment are the larger size of the papillae and the horn tubules of the sole segment (see figure 29a). The sole epidermis is not pigmented, contrary to that of the pad. It appears brighter. The transition from the sole to the pad can be recognized by means of the missing stratum granulosum in the sole epidermis and the missing subcutis (see chapter 6.1.2 and 6.1.3.4). The changeover from the white zone to the sole can be identified by the linear arrangement of terminal horn rows and the existence of horn leaflets between these rows, visible in transverse sections.

The size of this segment ranges from around 9.0 cm for the width to 1.5 cm for the length in the front hoof and 3.0 cm in the hind hoof (see chapter 6.1.2 and 6.1.3.4), measured microscopically.

The sole papillae are inclined in the first few papillae where they adjoin the terminal papillae. There, the corium is still slightly perpendicular. Then, where the corium runs horizontally, the horn tubules become perpendicular (see figure 28h).

6.3.4.1 Corium

Contrary to the leaflets of the wall segment, the corium of the sole segment forms only papillae. It is composed of a stratum reticulare and papillare. Here too, both parts have a crossed course of fibres. In the stratum reticulare, the connective tissue is tight, on the other hand in the stratum papillare, the connective tissue is looser. The stratum reticulare is rather broad in this segment.



29b

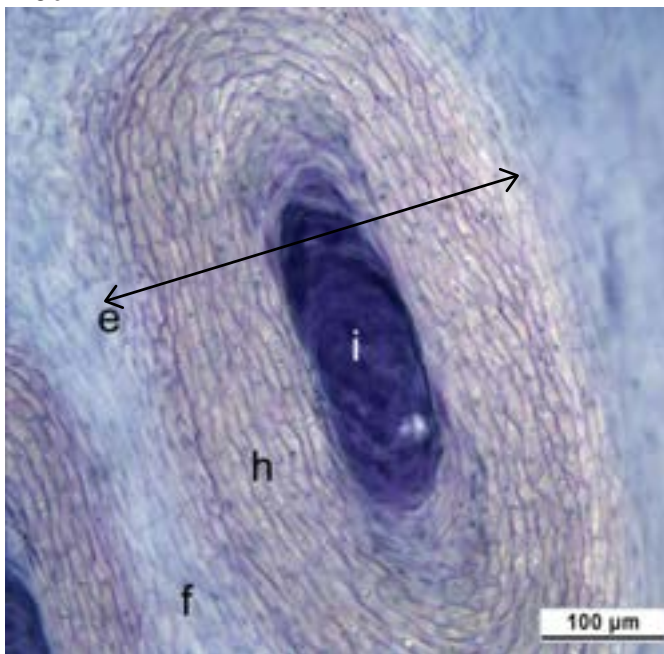


Figure 29: Histological sections of the sole segment
a: longitudinal section through the transition of the sole and pad segment, Asian elephant, AB-PAS staining
b: transverse section of a horn tubule of the sole horn, African elephant, AB-PAS staining
a1/a2-stratum reticulare of the corium, b1/b2-stratum papillare of the corium, c1/c2-stratum spinosum of the epidermis, d-stratum granulosum of the pad segment, e/e1/e2-horn tubules, f/f1/f2-intertubular horn, g-transition from the sole to the pad segment, h-cortex of the horn tubule, i-marrow of the horn tubule
1 with a1, b1, c1, e1, f1: sole segment; 2 with a2, b2, c2, d, e2, f2: pad segment

As in the periopic and coronary segments (see chapter 6.3.1.2 und 6.3.2.2), some papillae are big and subdivided by cords built by the surrounding stratum germinativum (see figure 26). From that, several smaller papillae (100 to 400 μm in diameter) arise and finally the horn tubules arise from the adjacent epidermis. All this gives the impression of a group formation. The papillae themselves are round to oval in shape and vary in size. An important feature is the fact that the corial papillae usually reach close to the undersurface. The papillae reach into the stratum corneum by about 3.7 mm, where they are separated just by a few cells of

the germinative layer from the horn layer. The relation of the papillae to the pure horn part is 1:1. The bases of the papillae measure approximately 0.5 mm. The course of the papillae is more or less perpendicular, but inclined slightly towards apical initially and then towards the palmar/plantar and even slightly sideways.

6.3.4.2 Epidermis

The epidermis consists of a stratum germinativum and a stratum corneum; a stratum granulosum is missing. This is another big distinction to the pad epidermis (see chapter 6.3.5.3 and figure 29a). A cubic shape can be seen in the basal cells. The depth of the stratum spinosum reaches 1.2 mm.

Due to the special arrangement of the big papillae, smaller ones arise out of them and therefore a group formation of horn tubules is created in the stratum corneum, as well. Bigger horn tubules are surrounded by smaller ones (see figure 26). The diameter of such a pattern ranges from 1.1 x 1.3 mm downwards. Single horn tubules measure approximately 90 x 150 μm to 250 x 360 μm in the transverse section. The individual horn tubule consists of a marrow (40 x 60 μm to 90 x 140 μm) and of a cortex (depth in the transverse section: 30 to 130 μm), see figure 29b. The sole of the African elephant's hoof has slightly larger horn tubules in the stratum corneum. While the marrow is made up of decaying material (PAS positive), the cortical cells are spindle-shaped (type 2, Bolliger, 1991). The intertubular horn cells are larger than the cortical ones and their shape changes from polygonal to lengthwise elliptical. Thus, the differentiation between the cortical cells and the intertubular horn cells works well. The very thin sole horn appears conspicuously in many of the captive elephants' hooves (see chapter 6.5).

In summary, it can be said that the sole horn is rather equivalent to the coronary horn, even though the cortical cells are of different types. The sole can be additionally differentiated by the lack of a stratum granulosum in comparison to the pad.

6.3.5 The pad segment

The pad segment flanks the periople, the coronary and the sole segment (see chapter 6.1.3.5 and 6.3.1, 6.3.2 and 6.3.4). With the help of the stratum granulosum and the existence of a large subcutis, which is called a digital cushion, the above mentioned segments can be differentiated. Only the demarcation to the periople is difficult due to their similar features.

The measurements of this segment can be verified in chapter 6.1.2.1 and 6.1.3.5, but it should be emphasized that the pad segment shows the largest extension.

6.3.5.1 *Subcutis*

As mentioned in chapter 6.1.2.1, the pad segment has a big digital cushion made out of connective and fat tissue (see also chapter 6.3.6). The special feature of the subcutis is the fact that it is modified into highly elastic cushions (see chapter 6.1.5).

6.3.5.2 *Corium*

The corium of the pad consists of the stratum reticulare and the stratum papillare (see figure 30a). The latter constructs papillae and is filled with loose connective tissue and longitudinal cut fibres. The stratum reticulare contains tight connective tissue and a crossed course of fibres. It shows an large extension of about a thickness of 5.0 mm.

The appearance of some papillae turns out to be the same as in the periople segment (see chapter 6.3.1.2). They are also larger than elsewhere, very proximal and dissected by cords built by the surrounding stratum germinativum (see figure 26). Out of this, a number of smaller papillae are created and then the horn tubules. A group formation of the papillae and the horn tubules can be seen.

In the Asian elephant, the pad segment papillae are thinner (330 μm on average) than those of the sole segment (see figure 29a), but for the African elephant the pad segment papillae are thicker (530 μm as a mean) than those of the sole segment. The papillae are shorter (3.0 mm in average) in the Asian elephant's pad segment and longer (4.4 mm as a mean) in the African elephant's pad than those in the sole²³. The shape of the papillae is round rather than oval. Therefore, their arrangement is relatively regular.

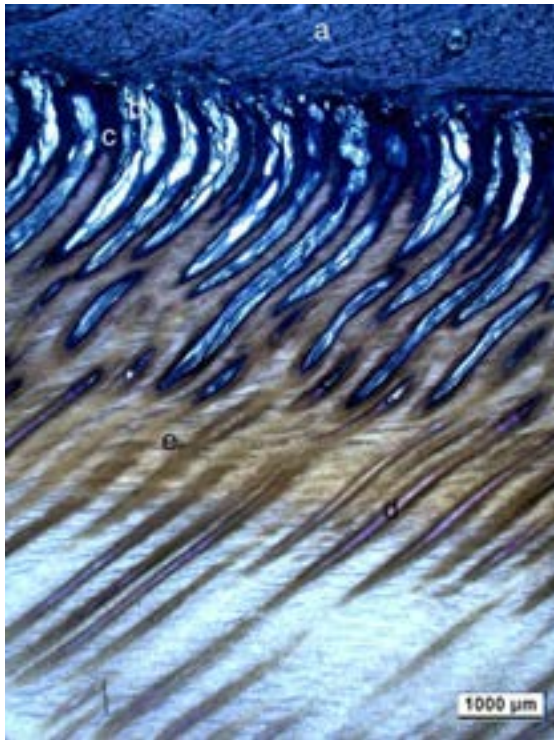
The course of the papillae is towards the side (see figure 30a), which means that they do not grow in a straight line, but they are also inclined towards apical, except at the palmar/plantar part. There the papillae are bent towards palmar/plantar but it has to be said that the course can vary from this general description. The basis of the papillae is nevertheless perpendicular on the stratum reticulare, but it can occur that even the basis is not vertical.

6.3.5.3 *Epidermis*

The epidermis is comprised of a stratum germinativum, granulosum and corneum. The stratum spinosum of the germinative layer is about 1.6 mm for the Asian elephant's pad epidermis and 1.3 mm for the African elephant's pad. In comparison, the stratum granulosum is mostly just a few layers thick and consequently thinner than that in the periople. Especially around the apex of the papillae it is small, but between the papillae there are several layers.

²³ It has to be mentioned that the size of the papillae in both species varies enormously in width and length between individuals (this is also valid for the horn tubules) and the measurement data are difficult to collect due to the course of the papillae (see below). The samples are not always cut at the same position in each hoof.

30a



30b

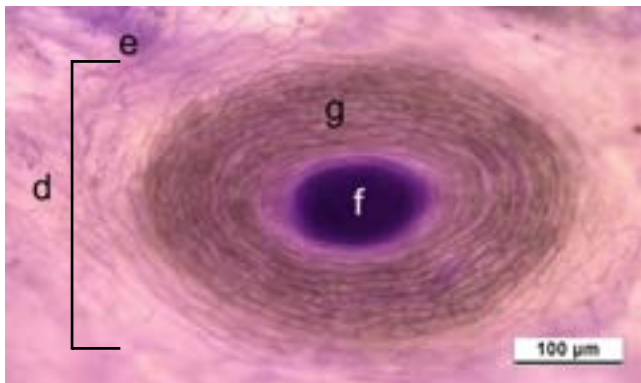


Figure 30: Histological sections of the pad segment
a: longitudinal section through the middle part of the pad, African elephant, AB-PAS staining
b: transverse section of a horn tubule of the pad horn, Asian elephant, AB-PAS staining
a-stratum reticulare, b-stratum papillare, c-stratum spinosum, d-horn tubule, e-intertubular horn, f-marrow of the horn tubule, g-cortex of the horn tubule

As in all segments, except for the leaflets in the wall segment, the pad horn is composed of horn tubules (275.5 μm for the Asian elephant's tubules and 352.0 μm for the African elephant's), whose course is the same as that of the papillae (see chapter 6.3.5.2 and figure 30a), and intertubular horn that is stained bluish as in the periople. The horn tubules are made up of a cortex (73.5 μm for the Asian elephant's cortex and 114.5 μm for the African elephant) and a marrow (120 μm for the Asian elephant's marrow and 113.5 μm for the African elephant), which is mainly composed of decaying material (PAS positive staining that begins even after the production of the marrow), see figure 30b. In numbers, there are not so many horn tubules per mm^2 , but they are all roughly the same size, except for some small ones, and all are fairly round in shape. The cortical cells are formed like those in the horn tubules of the sole (spindle-shaped) and are therefore easy to differentiate from the intertubular horn cells (polygonal and even honeycomb shape). Unexpectedly, the horn is

rather thin. The stratum corneum reaches an average thickness of about 10 mm in captive animals (see chapter 6.5). Another reason for the thin horn layer is the relation of the papillae to the pure horn part. This ranges from 1:1 to 1:2 for the Asian elephant, but about 1:3 for the African elephant.

Cracks reaching close into the papillae (see figure 30a) and a dissolved horn layer next to the undersurface, as well as a few vacuoles in different structures, have to be judged as normal. They were found in all examined hooves, even in the examined wild African elephants. The pad horn of the Asian elephants also gives the impression that it is more decayed distally on the surface than that of the African elephants, especially the intertubular horn.

For the epidermis of the pad segment, it can be said that it resembles the periopic segment in many ways, for example the staining and the assembly of the horn, but also the composition of the segment and the regular occurrence of cracks below the undersurface. Nevertheless, it should be noted that the horn in the pad is much tougher than that in the periople. Also, the three parts of the pad (apical, middle and palmar/plantar) show similar structures and measurements.

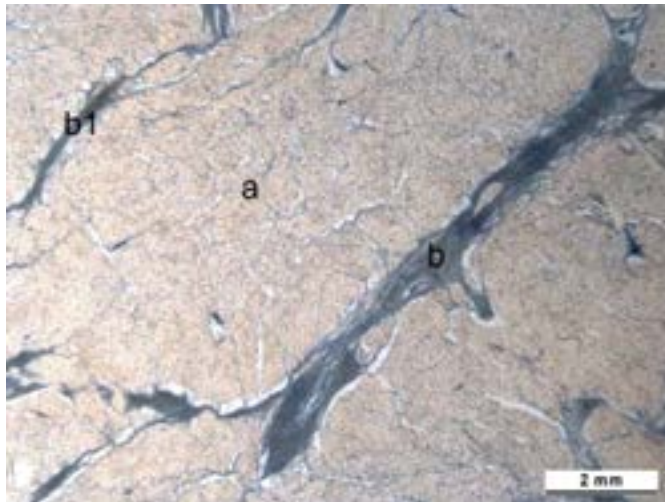
In summary, the corium with the papillae reaches deep into the horn material, but not as deep as in the sole segment (see chapter 6.3.4.2). **The pad horn also has a very thin horn layer in relation to the weight of the elephant and compared to the wild animals examined** (see chapter 6.5). This was considered as one of the most important findings. Micro cracks reach close to the papillae as a matter of course. This is the case even in the wild animals.

6.3.6 The digital cushion

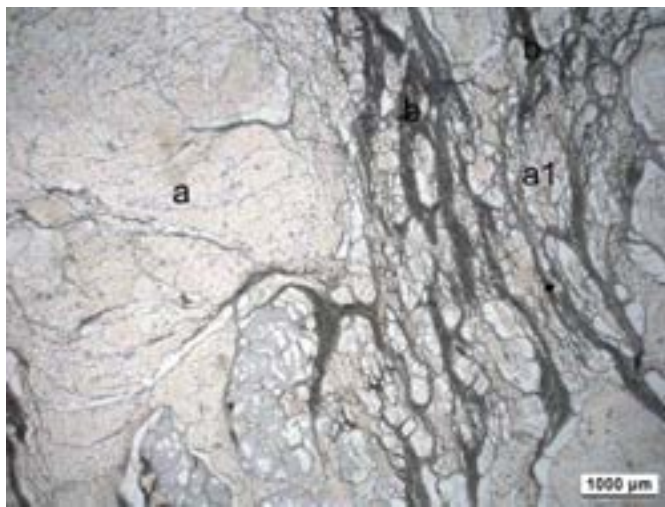
The subcutis consists of loose connective tissue, vessels, nerves and fat tissue. The course of fibres is longitudinal and not crossed.

The description will be subdivided into three parts: apical part, middle part, palmar/plantar part of the cushion, subcutis and "fat tissue" of the pad, respectively.

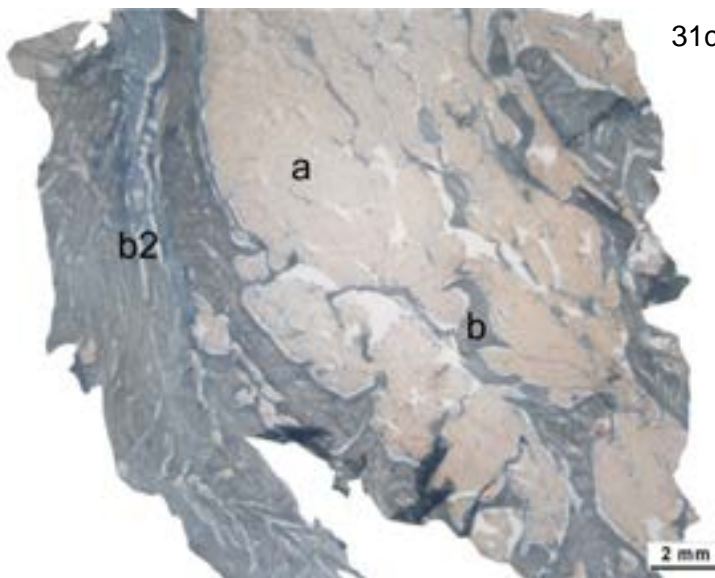
This special structure is well innervated and has quite a number of vessels. In almost all examined feet, mononuclear infiltrates of different dimensions could be found in the connective tissue of all three parts of the digital cushion, which consists of tense and collagen as well as elastic fibres. Among the feet examined, there was one of an 11 month-old Asian elephant. It was apparent that there was more connective tissue in its cushion than in those of the adult elephants. The cushion of the Asian elephant has generally more connective tissue.



31a



31b



31c

Figure 31: Histological sections of the digital cushion
a: longitudinal section of the apical part of the digital cushion, Asian elephant, Oil red staining
b: longitudinal section of the middle part of the digital cushion, Asian elephant, Oil red staining
c: longitudinal section of the palmar/plantar part of the digital cushion, Asian elephant, Oil red staining
 a-fat tissue, a1-fat islands, b-connective tissue, b1-cords of the connective tissue, b2-septi of the connective tissue

6.3.6.1 Apical part of the digital cushion

The connective tissue is arranged around the fat tissue and its quantity is small. The texture is rather loose. There is more fat tissue than connective tissue. The connective tissue acts as a support by means of its separate cords. See figure 31a

6.3.6.2 *Middle part of the digital cushion*

Unlike the apical part, the fat and connective tissues are both plentiful. The fat portion is enclosed like islands by the connective tissue, which works as supportive tissue for the fat tissue. The fibres of the connective tissue are rectified and the tissue itself is tense and tight. See figure 31b

6.3.6.3 *Palmar/plantar part of the digital cushion*

In this part, most of the connective tissue is fairly tight and also rectified, as in the middle part. Here the fat tissue can really be called a fat-cushion because the ample connective tissue encircles the fat tissue, which is rather small in quantity in this “cushion”. So, both tissues are grouped at single places and the septi of the connective tissue are quite voluminous (like a “pillar”). See figure 31c

6.4 Histopathological alterations in the different hoof segments

Although the samples used for the description of the normal histological structure were macroscopically healthy (especially the African elephants showed no macroscopic pathological findings), various changes to the normal state could be found in every segment and there was no hoof among the 24 histologically examined feet without any alterations at any locations. The alterations that occurred in the examined wild African elephants have already been mentioned in chapter 6.2. The other changes occurred mainly in their captive relatives. Because the divergences did not occur in every hoof and also not always in the same degree, the alterations cannot be described as normal for the species (see chapter 7.1). These modifications represent the deviations from the normal histological structure of the horn. Vacuoles, cracks and PAS positive reactions (especially in the horn tubules of the cortical cells and in other locations) were the predominant changes. All these changes and the other mentioned alterations give an appearance of poor horn quality of the respective hooves.

A few vacuoles in the cells of the stratum spinosum can be regarded as normal, but too many of them can be considered pathological. So, when they are mentioned in the following chapters, it means there was an excessive number of them. In the African elephant hoof, they are sporadically distributed in groups rather than regularly spread as in the Asian hoof segments. This leads to a degeneration of the cells, which results in them being coloured differently.

6.4.1 Summary of the histopathological alterations found in all segments

Here all the histopathological changes that occur at least partially in all segments are listed, except for the special arrangement of the leaflets of the wall segment.

1. The stratum spinosum (and sometimes also the stratum granulosum) is sometimes broadened and the stratum reticulare and papillare of the corium can show some mononuclear infiltrates, mostly around the vessels. These alterations are not found in the perioplic and wall segment.
2. Vacuoles can be found in the stratum spinosum, the cortical cells, the marrow and intertubular horn (see figure 32a).
3. Dispersed and disintegrated intertubular horn and sometimes horn tubules near the surface can occur in the stratum corneum of the epidermis, occasionally resulting in large fissures. In the segments of the undersurface, a decay of the structures (see figure 32b) and even holes can be observed. Especially in the pad segment, the undersurface is roughened due to these changes.
4. Micro cracks can appear within the intertubular horn, in part next to the apex of the papillae (see figures 31a and 32c), but also around or in the horn tubules (see figure 32d) as well as beside them.
5. The decay of the first two rows of the cortex is detected in many horn tubules and ends in a positive AB-PAS staining of the cortical cells (see figure 32e). Other PAS positive reacting structures in all segments (except for the periople) include the intertubular horn, and sometimes the stratum spinosum and granulosum.
6. Dilated and decayed marrows within the horn tubules exist in all segments (see figure 32f).
7. Increased numbers of pyknosis of the nuclei in the marrows and in other horn cells, especially near the horn surface, point to an increased rate of disintegration of the cells.
8. If the papillae are opened at the apex and the marrow is dilated and dissolved, blood appears in the papillae and tubules.
9. Where the cells and the superficial layer of the horn layer are loose and fissures reach the surface, bacteria are often found.

6.4.2 Specific alterations to the different segments

Some characteristic alterations of the singular segments are described below and the appearance of all alterations for the respective segment is assessed.

6.4.2.1 The perioplic segment

The subcutis sometimes included some mononuclear infiltrates around the vessels. Vacuoles in the germinative layer of the epidermis appeared, particularly in the region of the fold.

Assessment of the alterations: Most of the perioplic segments of the examined hooves showed a variety of pathological defects. The vacuoles in the stratum spinosum of the epidermis were multiple in more than 50% of all hooves and not present in just about a quarter of the total, but there were altogether more in the Asian than in the African hooves. Micro cracks in the intertubular horn were present in every hoof (see figure 32g), but in only a

few they were not extended. At times, they even reached the area near the apex of the papillae. In 16 out of 24 feet, the cortical cells of the horn tubules were PAS positive. The decaying of the marrow could be observed in almost 50% of the examined perioplic segment.

6.4.2.2 The coronary segment

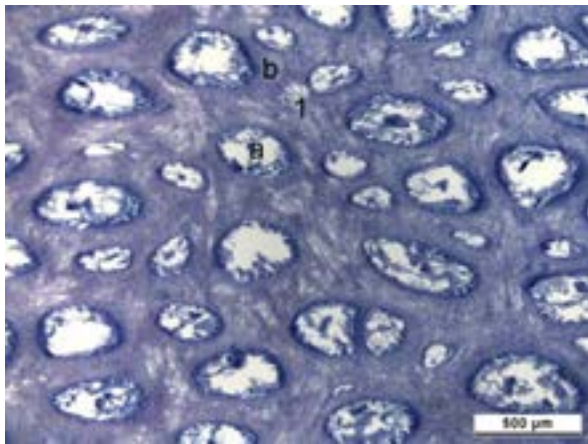
Vacuoles were found in the stratum papillare as well so that the connective tissue seemed to be destroyed. Micro cracks around the cortex of the horn tubules were found (up to 2.0 mm underneath the surface depending on the height in the horn wall and sometimes close to the apex of the papillae). Dilated marrows occurred especially in the outer and middle zone of the coronary horn tubules. Decay of the horn material was found. Another observed alteration was the abrasion of the coronary horn distally that could be so pronounced that the terminal horn of the wall segment was directly next to the horn wall surface (see figure 32h).

Assessment of the alterations: Pathological changes occurred mainly in the distal part of the hoof, though they can even appear in the proximal part of the coronary epidermis. Again, the vacuoles in the stratum spinosum of the epidermis were one of the most common features of all. Just less than a quarter showed no vacuoles. But of the African elephants, only 50% showed multiple vacuoles. Many hooves also showed hollow spaces in other structures of the coronary segment. Micro cracks were not as common as in the perioplic segment. In comparison, dilated marrows and PAS positive reactions of the cortex of the horn tubules appeared regularly. In about 50% of the examined coronary segments, blood in the horn tubules existed: this was mostly in the Asian elephants. Pyknosis of the nuclei in the horn cells and the decaying of the marrow occurred in many of the hooves. In less than a quarter of the samples, holes or fissures were found in the horn material. Another important feature was the abrasion of the coronary horn distally, which appeared in less than 50% of samples.

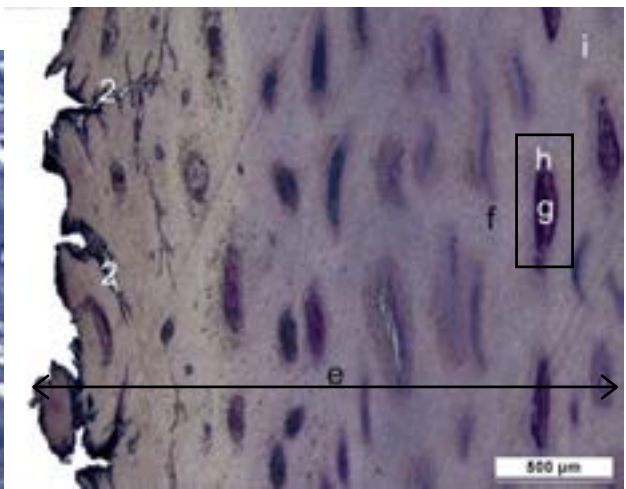
6.4.2.3 The wall segment

Most divergences in this segment appeared in the terminal horn and just a few changes were observed in the leaflets. Vacuoles occurred in the stratum spinosum of the soft leaflets and in the horn leaflets (see figure 32), whereas cracks only occurred in the horn leaflets. Vacuoles were also found in the cap horn cells, where blood and cells dropping out of the cap horn tubules appeared now and then. Even PAS positive reactions were seen in the stratum spinosum of the epidermal leaflets and in the horn leaflets, as well as in the above mentioned cracks. The secondary leaflets were sometimes absent and at times unequal. The cortical cells were equally affected by fissures. Furthermore micro cracks in the white zone could be detected towards the bearing surface.

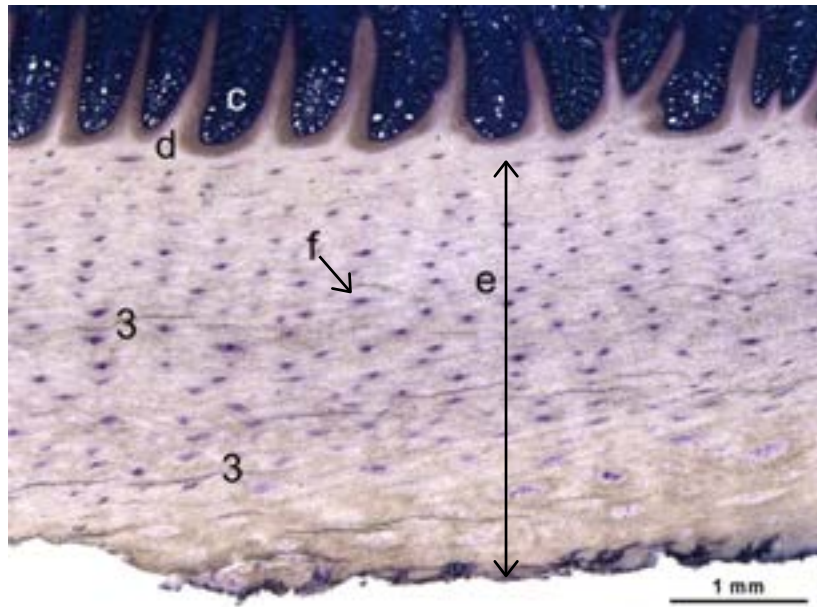
32a



32b



32c



32d

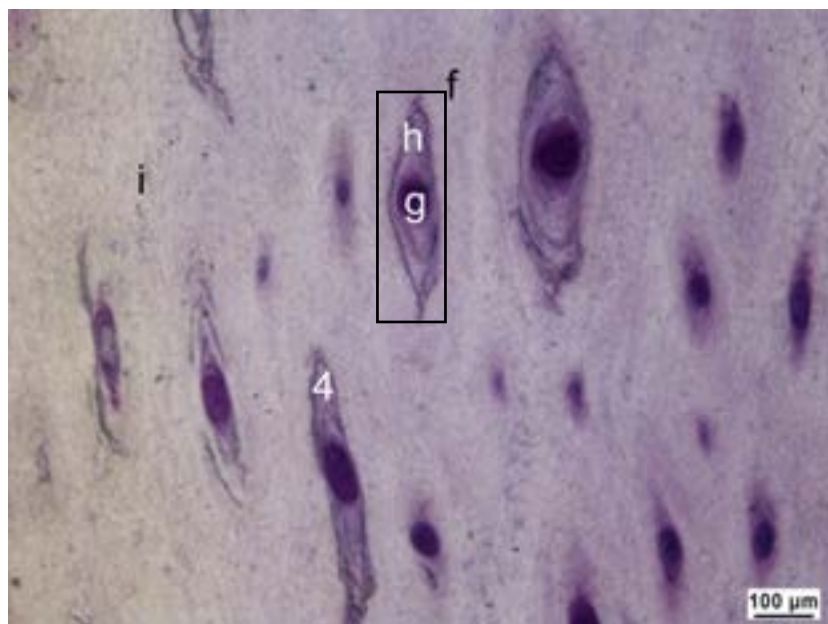
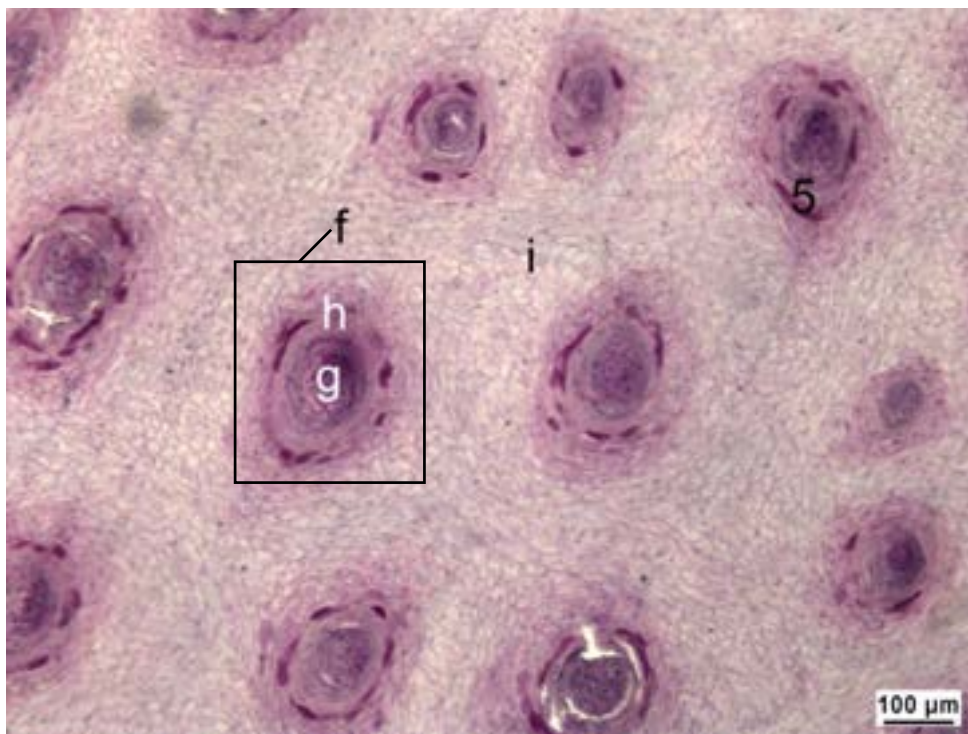


Figure 32: Histological sections of histopathological alterations in the different hoof segments

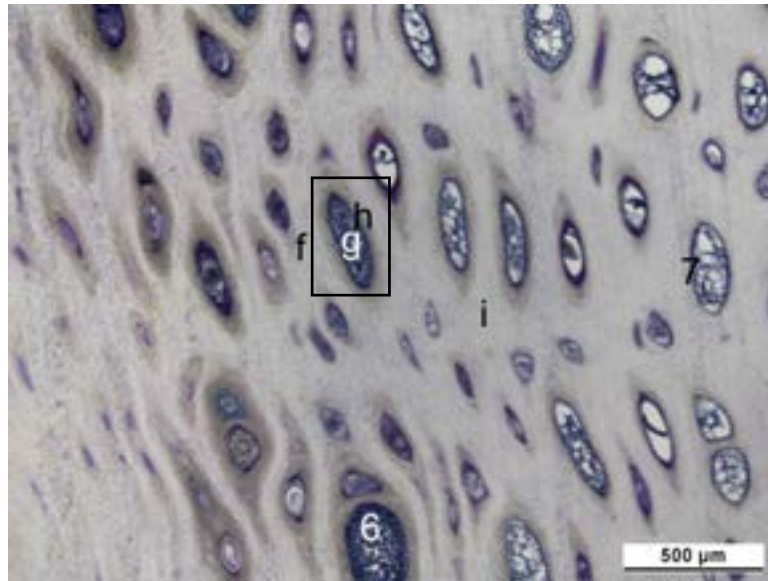
- a:** transverse section through the proximal part of the pad segment with vacuoles in the stratum spinosum (1), Asian elephant, AB-PAS staining
- b:** transverse section through the proximal part of the horn wall with decayed horn material on the surface (2), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 27d)
- c:** transverse section through about the middle part of the horn wall with micro cracks in the intertubular horn of the coronary horn (3), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 27d)
- d:** transverse section of coronary horn tubules with micro cracks around the cortex of the horn tubules (4), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 27e)
- e:** transverse section of pad horn tubules with PAS positive reaction in the cortical cells (5), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 30b)
- f:** transverse section of coronary horn tubules of the middle zone with dilated (6) and decayed (7) marrow of the horn tubule, Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 27d)
- g:** transverse section through the periopic segment of the horn wall with cracks in the intertubular horn (8), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 25)
- h:** longitudinal section through the distal part of the horn wall and the weight bearing border with abrasion of the coronary horn (9) and a-decay of the horn material within the horn layer (10), Asian elephant, HE staining (as a comparison see the normal structure in figure 28h)
- i:** transverse section through the distal part of the wall segment with micro cracks in the horn leaflets (11), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 28e)
- j:** longitudinal section through the proximal part of the pad segment with a broadened basis of the corial papillae (12), Asian elephant, AB-PAS staining (as a comparison see the normal structure in figure 30a)

a-papilla, b-stratum spinosum, c-corial leaflets, d-horn leaflets, e-stratum corneum of the coronary horn, f-horn tubule, g-marrow of the horn tubule, h-cortex of the horn tubule, i-intertubular horn, j-terminal horn, k-terminal papillae, l-terminal horn tubule, m-stratum granulosum, n-stratum reticulare

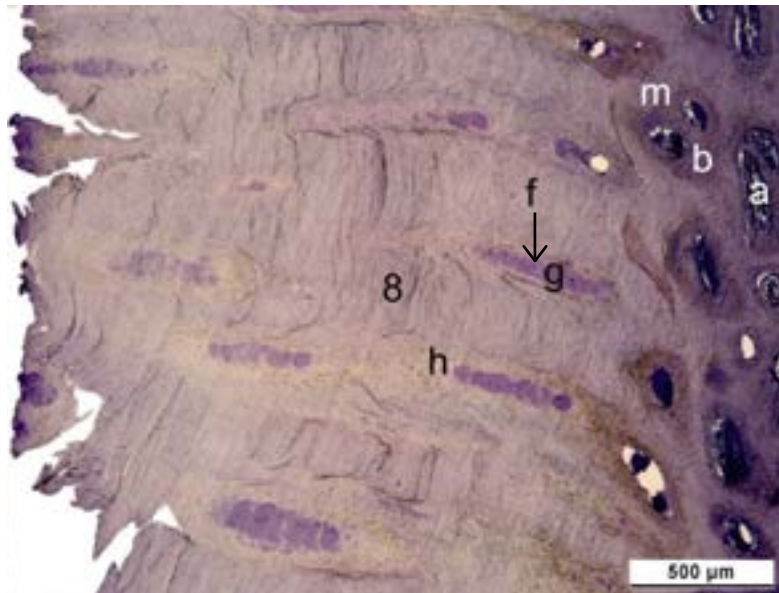
32e



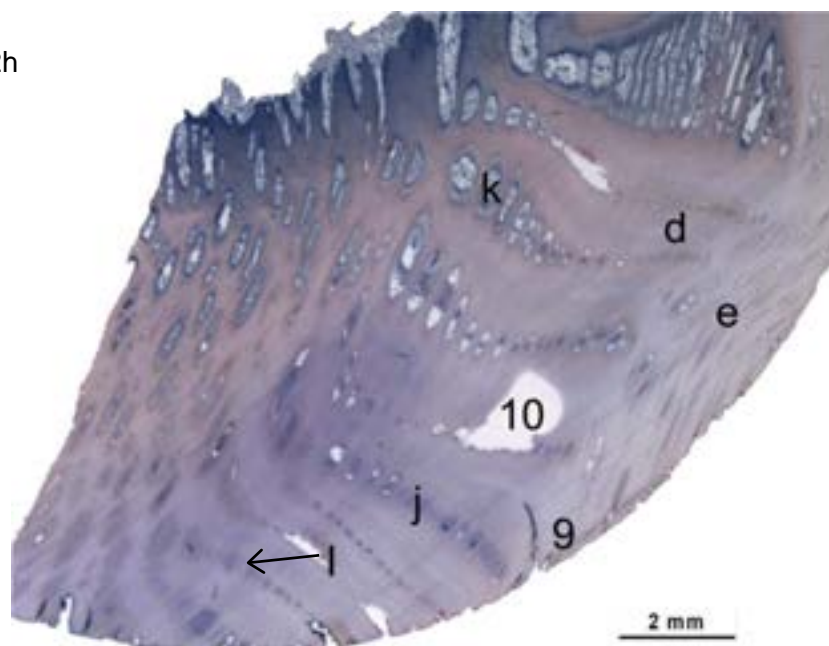
32f

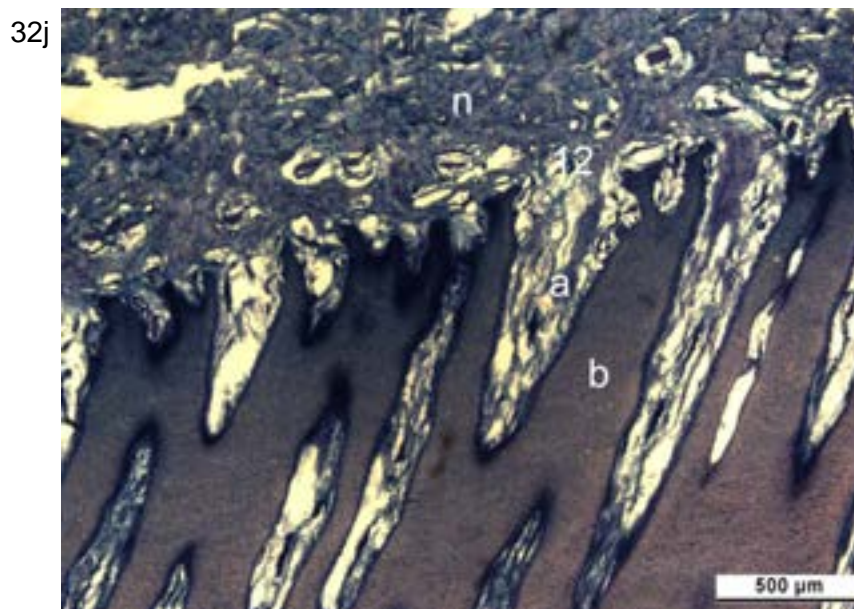
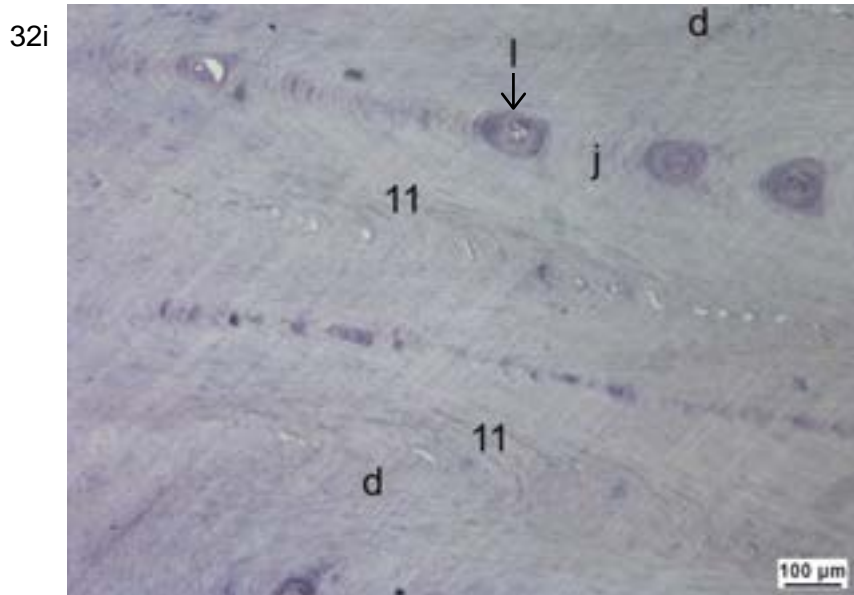


32g



32h





Assessment of the alterations: The most common features especially affected the terminal horn, except for the micro cracks that were present in the horn leaflets. Again, vacuoles in the stratum spinosum of the epidermis were seen in almost every examined hoof, the wild African animals included. Over 50% also showed vacuoles in the cortical cells of the horn tubules. Micro cracks in the intertubular horn of the terminal horn appeared in 14 out of 24 hooves whereof just 3 were from African elephants, including the wild ones. As in the coronary segment, the dilated marrows played a certain role in the terminal horn, at any rate in more than half of the examined wall segments. Especially the PAS positive reaction of the cortical cells of the horn tubules was a conspicuous feature that existed in almost every terminal horn (in only 5 hooves for each structure is it not to be found). A general decaying of the horn material occurred in 15 out of 24 hooves.

6.4.2.4 *The sole segment*

The cortical cells were partly disintegrated.

Assessment of the alterations: In the sole segments of the Asian elephants, vacuoles could be detected in every hoof, except for one, contrary to the African elephants where it was visible in 50% of the examined hooves. In less than 50%, vacuoles in the intertubular horn and in the cortical cells were common, but in just two of the African elephants. Micro cracks in the intertubular horn were as frequent as in the other segments, exactly in 18 out of 24 hooves. Conspicuously, fissures in the stratum spinosum of the germinative layer of the epidermis were detectable in 8 out of 17 Asian elephants' soles. Dilated marrows affected almost every hoof. In about two thirds of the examined sole segments, the cortical cells showed a PAS positive reaction. Equally, the disintegration of the marrows and of the horn material, especially underneath the surface, was quite often seen, especially in the Asian elephants.

6.4.2.5 *The pad segment*

In some hooves, the corial papillae were enlarged in width and length (see figure 32j) and were greatly degenerated. There were also cases where the intertubular horn was filled with cracks proximally, but distally it appeared uncracked. A lot of fissures could be found in a certain segment, although the horn looked healthy below and above this segment. Even in the stratum spinosum of the epidermis, micro cracks were found. The PAS positive reaction was seen in the area of the fissures and underneath the surface where the decay of the horn cells took place.

Assessment of the alterations: The same could be said about the vacuoles of the sole segment as about those in the stratum germinativum of the epidermis. In more than 50% of all the examined pad segments, vacuoles could be found in the intertubular horn and in the cortical cells. There was not one hoof without any cracks in the intertubular horn (the wild African relatives included). Dilated marrows was also found in almost every hoof. The PAS positive reaction was very common in the cortical cells of the horn tubules (22 out of 24). Decaying of the marrows and decaying of the horn material on the surface or bigger cracks near the surface or in the horn material were seen in almost 50% of all examined pad segments.

6.4.2.6 *Young captive elephants*

Even in the feet of young captive elephants (i.e. 1 to 3 years old) and of wild-born animals, the following already mentioned alterations were found: in both cases, PAS positive staining of the cortex, cracks in the intertubular horn next to the area of the papillae and decaying of the marrow of the horn tubules as well as dilated marrows within the horn material and vacuoles in various structures.

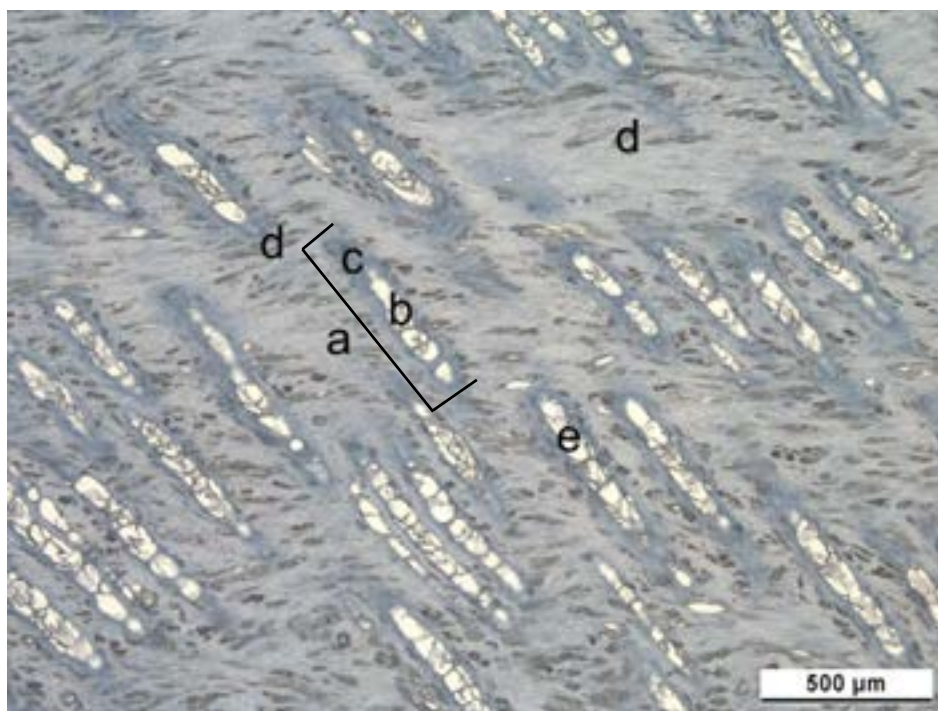


Figure 33: Longitudinal section through the horn part of the pad segment of a dead born neonate, Asian elephant, AB-PAS staining
a-horn tubule, b-marrow of the horn tubule, c-cortex of the horn tubule, d-micro cracks in the intertubular horn, e-decayed marrow of the horn tubule

6.4.2.7 *Still born elephant neonate*

What is noteworthy for these specific samples were the microscopic findings already to be found in such a young elephant: The periople already has micro cracks in the intertubular horn, where only a few horn tubules and no vacuoles were observable. In the coronary horn, vacuoles and dilated marrows of the horn tubules occurred. Decaying marrows were visible in the weight bearing border. The cortical cells and the intertubular horn of the terminal horn had some vacuoles. The horn tubules of the terminal horn were quite big in parts. The coronary horn reached to the weight bearing border. In general, the subcutis was much looser. The papillae of the pad segment were slightly smaller than in an older animal. The pad horn contained quite a number of vacuoles in the stratum spinosum and micro cracks in the intertubular horn (see figure 33). It showed a reticular horn with polygonal cells (eponychium). The marrows of the pad horn tubules were decayed and dilated.

6.4.3 **Histological description of some macroscopic alterations**

A selection of macroscopic alterations were histologically processed (see chapter 5.2.2.5) and are microscopically described. Measurements are given as well, which should assist in visualising the relations between the different structures. They are to be understood as guidelines in the way of descriptive statistics.

The macroscopic state is described in chapter 4.6.2, in the figures 3 and 23 (see also chapter 4.6.2). In the microscopic description of the alterations, only the histological changes from the normal state are mentioned.

Because there were just some small alterations in the histologically examined African elephants' feet (such as some overgrowth of the periople and small fissures in the horn wall), all the alterations presented here refer to Asian feet.

6.4.3.1 Overgrowth of the periople

The subcutis contains some mononuclear infiltrates, as well as the corium. At the same time, there are some blood aggregations in the papillae, which come from small, filled vessels. A lot of vacuoles are seen in the stratum spinosum of the periopic segment, which is rather thinner than usual. Vacuoles, but also micro cracks are part of the stratum corneum. Not just the papillae, but also the horn tubules appear longer than normal and curved as well as curved (see figure 34). The marrows of the horn tubules are dilated in part and give the impression of being inflated and therefore at times decayed. There are also some PAS positive reactions, partly in the cortical cells of the horn tubules, but also in the intertubular horn and even in some parts of the stratum spinosum. Most parts of the epidermis show disintegration.

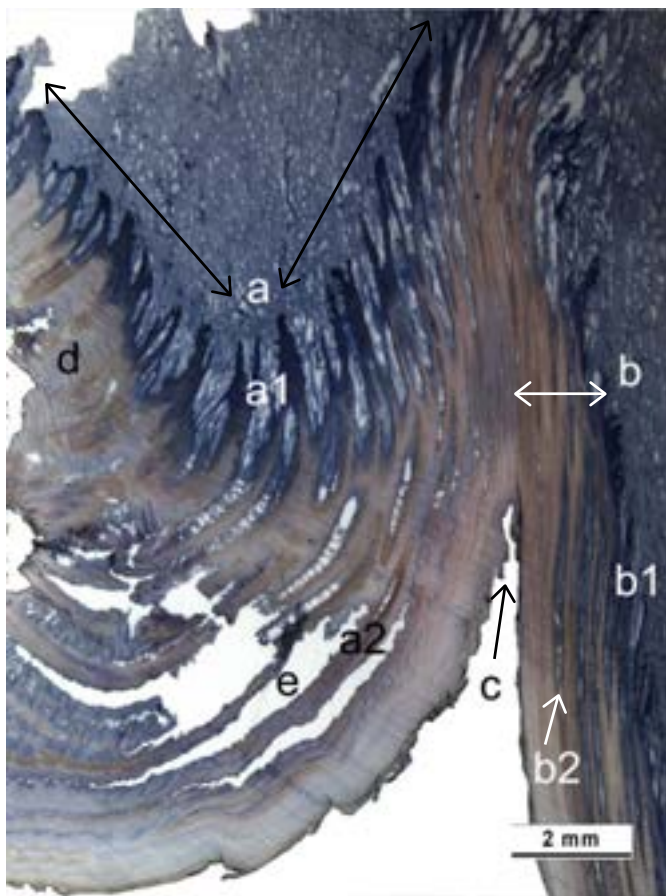
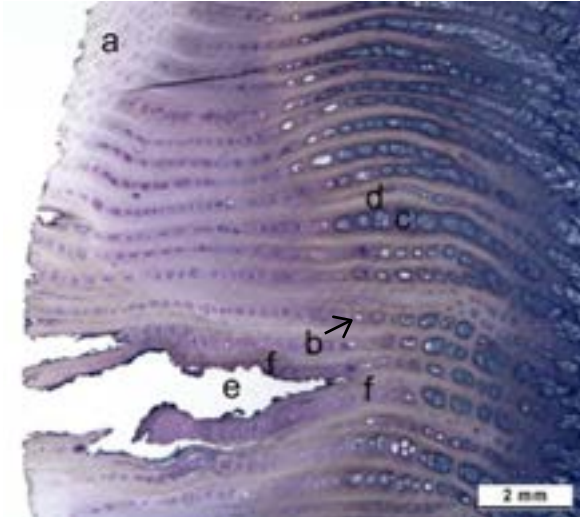


Figure 34: Longitudinal section through the proximal part of the horn wall with an overgrowth of the periople, Asian elephant, HE staining
a-periopic segment, a1-stratum papillare, a2-horn tubule, b-coronary segment, b1-stratum papillare, b2-horn tubule, c-fold, d-micro cracks, e-disintegrated material in the epidermis

35a



35b

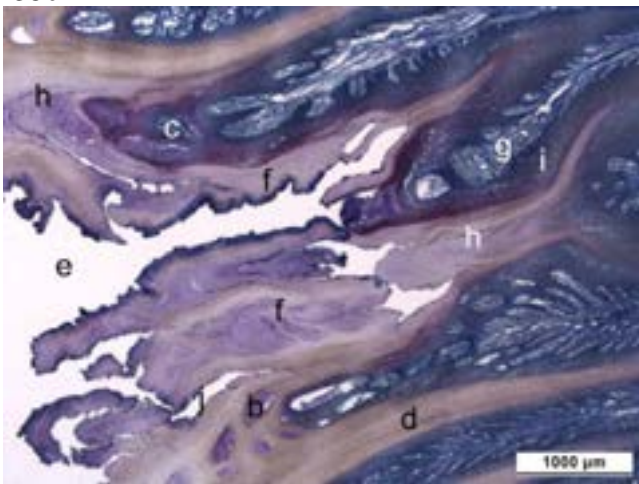


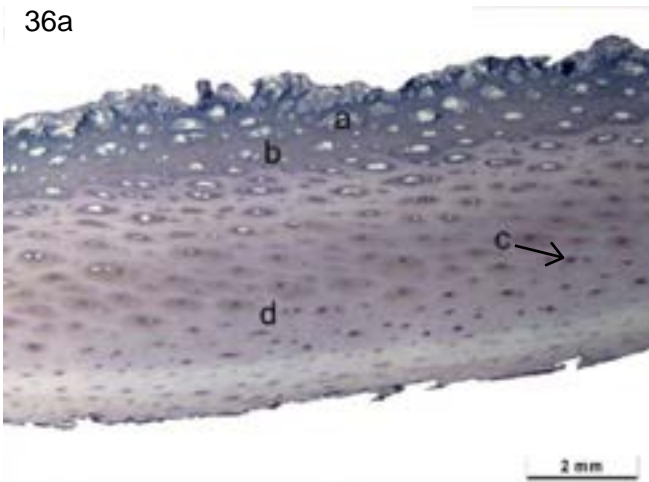
Figure 35: Histological sections of cracks in the horn wall

a: transverse section of the distal part of the horn wall with a crack, Asian elephant, AB-PAS staining

b: transverse section through the middle part of the horn wall with a crack, Asian elephant, AB-PAS staining

a-coronary horn, b-terminal horn tubules with dilated and decayed marrows, c-terminal papillae, d-horn leaflets, e-crack, f granulation horn tissue, g-corial leaflet, h-micro cracks in the intertubular horn, i-vacuoles in the stratum spinosum of the epidermal leaflet, j-decayed horn material on the surface

36a



36b

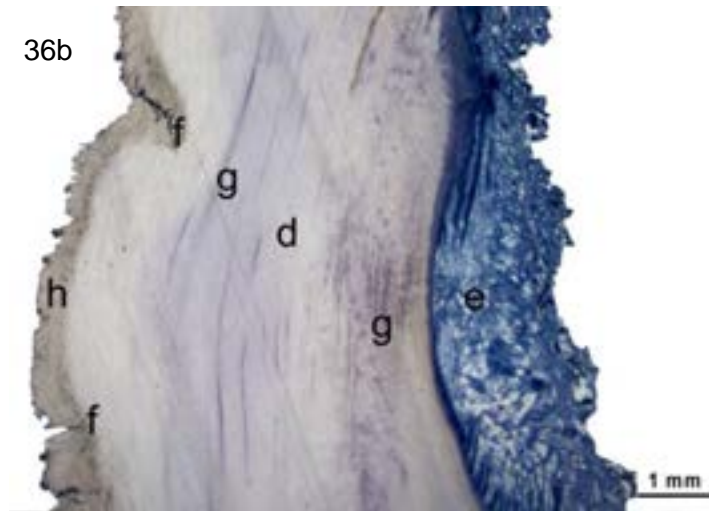


Figure 36: Histological sections of horn rings in the horn wall

a: transverse section through the proximal part of the horn wall with horn rings, Asian elephant, AB-PAS staining

b: longitudinal section through the middle part of the horn wall with horn rings, Asian elephant, AB-PAS staining

a-stratum papillare of the coronary segment, b-stratum spinosum of the coronary epidermis, which appears undulatory, c-horn tubule, d-intertubular horn, e-corial leaflet, f-lacerations of the horn material next to the horn ring, g-curved coronary horn tubules (especially in the inner zone), h-uneven surface of the horn wall

37

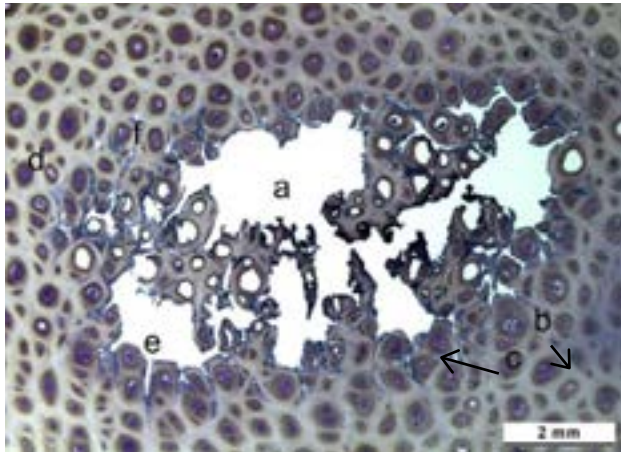


Figure 37: Transverse section through the distal part of the sole horn, Asian elephant, AB-PAS staining
 a-crack in the sole, b-horn tubule, c-dilated and disintegrated marrow of the horn tubule, d-intertubular horn, e-fissure from the crack into the intertubular horn, f-micro cracks in the intertubular horn

38



Figure 38: Transverse section through the distal part of the horn wall with pododermatitis, Asian elephant, HE staining
 a-coronary horn, b-horn tubule, c-dilated marrow, d-intertubular horn, e-horn leaflet, f-stratum germinativum of the terminal horn, g-decayed horn material

6.4.3.2 Cracks in the horn wall

The cracks noted in the horn wall were limited to the horn part, but they reached close to the corium (see figures 35a and b). The epidermis protects the corium by keratinization in the region of the crack (granulation tissue) and so as a general rule, none of the cracks appear without any horn. This also explains the fact that directly around the crack, the keratinization of the individual segments looks further advanced than in the residual surroundings (see below). The crack can extend to 6.5 mm horizontally, in the weight bearing border or the sole area even longer. It was not clear in every sample how far the crack actually reached towards the deep sensitive layers of the tissue.

The subcutis and corium contain a lot of mononuclear infiltrates. Proximal from the crack, the papillae of the coronary segment are larger than normal and the horn tubules are extremely big. The marrows of the coronary horn tubules are dilated. An PAS positive reaction is seen in the stratum spinosum of the coronary segment, the intertubular horn and several layers of the cortical cells of the horn tubules. As with the subcutis and corium, the stratum papillare of the corium of the wall segment contain some mononuclear infiltrates.

The structures which were found at the widest distance from the crack show the normal histological construction including some alterations described in chapters 6.4.1 and 6.4.2. In the region of and next to the crack, there are increased numbers of vacuoles to be found, e.g. in the stratum spinosum of the epidermal leaflets and in the intertubular horn around the crack, and also increased numbers of big horn tubules with dilated and decayed marrows, at times blood in the tubular marrows and disintegrated cells on the surface and in the area of

the crack, increased PAS positive reactions in the germinative layer of the epidermal leaflets, intertubular horn and cortical cells of the coronary horn tubules and increased pycnosis of the nuclei in the horn cells. The epidermal leaflets caudally situated from the crack are also more bent to the side.

The coronary horn shows the normal width far from the crack, but next to the crack, it decreases in width enormously, especially towards the distal part. This means that there is actually no coronary horn around the crack. It is surrounded purely by terminal horn. The epidermal leaflets beside and caudal to the crack are pushed back. So, the keratinization is progressed. But the horn directly around the crack contains a lot of micro cracks. Sometimes, the crack itself is quite near the stratum germinativum (the nearest distance measured was about 100 μm and, the more distal, the more distant the crack is from the germinative layer or corium). So, it can be said that the cleft almost reaches the epidermal leaflets, but there is usually some horn before it reaches the corium.

Further distally, the structures are in total disorder around the crack. Everything is distorted, bent to the side, deformed and driven back (especially the structures of the terminal horn). A lot of vacuoles are present in the horn cells and fissures in the horn leaflets. In the terminal horn close to the crack, the marrows are decayed and vacuoles are visible in the cortical cells, the marrows of the horn tubules and the intertubular horn. Dilated marrows in the horn tubules and micro cracks in the intertubular horn are other features. Next to the crack in the terminal horn, the stratum germinativum, the cortical cells of the horn tubules and the intertubular horn show PAS positive reactions. The terminal horn tubules contain some blood in parts. Bacteria are found on the surface of the cleft.

The crack can reach to the sole horn, where the horn tubules also have dilated and decayed marrows and big tubules. Vacuoles are seen in the cortical cells and the marrows and the intertubular horn is stained red in the AB-PAS-coloration.

Summarized, it must be stressed that a crack involves more than just the crack itself. Surrounding and proximally situated structures are also affected (e.g. coronary papillae and horn tubules, epidermal leaflets and so on, as described above). The surrounding of the crack shows micro cracks. The cleft is like a wedge that disperses the structures. Remarkably, there is no inflammation close to the cleft.

6.4.3.3 *“Horn rings” in the horn wall*

The first obvious alteration is found in the stratum spinosum, where the surface of the stratum spinosum appears undulatory (see figure 36a) and represents the surface of the horn wall. Therefore, in the stratum spinosum, which also shows a lot of vacuoles, a thickening of this layer can be seen where macroscopically the “rings” are. These “rings” are bordered by lacerations of the horn material (up to about 1.2 mm deep) and so the top of the horn wall appears very uneven in a longitudinal section. It also has a lot of decayed horn

cells. Thus, the tori are characterized by detachments and symptoms of decline of the horn material where bacteria and fungi can also be detected. In this area, there are hardly any horn tubules detectable or then just big ones with dilated marrows. Pycnosis of the nuclei increases in the horn cells towards the surface, especially in the cortical cells and also in the marrows of the horn tubules.

The horn tubules, especially in the inner coronary zone, are curved and protrude to the outside (the papillae are also already wormed), see figure 36b. So the tubules build "horn rings". In the transverse section, they are lengthwise elliptical and flat. A few horn tubules of the outer coronary zone have dilated marrows. Vacuoles are found only in the marrows of the coronary horn tubules of the inner zone and an PAS positive reaction is found in the intertubular horn of the same zone, on the surface of the coronary horn, in some cortical cells of the coronary horn tubules and in the apex of the epidermal leaflets. The intertubular horn generally displays decaying epithelium in the coronary segment. The coronary horn contains quite a number of horn tubules and is quite thick because of the "horn rings". The stratum spinosum of the epidermal leaflets, even of the horn leaflets and of the terminal horn, shows a lot of vacuoles, as does the intertubular horn of the terminal horn, which also has some micro cracks. Vacuoles are also present in the cap horn.

Discoloration of the horn cells represent a low quality of horn. The horn has lost absolute resistance through all these alterations.

6.4.3.4 *Crack in the sole*

Proximally, still in the unaffected area of the papillae where the crack lies distally, there are many micro cracks and vacuoles in the intertubular horn. Conspicuously, the first horn tubules are already ordered around the papillae in the same region. The stratum germinativum gets by without many vacuoles. The PAS positive reaction happens exactly there in the intertubular horn where the crack is in a more distal position and in the cortical cells of the horn tubules.

Distally in the sole horn, the crack is very visible (see figure 37). Big horn tubules and dilated marrows, whose content is disintegrated especially around the crack, are seen. Fissures from the crack into the intertubular horn, as well as bacteria and fungi next to the crack are other alterations. On the surface of the crack there are just the horn tubules left and these extend into the crack.

6.4.3.5 *Pododermatitis chronica suppurativa et proliferativa - foot rot*

The histological slides for this pathological alteration were taken from horn samples of an elephant currently showing this kind of pododermatitis.

It was nearly impossible to recognize any structures (see figure 38); in particular the apex of the terminal horn is not well delimited from the coronary horn, which shows dilated marrows. The marrows are decayed or contain vacuoles. The latter are also found in the cortical cells,

which are loosened. The intertubular horn looks undisturbed except where vacuoles are detected. Micro cracks appear around the horn tubules.

Although these are horn samples, the stratum germinativum of the terminal horn is still visible. Vacuoles often occur in the horn tubules and intertubular horn of the terminal horn. Micro cracks and vacuoles are present in the horn leaflets. Pycnosis of the nuclei turn up in the horn tubules of the terminal horn. The terminal horn tubules are more lengthwise elliptical and have some dilated, as well as decayed, marrows. They differ in size.

The AB-PAS-reaction is positive in the cortical cells and the intertubular horn of the coronary segment and just some parts of the stratum spinosum of the terminal horn.

It is also obvious that most of the horn is decayed or torn, especially on the surface. There are some indications of increased disintegration of the marrows and therefore an increased rate of regeneration of the epithelial cells.

6.5 Microscopically measured thickness of the horn layer at defined locations

An arithmetic mean was calculated from the measurements taken from each foot where a measurement at the appropriate location was possible according to the descriptive statistic method. Sometimes, the thickness at a certain location varied so much that these margins of deviation had to be included. Especially in the pad, the wide range of values comes from the fact that each horn has an individual thickness, and even more so from the macroscopically visible furrows. So, they were incorporated, where possible, by measuring the thinnest and thickest parts at a defined location in the pad and they are included in the arithmetic mean. Because there were no differences to be found between front and rear feet in both species, they have not been treated separately.

It has to be taken into consideration that it is difficult to cut at the same location each time for an exact comparison of the thickness of the horn material in every hoof, but the measurements were taken more or less at the same height in each nail.

The height measurements at times involved different portions of the horn (see figure 39). In the first position for assessment of the horn wall, the coronary horn just builds the horn material. In the middle of the horn wall, the coronary horn and the horn leaflets are the major part of the horn thickness but sometimes the cap horn or even the terminal horn are already reached. Coronary horn, horn leaflets and certainly cap and terminal horn are involved in the distal part of the horn wall. The measurements from the white zone to the pad horn are taken perpendicularly. So, the horn leaflets and the terminal horn build the horn material for the height of the white zone. For the sole and the pad the same is valid: horn tubules and intertubular horn of the respective segment fill out the thickness from the undersurface to the living part of the segment.

6.5.1 Asian elephant

All examined feet from Asian elephants were of captive animals. The thickness of the horn wall remains constant from proximal to distal. Just underneath the fold, the wall is relatively thin with 1.7 mm for the adults and 1.5 mm for the young elephants. It is notable that the difference between the young and the adult horn depth is quite small. This changes in the distal direction. In the distal quarter of the horn wall, the horn mass reaches a size of almost 10.0 mm. From the undersurface, the height of the horn thickness in the weight bearing border area reaches about 10.0 mm for the adults and 6.9 mm for the young ones. The sole is about the same size and the pad begins with a lower height in the apical part and grows towards the middle before dwindling in the palmar/plantar part for the adults. Contrary to this, the sole of the young animals is smaller than the weight bearing border and the pad grows from the apical part to the palmar/plantar pad horn (see table 13).

Table 13: Thickness of the horn at different locations in the hooves of the Asian and African elephants examined, ranges according to the descriptive statistic method are given
 1st line = arithmetic mean, in parentheses = minimum – maximum of the measurements, n = numbers of measurements from different feet of different elephants, inclusive of the margin of deviation

		Asian elephant		African elephant		
		adult	young	adult-captive	adult-wild	young-wild
Horn wall	in the height of the fold	1.7 mm (0.7 - 3.8) n = 10	1.5 mm (0.9 - 3.8) n = 11	2.0 mm (1.7 - 3.0) n = 3	4.3 mm (3.2 - 5.3) n = 2	1.4 mm n = 2
	in the middle	5.8 mm (2.8 - 8.0) n = 8	3.6 mm (2.3 - 5.1) n = 8	9.3 mm (7.0 - 13.9) n = 3	7.5 mm (6.5 - 8.4) n = 2	1.8 mm n = 2
	distal quarter	9.2 mm (4.5 - 12.5) n = 8	5.4 mm (4.4 - 7.9) n = 8	9.0 mm (4.5 - 17.0) n = 3	6.8 mm (5.7 - 7.9) n = 2	2.6 mm n = 2
Weight bearing border	height of the white zone	10.0 mm (5.5 - 23.4) n = 7	6.9 mm (4.6 - 9.1) n = 12	13.0 mm (6.9 - 22.6) n = 3	14.5 mm (13.0 - 15.9) n = 2	5.0 mm n = 2
Sole	height of the sole horn	9.6 mm (7.5 - 13.0) n = 7	5.2 mm (4.1 - 6.9) n = 9	10.7 mm (10.1 - 11.3) n = 2	13.8 mm (13.0 - 14.5) n = 2	5.8 mm n = 2
Pad	height of the apical pad horn	8.9 mm (2.9 - 13.0) n = 10	4.8 mm (1.6 - 8.0) n = 13	7.4 mm (5.2 - 10.1) n = 3	14.0 mm (13.6 - 16.5) n = 2	4.8 mm (3.8 - 5.9) n = 2
	height of the middle pad horn	11.7 mm (5.7 - 17.4) n = 9	5.1 mm (2.9 - 8.3) n = 12	7.4 mm (2.9 - 10.9) n = 3	15.6 mm (8.3 - 26.1) n = 2	6.5 mm n = 2
	height of the palmar/plantar pad horn	8.8 mm (1.4 - 15.9) n = 8	6.0 mm (3.8 - 7.8) n = 11	11.3 mm (6.5 - 19.1) n = 3	19.2 mm (15.5 - 22.9) n = 2	2.9 mm n = 2

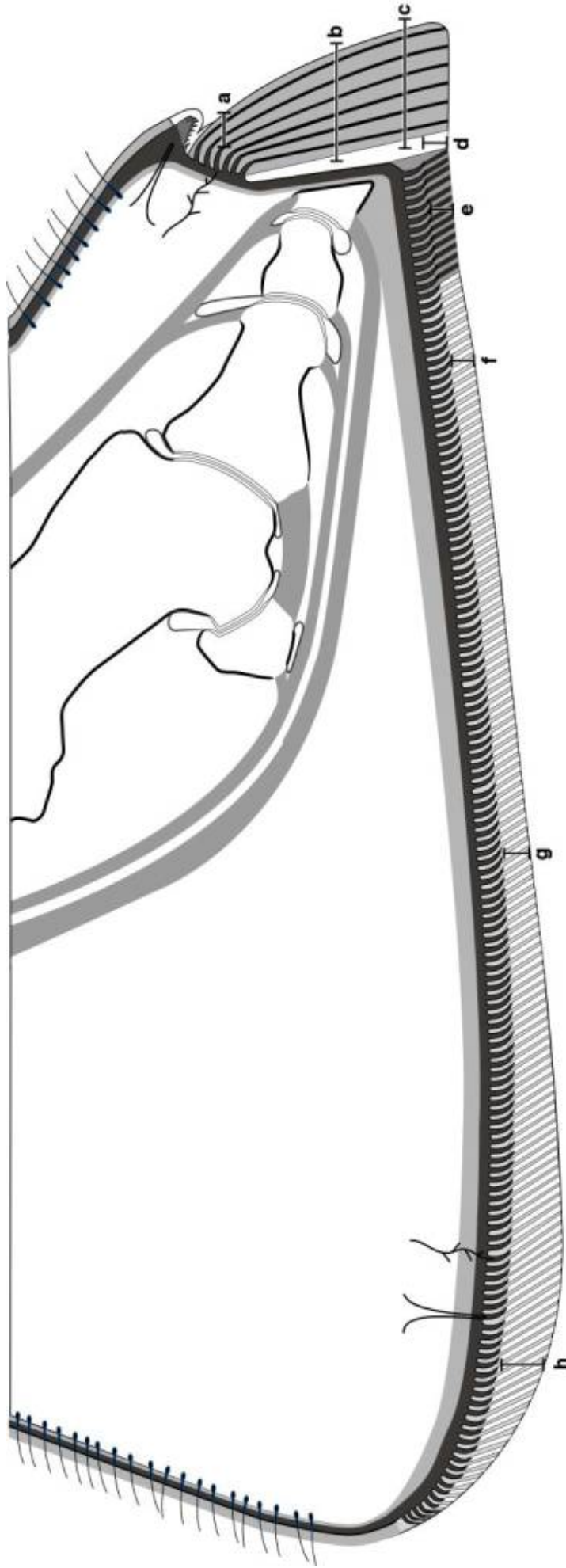


Figure 39: Designation of the horn portions, which are involved for the measuring of the horn thickness in the hooves of Asian and African elephants
 a – c: horn wall, a-in the height of the fold, b-in the middle, c-distal quarter, d-height of the white zone, e-height of the sole horn, f – h: pad horn, f-
 height of the apical pad horn, g-height of the middle pad horn, h-height of the palmar/plantar pad horn

6.5.2 African elephant

Although there is nothing known about the age nor the sex of some of the African elephants used for the histological processing, it can be assumed that all of them were adults (see chapter 5.2.1), except for two animals, where it is known that they are juveniles from the results collected and the macroscopic appearance of their feet. 3 are assumed to be captive elephants and 4 are definitely from the wild, including the two juvenile animals. Therefore, they are listed separately to give a comparison between captive and wild animals, as well as young and adult elephants. The young wild samples are mixed specimens from two different animals and therefore they are noted as $n = 2$.

In general, the thickness of the horn layer of the captive adult African elephant is about the same as that of the captive adult Asian elephant, with minor deviations. But the width of the horn layer of the wild African elephants is bigger than that of the captive Asian elephants. Remarkably, the horn wall width of the captive adult African elephants is at least equal to or thicker than that of the wild animals. The horn wall of the wild elephants is much more constant. But the weight bearing surface of the wild African species is invariably thicker than that of the captive animals, in the pad even almost double in size. This is even more astonishing because the feet of the wild animals are the dried specimens from the "Naturkundemuseum" Berlin. Having lost water, they have probably shrunk and could have been even thicker. The comparison of the young captive Asian and wild African elephants results in about the same thickness of the horn layer, with some exceptions.

In the histological samples of the wild African elephants, there was no abrasion of the coronary horn distally, as in many of the captive animals.

6.6 Biotin concentration in the blood plasma of elephants both with and without biotin supplementation

The measurements of the biotin plasma concentration in all elephants examined were divided into two groups: supplemented and non-supplemented animals (see table 14). According to the Mann-Whitney-Test, the comparison between the elephants supplemented and non-supplemented is significant ($p = 0.0491$). The mean for the treated animals is 1589 ng biotin/l plasma and for the untreated elephants 390 ng biotin/l plasma. According to the analysis of variance according to ANOVA, the comparison is close, but not significant ($p = 0.0620$). During the statistical analysis, it turned out that the border between supplementation and non-supplementation lies around 500 ng biotin/l plasma.

Table 14: Biotin determination in the blood plasma samples of 6 different Asian elephants supplemented and non-supplemented from two different zoos (ng biotin/l plasma)

Zoo	Elephant	Biotin (ng/l)	
A	1	supplemented for 8 months	410
		supplemented for 11 months	7800
		supplemented for 13 months	300
	2	supplemented for 8 months	640
		supplemented for 11 months	1760
		supplemented for 13 months	450
B ¹	3	supplemented for years	580
		supplemented for years	779
	4	supplemented for years	589

Zoo	Elephant	Biotin (ng/l)	
A	1	not supplemented	360
		not supplemented	310
		not supplemented	480
	2	not supplemented	350
		not supplemented	280
		not supplemented	660
B ²	3	time before supplementation	289

Notes: ¹ Elephant 4 of Zoo B was not taken into the statistical analysis because there is no sample of non-supplementation; ² This elephant is the same as the supplemented elephant 3, but here the measurements are from the time before biotin supplementation.

As table 14 shows, the initial biotin concentration in the blood of non-supplemented animals is generally below 500 ng/l. In supplemented animals, the concentration measured depends on the time elapsed between biotin feeding and blood collection. 5 to 10 minutes after biotin feeding (when zoo B always takes the blood), the concentration had already reached more than 500 ng/l. The highest measured value achieved 7800 ng/l (zoo A), but the time after the biotin feeding was not given.

3 samples of supplemented animals from zoo A did not reach 500 ng/l. But, as mentioned in chapter 5.3, the supplementation and time of collection were not communicated very clearly by zoo A. In the unsupplemented plasma collections, all samples are below 500 ng/l, except for one sample of 660 ng/l.

7 Discussion

The present study describes the elephant's hoof epidermis, corium and subcutis. This should help for the understanding of the structures and the location of horn production as well as for assessing good and healthy horn quality. Knowledge of the normal hoof structure and the rate of horn production are also important for understanding pathological conditions and providing a prognosis for them. Therefore, the normal macroscopic and microscopic horn structures of the hooves of elephants for which data measurements were obtained are discussed and possible differences between the Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephant are explained. The elephant subspecies are not differentiated. There is much speculation about the subject that the African species seems to be less susceptible to foot problems (Ramsay and Henry, 2001). Comparisons between captive animals and their wild relatives of both Asian and African species are given, together with an analysis of the differences. The macroscopic and histological structures are explained by comparison with well studied hooves of e.g. cattle, horses and even rhinoceroses to provide a basis for assessing histopathological changes and especially horn quality. Additionally, the occurrence of common macroscopically and microscopically pathological findings is examined. The reasons for foot problems (except for traumatic incidents) remain largely unknown and speculative. However, as a consequence of the findings, some considerations are given to the causes and occurrences of foot diseases.

The importance of the elephant's foot and its diseases was finally raised by the book "The Elephant's Foot" (Csuti et al., 2001), which quickly became very popular among experts in elephant husbandry. But even in this book, there was one concluding remark on investigating the histology of horn structures of elephants' foot (Fowler, 2001; Ramsay and Henry, 2001). In addition, the concentration of biotin in the blood plasma is examined and discussed as a small basis for biotin supplementation, which tries to improve the hoof horn quality and to prevent foot diseases.

7.1 Material and Methods

The choice of the zoos visited was more or less arbitrary and limited to 10 institutions. It did not matter whether the institution kept African or Asian elephants. The make-up of 6 zoos holding Asian elephants, 2 zoos holding African elephants and 1 zoo holding both species approximately reflects the figure of elephant husbandry in European zoos. An opportunity arose to collect data from captive and semi-wild animals from Sri Lanka, and so these data were also incorporated. Because it was not certain whether enough material could be collected, every foot offered was taken for both macroscopic and microscopic investigation. The numbers of front and hind feet examined are about equal.

The measurement of the horn growth rate in the horn wall turned out to be a delicate process in some cases as the notch cut into the horn wall for observing the growth rate quickly disappeared. Some people performing these measurements reported difficulties in reading the growth rates. The notches were trimmed away by the elephants themselves (abrasion of the horn wall by rasping away by the elephants when using their feet for playing or as instruments for preparing bark or leaves as food) or during daily foot care. Another possibility could be that they simply grew out because they were not cut deep enough. This can happen if the cut is just measured once a month and not checked daily to see whether it is still visible. Zenker (pers. comm., 2004) explained that this is quite remarkable and that he did not experience the same problem when researching the Lippizaner horses of the “Spanish Riding School” in Vienna. He also stated that some elephants had an oblique horn growth instead of a perpendicular one. This corresponds with Seilkopf (1959), who declared that the horn of the elephant does not grow perpendicular in the horn wall as in the horn wall of the horse, but obliquely. But to confirm this statement, obliquely dispersing horn tubules should be found in the horn wall. As described in chapter 6.3.2, the course of the horn tubules is inclined, but not oblique, and it proceeds in a generally perpendicular direction towards the weight bearing border. The suggestion is rather that the oblique course of the horn growth is a sign of an unhealthy horn wall because it should actually grow perpendicular. Because the growth rate was not measured by the author himself, he depended on the reliability and accuracy of the indicator. Therefore, it is more exact to convert the horn growth rate from the whole period of measurement to 28 days to iron out any measurement errors and to incorporate individual and seasonal variations (Geyer, 2004). This is because the growth rate can be influenced by the blood supply to the corium (meaning the growth rate is diminished in winter time), the air temperature (Wheeler et al., 1972), the amount of exercise the animals receive (prolonged movement increases the blood supply) and the age of the animal (Leu, 1987; Josseck, 1991; Schmid, 1995; Stern, 2000).

The exungulation of the individual hooves was better achievable with the method described than had been expected, which helps in describing the structures of the hooves.

Due to the kind help of a number of veterinarians, it was possible to collect some figures of feet of wild African elephants and of semi-wild and captive animals of the Asian species still living in their countries of actual origin. Although the thickness of the horn wall was not measurable, the much thicker horn layer in the weight bearing surface was obvious. The state and appearance of the feet was certainly describable.

Unfortunately, the collecting of dead elephants' feet was a complicated exercise and so it was not possible to determine the investigative material in advance. Any foot that was available has been taken, even when the age and origin of the animal was not known. It would have been ideal to have one front and one hind foot from a certain amount of animals

and species. However, it was not possible to choose the material in a systematic way. Having said that, the number of front and rear feet is approximately equal.

Horn samples, cut during foot care, are not of great use because the pieces are small and superficial. It is seldom that the samples are cut deep into the "living" tissue in these circumstances, which would have been necessary for gathering reliable data.

The methods for preparation and processing of histological material are well established (Fürst, 1992; von Houwald, 2001; Monhart, 2002). The only disadvantage was the fact that some samples were difficult to cut in the cryostat and in almost every histological slide cuts of the cryostat knife are visible. The dried material from the „Naturkundemuseum“ Berlin were particularly tricky to deal with in several steps of the preparation process due to their dryness. Although the blocks were not too difficult to cut, it became obvious after staining and mounting that, in every sample, notches and some crystals could be seen in the histological slides. Possible causes for the latter problem could be the denaturation of the proteins during drying out (e.g. conservation process by heating or handling with salt), although there were no statements about the conservation process given, the presence of ice crystals because the samples were watered before cutting in the cryostat or the presence of bubbles once the slide had been covered with the cover glass. Finally, it must be said that the results of the processing of this material were not really satisfying in the microscopic view.

Because it is not always possible to cut every block sample at the same height and at exactly the same location, the measurements that have been given cannot be taken as completely exact data, but rather as guidelines in the way of descriptive statistics. The reasons are the individual differences of the hooves and of the section, which was not always horizontally situated.

The advantage of the AB-PAS staining was the good differentiation of sound and diseased material. Degeneration is recognizable from the red staining of the cell membrane or cell body. Sound horn is indicated by narrow marrow and just a few red stained, decayed horn cells (Schmid, 1995).

The biotin examination was difficult because the application of the vitamins was not controllable and was given by other people than the author. This presumes good communications between all parties and consistency in the daily supplement does. The results do not entirely support these presumptions.

7.2 Macroscopic examination of the elephants' feet

The measurements of the feet were taken to have a better idea of the shape of the feet and to recognise early indications of pathological events through divergences from the normal

appearance, as well as to give signs for stresses in the feet. There are several influences on the shape of the feet (genetic and environmental), but also foot disorders may have an effect.

7.2.1 The foot

The position of the whole leg is perpendicular, contrary to the bones of the foot, which are oblique and to the digits, which are even almost horizontal. This perpendicular position is explainable by the huge weight that an elephant has to carry around (Altevogt et al., 1987). This construction enables the legs to hold up under their load. If the joints were positioned at an angle, the elephant would have to apply great force to maintain its position. When standing very straight, the traction of the weight rests directly on the feet. The stance of the hind feet helps to distribute the weight and to keep its position with minimal expenditure of energy. A comparison of the stance of an elephant with that of a rhinoceros (von Houwald, 2001) or of cattle is not possible because the legs of the other animals are not straight. Instead, elephants have a similar leg positioning to horses.

The enormous weight resting on an elephant's feet should be kept in mind. Fowler (2001) judged the weight distribution of an African elephant weighing 13'200 pounds, and concluded that, while standing, 3'300 pounds are borne by each foot, which equates to 12.99 pounds per square inch. This is also always to be kept in mind when discussing foot diseases and elephant husbandry.

The elephant belongs to the suborder ungulate (as horse, cattle, rhinoceros and hippopotamus) and is even classified as subungulate. The horse and all the other animals belonging to the perissodactyla (e.g. tapir, rhinoceros) are close relatives of the elephant (Fowler, 1980; von Houwald, 2001; White and Santos, 2004; IUCN/SSC/African elephant specialist group, 2005). The horse, cattle, hippopotamus and tapir are digitigrade like the elephant in the fore foot. So, they have similar foot constructions.

As Ramsay and Henry (2001) confirmed, the normal macroscopic anatomy of the feet of African and Asian elephants does not differ much. But there are some small but important differences between fore and hind feet and between Asian and African elephants. Apart from the already known distinctions (e.g. number of nails) and appearances, some obvious differences were found: (1) the circumferences of the front feet are larger than those of the hind feet for the Asian species, but they do not differ very much for the African species; and (2) the African elephant displays a narrower rear foot shape than the Asian elephant.

(1): There are two remarkable facts about the circumference of the feet, comparing African and Asian elephants. Firstly, the difference in size between front and rear feet is much smaller in the African species, if not almost the same. So, the weight of the body must be distributed differently in the African elephant. The reason for this could be the different habitats in which the species live. *Loxodonta africana africana* walks on hard underground and the Asian elephant on soft soil in the wild but in captivity mostly on hard surfaces.

Secondly, the circumferences of the front feet of both species are approximately the same, although the African elephant is taller and heavier than the Asian elephant. This statement could be explained by the much bigger and probably heavier front part of the body of the Asian elephants (massive head and neck). This is a confirmation of the statement by Virchow (1910) who also recognised the much bigger load on the fore feet because of the heavy head. Both facts could be an explanation for the greater amount of foot problems in the captive Asian elephant and for the observation that foot diseases (especially cracks) occur mainly in the front feet (West, 2001). Actually, it is generally accepted for quadrupeds that the front feet have to carry more than half of the body mass and therefore the forelimbs are subjected to more disorders than the rear limbs (Adams, 1979).

(2): The difference in shape is understandable given that the hind feet are much larger in the African elephant than in the Asian species and the African elephant is heavier than the Asian elephant. Because the males are generally heavier than the females, their feet have a greater circumference in comparison to the females. As a logical consequence of the much heavier part that has to be carried by the front feet (head, neck, shoulder and breast), they are in general much bigger than the rear feet.

7.2.2 The hoof

The construction and exterior of this component of the foot looks approximately the same in both species. But also here are some small, but not unimportant differences.

The shape of the nails is similar to those of human beings. The angle of the horn wall against the floor is generally constant in each species and the size of the horn walls differs between both species and sexes. This might reflect the different origins and habitats of the two species and the different substrates on which they live. This might be considered for the husbandry. A conspicuous feature in the nail is the fold, which does not exist in all animals, but e.g. in the bovine hoof (Fürst, 1992) or in the dog's nail (Geyer, 2005), see chapter 7.2.3.1.

In the macroscopic view, the sole looks as if it belongs to the nail. Indeed, Seilkopf (1959) spoke of the "sole of the nail". However, as the microscopic investigations showed, the sole is an independent component (see chapter 6.3.4 and 7.4.4). The big difficulty macroscopically is to define the transition to the pad horn. In some elephants, it is easy because a furrow separates the sole from the pad, but such a demarcation is not found in every animal. If it is not present, the measurement of the sole is rather difficult. As it turned out in a comparison, the length of the sole varies between a determination from the undersurface and a measurement with the help of the subcutis that is visible in the longitudinal slice and starts with the pad segment (see chapter 7.2.3.4 and 7.2.3.5). The assessment when measured from the undersurface was too large in most cases. Anyway, the length of the sole differs between individuals.

The pad has some special features as well. Unlike the perimeter of the feet, the length of the pad is larger in the hind than in the front feet. This derives from the shape of the respective feet. In order to accentuate the different forms of the hind feet between the species, the much longer pad length of the African elephant is to be emphasized. The undersurface of the pad area does not look the same in both species. In the captive African elephant, the surface is generally smooth with just a few furrows, but in the captive Asian elephant, a few too many ditches are to be seen. The reason for this is difficult to localize, although probably connected with the differing appearances of the species and differences between captive animals and wild relatives (see chapter 6.1.4 and 6.2.4). But all these considerations would have to be proved via future studies. Neither the reasons for the development of these furrows (see chapter 7.4.5) nor their function are clear. Fowler (1993) and Rüedi (1995) noted the furrows and the circle-shaped horn pieces as a portal of entry for infectious agents and a possibility for injuries by foreign bodies and concluded that their occurrence has to be prevented or removed. But this is put in doubt by the fact that the furrows are seen in the wild animals as well (Altevogt et al., 1987). The same author described the pad horn as being extraordinarily resistant due to these furrows. Therefore, they must belong to the normal anatomic appearance of the species and cutting them away is an intervention into the natural anatomical exterior. The pad horn of most captive animals looks quite thin, especially after foot care and not all of them display furrows (see chapter 7.2.4).

Neither of the declarations of Seilkopf (1959), that the Indian elephant has a harder and stronger horn than the African elephant and that the nails of the hind legs are softer than those of the front legs, could be established and it is only possible to speculate on this (see chapter 7.2.1).

7.2.2.1 Subcutis

The subcutis is structured in a similar way to the horse (Bolliger, 1991) and cattle (Fürst, 1992). It enables the change from the elastic skin to the less-movable horn capsule and serves as an effective buffer and protection of the underlying structures (Bolliger, 1991). The digital cushion of the pad reaches an extension in the elephant that is probably the greatest of all terrestrial mammals. The digital cushion is also responsible for supporting the elephant's weight, similar to cattle (Fürst, 1992), but contrary to the horse, which bears its weight over the wall segment (Bolliger and Geyer, 1992). In the elephant, the cushion is constructed of elastic, connective and fat tissue, in general joined by retinacula. This construction enables the shift in weight from the elastic skin to the less-movable hoof capsule. Another feature of the digital cushion is that it effectively enlarges the elephant's feet when the whole weight is put on the feet, either when standing or walking. But it cannot extend arbitrarily through the present retinacula. Therefore, the "fat tissue" has the effect of a

shock absorber, it protects the underlying structures and it contributes to cushioning the enormous weight the feet have to bear. In fact, it takes over the biggest part of this job.

The fact that the fat tissue of younger animals is much whiter than that of adults leads to the assumption that there are not the same fat acids in these two different age groups or more connective tissue in the young animals, comparable to cattle (Räber, 2000), which could be confirmed by further investigations. This might be an important part of the continuing study of the pathological alterations to elephants' feet and the causes.

Underneath the phalanges, the subcutis is well developed, so that the corium is well protected against the compression load. This is different for cattle (Fürst, 1992; Koller, 1998). Virchow (1910) once proclaimed that the bones bend under the body weight. This he concluded from the fact that the digital cushion becomes larger in size under pressure. His conclusion seems to be very unlikely. A movement of the bones within the whole foot structures would persistently provoke friction and pressure on the underlying structures.

In the wall and sole segment, there is no subcutis because no absorption or padding is necessary. Therefore the corium is well developed and takes on the traction and pressure in this area.

7.2.2.2 *Corium*

Although the nail does not seem to have such a weight bearing function, as in the horse (Bolliger and Geyer, 1992) or in cattle (Fürst, 1992), the corium is well developed in all segments (see below). Especially in the pad segment where the biggest part of the weight is cushioned, the corium is quite thick and provides a good distribution of the body weight. The reason for a well developed corium in the horn wall might be the fact that the elephant also places the feet over the weight bearing border when it walks.

The corium is composed of papillae and corial leaflets as in other animals with a horn capsule on their feet, e.g. in the rhinoceros (von Houwald, 2001), horse (Fürst, 1992) and cattle (Bolliger, 1991). The existence of laminae in elephants' hooves has long been recognised (Steel (1885), whereas Seilkopf (1959) wrote once that there is no connection between phalanges and Fowler (1993) denied their occurrence, although he has apparently revised his views more recently (Lahiri-Choudhury, 2001). Actually, a tight connection between epidermis and corium through the primary to tertiary leaflets is present particularly in the wall segment. Papillae and leaflets are a very important construction, especially for the digital organ, because it helps to attach the epidermis firmly to its underlying tissues. This seems to indicate that the nail takes over a certain part of the weight bearing function or that the elephant puts some pressure on the nail while walking, so that such a tight bond is needed.

Nevertheless, the strong connection between corium and periosteum of the phalanges shows that the nails are not in loose contact with the underlying structure, but actually tightly

connected with the periost (see chapter 6.3.3). One task of the horn walls certainly seems to be the protection of the underlying tissue and the matching phalanges (Ramsay and Henry, 2001), e.g. because at times the elephants use their feet for preparing their feeding. The statement of Fowler (1978) that elephants have rudimentary nails on their feet, can definitely be denied.

7.2.2.3 Epidermis

The structure of the epidermis has the same general construction as in other animals and there is also no difference between the two species. The horn layer is strengthened by the special arrangement of the horn cells with the horn tubules. Even a coronary and a weight bearing border can be seen as in other ungulates.

7.2.3 The hoof segments

The subdivision into five different segments is based on the different morphologies of the subcutis, corium and epidermis and on the anatomy of domesticated animals (Bolliger, 1991; Fürst, 1992; von Houwald, 2001). It simplifies the comparison of the elephants' hooves with other animals and among elephant species and individuals.

7.2.3.1 The perioplic segment

The perioplic horn on the horn wall does not reach the weight bearing border, as it does in the horse (Bolliger and Geyer, 1992), but rather behaves as in the bovine hoof (Fürst, 1992), where the perioplic horn lies on the coronary horn. The reason that it does not reach the end of the nail could be the use of the foot as an instrument for preparing food. Therefore, the widely held opinion that the nail should not be trimmed due to the violability of the periople is not justified.

The special appearance of the periople is the fold. This structure describes the part of the corium of the periople that reaches over the coronary segment towards the distal part and then bends towards proximal. Finally, it connects with the corium of the coronary segment. The similarities to the fold in the dog's nail are obvious (Geyer, 2005).

It seems that the horn of this segment goes around the foot as a transition between the hairy skin and the pad horn. This resembles the periople of the bovine hoof (Fürst, 1992), where the horn also extends to the palmar/plantar part of the hoof and adjoins to the pad horn.

7.2.3.2 The coronary segment

The macroscopic expansion of the coronary segment attains approximately the same form as that of the cattle's hoof (Fürst, 1992), but is much steeper than in cattle. It can be compared with the coronary segment of the hoof concerning its thickness (Bolliger and Geyer, 1992). Due to the hardness and strength of the horn, it fulfils the function of protecting the underlying tissues.

7.2.3.3 *The wall segment*

The wall segment has the same form as the whole horn wall. It is also reminiscent of the wall segment of the bovine hoof (Fürst, 1992). The wall segment of the African elephant is on average bigger in its extension, although the nail as a whole is slightly smaller. The reason for this is difficult to find. It could be connected with the different portions of the wall segment at the suspension of the phalanges on the horn capsule, but this is just speculation. The existence of primary to tertiary leaflets as a strong connection between epidermis and corium could support this hypothesis (see chapter 7.2.2.2 and 7.4.3). However, according to the assumption that the main part of the weight is borne by the pad segment, the traction forces are much smaller in the wall segment, compared with e.g. those of horses and therefore the wall segment does not reach the same extension as in the horse's hoof (Bolliger and Geyer, 1992). Therefore, the white zone, *zona alba*, does not have the same importance as in other animals, but it does also appear in the elephant's weight bearing border.

7.2.3.4 *The sole segment*

The sole is just a small part of the weight bearing surface of the elephant's foot, compared with the pad. The sole is defined by the fact that the corium lies directly on the periost. It is difficult to describe the sole from the palmar/plantar angle, as Seilkopf (1959) determined. Evans (1910) had already described the papillae of the "sole" after removing the horn capsule. A remarkable finding is the very thin horn layer, which is even macroscopically visible. The statement of Seilkopf (1959) that there is a sole only in the claw-shaped curved nail has to be contradicted because a sole could be found in all of the investigated hooves, although some were very small.

In colloquial language (used by vets and keepers in day to day practice) as well as in the paper of Smith (1890), the term "sole" means the whole undersurface of the foot without any specific differentiation. But this is not strictly correct, as can be seen in different chapters on the sole and pad segments. There are a few differences between sole and pad so that, although they are difficult to distinguish macroscopically, they should be named separately.

7.2.3.5 *The pad segment*

First of all, the definition of the pad should be clarified. The pad is the part of the weight bearing surface that has a subcutis underneath. Although there are different opinions about the distinctive features of the sole and the pad (Fürst, 1992), the subcutis and the digital cushion are taken as the decisive features for the subdivision of the weight bearing surface into sole and pad. The digital cushion is the largest segment of all and its subcutis acts as a shock absorber. In addition, in order to clarify the different extensions of the pad compiled by Seilkopf (1959), the pad is seen as a mutual pad in this study resulting from the fusion of all digital pads.

Contrary to the bovine pad, where the horn grows from palmar/plantar to apical (Fürst, 1992), the horn is pushed downwards but with a slightly oblique incline. If the pad horn overgrows, it does not grow over the nail or the sole.

The thickness of the pad epidermis (which includes the stratum germinativum, granulosum and corneum) is remarkable with a mean thickness of only 12.5 mm. The pad horn layer is therefore a bit smaller. This is astounding when the weight and the size of the animal are considered. It seems clear that the ground the elephants are kept on could be a cause for this fact, together with the foot care (see chapter 7.2.4) and is therefore very important.

The undulating course of the horizontal epidermal line could be an expression of the pressure caused by the enormous weight load affecting the corial and epidermal layer over the subcutis.

In summary, the appearance of the pad horn varies considerably depending on foot care, floor conditions and age.

7.2.4 Comparison of wild and captive elephants

During the visits to the different zoos, all of them have a free contact system, inside and outside enclosure and therefore give regular foot care. The undersurface of the feet of both species attracted particular attention due to their differences (see chapter 6.1.2 and 6.2.2). But after a study of figures of wild, semi-wild and captive (living in the country of actual origin) animals, the divergences between the species became obvious, and different from what was first observed.

Possible explanations for the natural differences of the pad surface between the species could be found in the natural habitat in which they live. The wild African elephant (only "*Loxodonta africana africana*" was considered) stays predominantly in the savanna or in other similar mainly dry areas, whereas the Asian elephant lives in forests and grassland. Therefore, it is understandable that the African species "needs" a resistant and extensive horn layer. The undersurface of the Asian elephant is adapted to the natural habitat where the elephant walks on softer and moister ground. This could also be the reason for the different appearances of the captive elephants. Most of the floors both species are kept on in European zoos (there are no actual differences in the husbandry between the two species) might be too hard for the horn of the Asian species, which is used to softer ground. This might be confirmed by the smaller relation of the papillae to the horn part in the captive Asian elephant (see chapter 7.4.5). With respect to the influence of the floor substrates on foot health, Buckley (2001) declared natural substrates as the preferred surface. She even went so far as to claim that "with the absence of concrete, nails and pads remain supple, not dry" and that "with a free-choice access, elephants spend the majority of their time engaged in activities on natural substrate." Frequent foot care plays a part in the appearance of the undersurface of both species. Other reasons for the differences could for example be a

different hardness of the horn material for the floor they are kept on, a better abrasion of the African pad horn material or faster growth rates in the Asian pad horn.

Another feature that stands out between wild and captive elephants is the apparently much thinner sole and pad horn layer of the captive relatives of both species in comparison to the wild ones. This was microscopically confirmed in chapter 6.5, where the thickness of sole and pad horn reaches approximately 10 mm. The few histologically measured and examined feet of wild African animals in this study showed a horn layer at least double that size. This could be one additional factor and a precondition for the foot disorders that occur in elephants, beside others (such as feeding, climate, foot care, lack of exercise and hygiene). There are two possible explanations for this: So far, the elephant specialists were concerned about the excessive horn building in captive elephants and the lack of horn wear, due to the lack of exercise for the elephants in a zoo or circus. For these reasons, they installed floors (concrete, asphalt, poured cements, plaster stones, rubber mats) intended to assist the abrasion of the sole and pad horn. Additionally, the foot care, particularly the removal of horn that is not abraded by walking on these hard floors was intensified (sometimes after foot care a reddish tissue – the corium – was visible underneath the horn layer). Therefore, the floor might be designed for too much abrasion of the sole and pad horn layer, and the foot care might be done to excess (Schanberger, 1990; Buckley, 2001). This would probably also explain the look of the undersurface of the feet of captive elephants of both species. In addition, Buckley (2001) stated that “even a moderately active elephant in a natural-habitat environment requires little foot care (as little as one to two hours each year).”

One possible consequence of the thin weight bearing surface could be that the pressure on the nails increases (Flügger (2002) also assumed this as a possible cause) and therefore cracks and other alterations become more likely because of the unnatural anatomy. Another effect could be a poor blood supply triggered by the thin horn layer, which cannot resist the enormous weight any more and, therefore, the blood vessels in the underlying corium are affected. This again leads to a diminished horn quality and results in a situation favouring pathological alterations. Therefore, the thin horn layer in the pad horn of captive elephants has been denoted as a „locus of minor resistance“.

According to the various talks with elephant specialists, it seems that zoos with a large enclosure and an offhand or at least protected-contact management, including no or only occasional foot care, have fewer problems with foot disorders.

7.2.5 Growth rate of the horn wall

There are several statements to be made about the horn growth rate of elephants. Firstly, Fowler (1993) mentioned a rate of 1 cm per month for the horn wall, not differing between African and Asian elephants, nor front and hind feet. Seilkopf (1959) declared the growth rate to be 2.2 to 2.5 mm per month and is accordingly well below the rate measured in this study.

He did not distinguish between the species nor the different feet either. Others have assumed that the time a defect in the horn wall takes to heal is the growth rate, which is not correct due to the different influences on the healing process and due to the location of the defect. In this study, it became clear that the two species and the front/rear feet have to be differentiated. The rate for the African elephant is slower than for the Asian elephant, and the difference is significant for the fore feet. In general, the growth rate of the rear feet tends to be lower than in the front feet (calculated for all individuals of both species). The reasons for the different growth rate are entirely speculative and therefore an attempt at an explanation will be omitted here. The growth rate for the horse (7 – 8 mm/28 d, Bolliger and Geyer, 1992) is about the same as for the Asian elephant front foot. In comparison, the rate for the horn wall of cows is about 4 mm/ 28 d (Schmid, 1995), which is a bit less than in any measured horn wall of the elephant.

The growth rate of the horn wall is important for the replacement time after a defect arises. The healing of a nail alteration will take at least as long as the new horn growth. If the length of a horn wall is about 75 mm, the regeneration of the coronary horn from the coronary to the weight bearing border on average takes more than 9 months for the front foot of an Asian elephant and usually almost 15 months for the hind foot of an African elephant. So, it needs time for the complete healing of a horn wall defect, especially if the disease affected lower lying structures and a normal nourishment of the horn layer is not ensured. This also means that attempts to improve the horn quality with a feeding supplementation, for example with biotin supplementation, have to be planned for this time span or even longer (the horn has to grow down to the weight bearing border). It also has to be taken into consideration that horn with already bad quality proximally will take longer to grow down than a normal, qualitatively good horn (Leu, 1987), see chapter 7.7.

In the zoo that was not included in the growth rate calculations, there was an elephant receiving biotin treatment that showed a growth rate double that of another elephant in the same zoo not receiving biotin supplements (see chapter 6.1.5). This could lead to the false assumption that biotin has an influence on the horn growth rate. Leu (1987), Josseck (1991) and Schmid (1995) showed that this is not the case although Buffa, (1992) claimed the opposite. In this case, it has to be mentioned that the supplemented elephant had displayed poor horn quality over a long time. So, it seems that the cause for the fast horn growth lies in the bad horn quality as Leu (1987), Josseck (1991) and Schmid (1995) confirmed. This is because fast horn growth does not mean good horn quality and good quality of the regrowing horn is more important than its quantity (Leu, 1987; Josseck, 1991).

Overgrown horn walls as a result of excessive horn growth are consistently a popular topic in zoos and in the literature. This growth is often assumed to be a general cause for foot problems (e.g. cracks, Fowler, 2001; Flügger, 2002). Excessive horn growth is usually

accompanied by fast horn growth. If the process of cornification is increased, the horn produced cannot be of the same quality as normally produced horn layers and is therefore of poor quality. In domestic animals, it could be shown that hoof overgrowth can be manifested by laminitis (Boosman et al., 1991). Laminitis however can be the result of hard enclosure surfaces or even the result of a feed with an overload of soluble carbohydrate, which leads to acidosis and finally to laminitis, as Clauss and Kiefer (2003) confirmed.

7.3 Pathological alterations in different husbandry systems

In all ten zoos in this study (with free contact management), cracks and nail abscesses were reported by all of those zoos keeping Asian elephants. Sole abscesses and overgrown nails were noted by every second zoo holding Asian elephants. It is impossible to compile meaningful results for the zoos keeping African elephants because only two of them answered the questionnaires. Nevertheless, even there, the two institutions listed some problems and others were observed during visits. It can be concluded that, despite slightly different husbandry systems in the different zoos, the same problems occur.

There are some signs that a free contact system attracts more foot problems than other systems (see chapter 7.2.4). Because there is no zoo with just a few or without any pathological alterations in this study, it is not possible to identify a husbandry system that guarantees no foot problems. This would need a test arrangement that allows a specific husbandry system to be followed without change during a test period and then it would need monitoring over a certain period of time to get reliable results. All other conclusions can only be speculative. Nevertheless, some statements concerning husbandry can be made.

A comparison between the macroscopic alterations found in the zoos visited and those in the questionnaires shows that there are some great divergences. Overgrowth of the periople and “horn rings” of the horn wall were not regarded as an alteration according to the questionnaires, although they were observed quite often in all zoos.

Additionally, it must be mentioned that there was an astonishing variation in the opinions of elephant keepers and the responsible persons who filled out the questionnaires regarding questions such as foot disorders, the recurrence of problems in the same elephant, the recurrence of problems in the zoo. There were also divergences between the observations of the author and the answers in the questionnaires. This shows that different views still circulate about feet disorders, which is confirmed by Dimeo-Ediger (2001), and much depends on how the interviewed person assesses the foot problems.

It has to be admitted that it is a matter of opinion what amounts to a pathological alteration or part of a disease. Many problems do not look too dramatic and so the impression arises that the disorder is not severe, although there might be typical signs for e.g. bad horn quality (see chapter 7.5). In this study, every divergence from the normal macroscopic and microscopic

state is taken as a pathological alteration, not only the changes linked to a clinical symptom such as pain, swelling or lameness. As mentioned in chapter 7.4, the sound, unchanged hoof is taken macroscopically and microscopically as representing the normal state.

The small percentage of totally sound feet in both species is noteworthy and shows the importance of evaluating the primary causes of foot problems. In the study of Mikota et al. (1994), 50% of the study population was affected by foot problems. This again emphasizes how common problems are. Distinct factors influence the state of the foot, such as the age and weight of the animals, but also genetic, environmental, feeding methodical and custodial factors as well as climate and adequate possibilities for movement. In general, it can be stated that unnatural foot shapes promote foot disorders (seen in some visited zoos) due to the fact that both the foot and the horn are unnaturally stressed, leading to a poor blood supply and e.g. to chronic laminitis.

In this study, it seems that African elephants exhibit a smaller range of foot disorders, but the number of examined African elephants was also much lower than the number of Asian elephants. So, the interpretation of this result has to be cautious. The fact that the captive African elephants show fewer foot problems than the Asian ones could also be explained by the fact that the African is more used to the hard floor environment found in most zoos, and which accords with his natural habitat. In addition, it is not possible to declare definitely from this study whether there are more foot problems in the front or hind foot. The front feet do appear to be the ones that are affected most, probably due to the heavy weight of the front part of the body (Adams, 1979; West, 2001).

It has to be kept in mind, and shows the importance of the topic and of the clarification of the reasons for alterations, that even in very young animals and in new born elephants, pathological divergences are found – some of them show “only” microscopic alterations (see chapter 6.4.2.6 and 6.4.2.7), but others even show macroscopic changes, particularly cracks. A genetic factor cannot be excluded in connection with foot problems and specifically with the existence of bad horn quality (see chapter 7.5). Indeed, in several zoos, the offspring of elephants that display foot disorders are reported to develop changes as well, even if they are not in the same zoo any more. But this could also be caused just by the husbandry conditions. At any rate, it is another indication for the bad state of captive elephants’ feet.

An examination form for the foot diseases occurring in elephants is given in the appendix. This should help keepers and zoo veterinarians to control and document feet and their health status. The development of diseases and their severity (grades) can be documented in an exact and simple way with this form. This could help to identify recurring diseases in the same elephants or the same diseases in different elephants or might help to identify possible causes of the problems.

7.4 Microscopic anatomy of the normal horn structures and some appropriate data

Before going into detail, it should be noted that minor pathological alterations are not considered as normal for the purposes of this study (with some exceptions, see chapter 6.3), but rather the unchanged, optimal state is taken as the normal structure. Actually, there are many alterations to be found in the different segments of the histologically examined hooves and there are only a few hooves and segments that show no alterations at all. Nevertheless, it would be a distortion of the figure to consider all these alterations as normal. In fact, all the histopathological changes have an impact on the state of the hooves of the captive elephants (see chapter 7.5). A good example for the fact that regularly occurring changes cannot necessarily be dismissed as irrelevant to a healthy state is furnished by the Lipizzaner horses of the “Spanish Riding School” in Vienna, where most horses showed alterations in the white zone (Josseck et al., 1995; Zenker et al., 1995).

The normal microscopic appearance of an elephant’s hoof showed little difference between fore and hind feet as well as between the species. There were no significant indications for the claimed differences in the occurrence of foot problems (Ramsay and Henry, 2001). Rather, a lot of similarities were found histologically with domesticated (Bolliger, 1991; Fürst, 1992) and other wild (von Houwald, 2001) ungulates. A lot of structures can be seen as reflected in them. The construction of the cells, papillae, leaflets and tubules show a large degree of regularity in the examined samples. In addition, it has to be admitted that the existence of interdigital (Lamps et al., 2001) and coronary glands (named as a disease process by one zoo according to the questionnaire) could not be established, nor were they cut in any samples.

Smith (1890) had already explained the nail of the elephant histologically, but he had spoken only of a wall and a sole, as well as of “laminae” and “foramina” and also of “papillae”. He made some comparisons to the horse’s hoof, but thought that especially the “horny laminae” are “unlike those of the horse”.

7.4.1 The periopic segment

The fold is also microscopically remarkable. It is built by the corium and epidermis of the periople and coronary segment, because the transition from the periople to the coronary segment happens in the elongation of the crease. The fold is something between a depression and a crease. The tubules around the fold are the largest. This is comparable with the giant tubules of the periople of cattle (Fürst, 1992).

A unique feature can be found in the periople: big papillae that are subdivided by epidermal cords and that form a group formation of papillae of different size. A group formation of the horn tubules has not been seen. But it has to be taken into consideration that the periopic horn tubules are not cut exactly on the horizontal in the transverse section and the presence

or not of a group formation is therefore rather difficult to judge. This phenomenon is also found in other segments (see below). Its function in the periople is not clear.

The stratum granulosum that occurs in the periople and in the pad is definitely found only where soft horn material is built (Warzecha, 1993). This emphasizes the similarity of both horn types and their actual consistency.

On the surface, both of the periople itself and of the horn wall where the periople lies on the coronary horn, no horn tubules are visible. Where horn tubules are found, the cortical cells are of type 2, which Bolliger (1992) describes as occurring in the inner zone of the coronary horn in the horse's hoof.

A few vacuoles in the stratum spinosum and granulosum and also some micro cracks in the superficially located stratum corneum can be regarded as standard. The exterior intertubular horn is older and is much more exposed to the influences of the environment.

7.4.2 The coronary segment

The coronary segment of the elephant's hoof almost reaches the extension of the coronary segment in cattle (Fürst, 1992). The length of the coronary papillae and horn tubules are rather difficult to measure because the corial papillae are inclined and curved. They should therefore be cut longitudinally from the basis to the apex for measurement. They do not run perpendicular as in the horse's hoof (Bolliger and Geyer, 1992). However, they are ordered regularly with the same length and orientation.

The horn part of the coronary segment is responsible for the shape of the nail. This because the horn tubules are inclined on their course towards distally as are the papillae. In this way, the different thicknesses of the nail can be explained. The differentiation of the coronary horn into three segments is not always very obvious (especially towards the distal end), but the varying size and shape of the horn tubules are always detectable. Although neither a group formation of papillae nor papillae with epidermal cords have been detected, in the middle zone of the coronary horn smaller tubules that surround a bigger one can be found. So, there must also be the same phenomenon as in the periopic horn (see above).

The cortex of the horn tubules are composed of cells of type 1 (Bolliger and Geyer, 1992) and the intertubular horn is built from polygonal shaped cells. So, contrary to the coronary horn tubules of cattle (Fürst, 1992), the cortical cells are easily distinguishable. Different types of cortical cells were not found in the different parts of the coronary horn, as Bolliger (1992) had described in the horse's hoof. Type 1 occurs in the outer and middle zone of the coronary horn of the horse (Bolliger and Geyer, 1992). Fürst (1992) showed that cloven-hoofed animals have this type in all segments.

The occurrence of the abrasion of the coronary horn in the distal part of the horn wall could be explained by the use of the feet for the preparation of or search for food (Buckley, 2001). Nevertheless they were not found in the histologically examined wild African animals (see

chapter 6.5.2). An explanation for this difference is difficult. No influence on the occurrence of macroscopic alterations could be established, contrary to von Houwald (2001), who described an abrasion of the horn wall of a captive Indian rhinoceros, with severe effects on the corresponding hoof.

As Fürst (1992) described the transition from the coronary segment to the wall segment, it also seems in the elephant's hoof that the corial papillae of the coronary segment build the corial leaflets of the wall segment, as the stratum germinativum of the coronary segments begins to build horn leaflets instead of horn tubules.

7.4.3 The wall segment

Smith (1890) described "horny laminae" in the wall of the nail and compared them with those of horses. But as already indicated in other chapters, the wall segment has to be regarded more in detail.

Evans (1910) and other authors cited in the study of Seilkopf (1959) recognized the strong connection between the corial and epidermal leaflets very early. This is confirmed in this study. Actually there is a strong bond between bones and nails through the corial and epidermal leaflets because the corium is directly associated with the periosteum of the digital bones and attached to the epidermis by the primary, secondary and even tertiary leaflets. Due to this arrangement of the corium and epidermis, it becomes clear that there is a big surface over which there is a strong connection between these two structures (sliding contact mechanism Bolliger, 1991) and this helps to fix the hoof epidermis firmly to its underlying tissues (Bolliger, 1991). Therefore, the third phalanx must be suspended in the horn capsule by the interdigitation of the epidermal and corial leaflets. This is contradictory to Fürst (1992), who explained that the cause of a lack of secondary leaflets, as in cattle hooves is based on the fact that the wall segments only have to bear a small weight. But the elephant is a typical example of an animal that puts most of its weight on the digital cushion and the pad segment. Therefore, the question has to be asked whether the elephant bears more weight on the wall segment than has been assumed to date (see chapter 7.2.2.2). The occurrence of tertiary leaflets was also described by von Houwald (2001) in her study on the Indian rhinoceros. Otherwise, the appearance of the wall segments with the primary and secondary leaflets is similar to those in the horse's wall segment (Bolliger and Geyer, 1992). The increase and decrease of the leaflets is similar to that in cattle (Fürst, 1992). The thickness of the stratum germinativum of the soft leaflets and of the transitional and connecting horn cells is also remarkable.

The cap horn with its construction of cells and tubules, which is built by the rim of the epidermal leaflets, is more reminiscent of the horse's hoof (Bolliger and Geyer, 1992) than of other species, but its extension is broader than that of the horse, but less than in bovine hooves (Fürst, 1992).

As distinct from the horse's hoof (Bolliger and Geyer, 1992), the elephant's terminal horn begins earlier in the wall segment and earlier in the Asian than in the African elephant's hoof. Otherwise, the construction is similar to that of bovine animals (Fürst, 1992), the horse's hoof (Bolliger and Geyer, 1992) and the rhinoceros' hoof (von Houwald, 2001). The terminal horn tubules are mostly cut transversely even in longitudinal sections. This shows that they must proceed in a sideways direction.

In the elephant's hoof, a white zone exists as in other animals such as cattle (Fürst, 1992), sheep and goats (Warzecha, 1993), horses (Bolliger and Geyer, 1992) and rhinoceros (von Houwald, 2001). The transition from the terminal corium and epidermis to the sole segment is rather difficult to identify. The horn leaflets, the linear arrangement of the terminal horn tubules and the fact that the corium of the wall segment passes perpendicularly and that of the sole segment horizontally helps to differentiate the two structures.

Micro cracks and vacuoles in the terminal horn were found in all examined samples of both captive and wild animals and has been taken as a common feature. Therefore, this part of the wall segment, which is exposed very strongly to external influences, is deemed to be a "locus of minor resistance" .

7.4.4 The sole segment

As in the perioplic corium, there are large papillae subdivided by epidermal cords from which several smaller papillae arise and finally several horn tubules of different sizes. So, the impression of a group formation is given. Here, as well as in the pad segment where it also occurs, a possible explanation for this arrangement could be a better distribution of the pressure, necessitated by the weight of the animal, and designed to enable the foot to better withstand the enormous pressure. Contrary to the papillae of the pad segment, the papillae of the sole segment run rather perpendicular and just slightly inclined in different directions.

The cortical cells of the horn tubules are of type 2, spindle-shaped cells, contrary to those of the horse's hoof, which are of type 3 (Bolliger and Geyer, 1992). The fact that the sole horn is very thin in many captive elephants' hooves and that the corial papillae reach close to the undersurface of the sole horn in captive, as the relation of the papillae to the pure horn part shows, and partially in wild animals' hooves gives the impression of a „locus of minor resistance“. Considering the weight and the thin sole horn, problems in this area are not surprising.

In the sole segment of elephants, there are no leaflets made out of horn in the transition of the terminal horn as can be seen in the sole segment of the cow (Fürst, 1992) or the Indian rhinoceros (von Houwald, 2001). This is because there are no linear arrangements of the tubules (see chapter 6.3.4.). If it occurs at all, the phenomenon in elephants is limited to just a couple of papillae at the border to the terminal horn.

It has to be stated that micro cracks in the intertubular horn near the undersurface can be accepted as standard due to the environmental influences, but not micro cracks near the papillae. In most of the examined hooves, the state of the sole segment was not very good (e.g. micro cracks, vacuoles, dilated marrows of the horn tubules, PAS positive reactions; see further chapter 7.5).

7.4.5 The pad segment

Similar to the corial papillae of the sole segment, the papillae are also subdivided by epidermal cords, which end in a group formation of the papillae and the horn tubules. The reason for this is probably the same as for the sole segment (see chapter 7.4.4). Very conspicuous is also the thick layer of the stratum reticulare. This could be a support for absorbing the enormous weight in the feet, together with the digital cushion (see chapter 7.4.6).

A very special aspect is the course of the papillae and the horn tubules. They are directed partly sideways, partly meandering, partly inclined towards apical. It gives the impression that both papillae and horn tubules yield to the weight of the animal and so it is not possible for them to stand perpendicular because the basis of the papillae would then actually lie in a perpendicular direction to the stratum reticulare. Another reason would be the softness of the horn material in comparison to the other segments of the weight bearing surface. The differences between the relation of the papillae to the pure horn part of the two species might reflect the different adaption to the husbandry condition (see chapter 7.2.4 and 7.6). This is meant in connection with the abrasion through the floor and a too abrasive foot care for the Asian elephant. Otherwise, it might reflect the different reaction of the horn part to the differing body structures, origins and habitats of the two different species as in the macroscopic appearance (see chapter 7.2).

The cells of the cortex of the horn tubules are spindle-shaped and therefore type 2 according to Bolliger (1992). Surprisingly, this is the same type as in the cortical cells of the sole horn. The thickness of the pad horn is also unexpected and reaches a mean value of only about 1 cm for the captive elephants (see chapter 7.6). Therefore, the pad is taken as another „locus of minor resistance“, although Evans (1910) thought that the weight bearing surface is comparatively thin because it is meant for walking on soft ground.

The three parts of the pad (apical, middle and palmar/plantar) show structures and measures of rather the same condition, contrary to the bovine or equine pad (Bolliger, 1991; Fürst, 1992).

The pad resembles the periople quite closely and therefore a microscopic differentiation where they come together (palmar/plantar) is rather difficult. The horn in particular is very similar in its staining. Nevertheless, it is noticeable that the horn in the pad is much tougher than that in the periople, contrary to the pad of the bovine hoof (Fürst, 1992).

In the pad horn, micro cracks, vacuoles and changed horn cells can be found near the undersurface. According to Leu (1987) and Zenker (1991), these are attributes for damaged horn. But these symptoms of decay of the horn near the surface can be declared as common near the surface due to environmental influences, as in the bovine pad (Fürst, 1992). These micro cracks in the intertubular horn near the surface might also play a role in the building of the macroscopic furrows. Due to the fissures, there is no longer an adhesion of the horn and so it decays uniformly. Because of the ground the animals walk, the furrows might then be built. Another explanation could be the existence of differently sized papillae in the pad segment as mentioned above. There are varying sizes and lengths of horn tubules arising from these papillae and so the furrows might be built. They might be comparable to the building of the “horn rings” that are formed by the different sizes of papillae depending on the blood supply to the pad corium.

In comparison, alterations have to be taken as a serious problem near the apex of the papillae. These are found in most of the examined feet of captive and wild elephants, and these mean early degeneration. Actually horn that has a lot of micro cracks is not very resistant and so the horn could be asserted as „locus of minor resistance“ due to a secondary cause (Fürst, 1992).

7.4.6 The digital cushion

Seilkopf (1959) compiled the different opinions about the digital cushion in his day. These ranged from an “accumulation of elastic tissue” to “a cushion of a gelatinous mass”. Neuville (1927) described the digital cushion as consisting of a trabecular framework of elastic fibres, which created a fat island in which small fatty globules were deposited.

The digital cushion of the pad segment contains fat tissue that is encircled by connective tissue in different ways. The connective tissue can be imagined as a “meshwork of dense connective tissue”, which “divides the cushions into compartments similar to the stitches of a mattress” (Räber et al., 2004). The three parts (apical, middle and palmar/plantar) differ from each other in some characteristics. In the apical part, the fat tissue predominates. There, the connective tissue has the task of maintaining the formation of the fat tissue by holding it together. So, this part might take over the task of cushioning the bones. In the middle part, the fat is enclosed in connective tissue islands and so this might be responsible for absorbing the animal’s weight. Against that, the palmar/plantar part has more connective than fat tissue due to much more pressure of weight in this area and the fact that there is the biggest extension of the digital cushion and longest distance to a supportive structure i.e. bones. Its function might be the supporting and holding of the digital cushion, because otherwise it would crash down under the heavy load. At the palmar/plantar part, the strain is probably the biggest on the whole foot. Therefore, the area where the foot is stressed most should have the biggest portion of connective tissue (Räber, 2000).

Because it was observed that there is much more connective tissue in young elephants, it can be assumed that as with other organs the fat tissue develops in the course of growing and in taking on its actual task. Räder (2000) showed that the age of the animals influenced the composition of the fat tissue in the digital cushion of cattle. Younger animals had more connective tissue, whereas the digital cushion of the adults consisted of much more fat tissue. So, the composition probably depends on the age and the number of lactations, as in cattle. The portion of fatty acids (saturated/unsaturated) depends on age, feeding and probably on the state of the animal's hormones (Räder et al., 2004).

The Asian elephants seem to have more connective tissue than the African species, which could be explained by the different substrates on which the species live. This should be considered when choosing the surface of zoo enclosures. This would mean that the foot of the Asian elephant cannot absorb weight as well as the African species. If the natural habitat of the Asians, consisting principally of soft ground, is taken into account, then the Asian elephant does not need the same weight absorbing possibilities as the African species living in the steppe. It could be assumed that walking on hard floors such as concrete or asphalt in captivity could have a bad effect on foot problems for the Asian elephant. But this hypothesis should be examined through further specific investigations of the digital cushion as Räder (2004) did with the digital cushion of cattle. The digital cushion of the elephants is one of the most important parts of the foot and also the whole body, but so far little examined.

In conclusion, fat and connective tissue enable the function of a stable cushion. The connective tissue has the task of preventing the extension of the fat tissue. This is therefore restricted by the connective tissue. This gives padding and elasticity as well as acting as a shock absorber for the enormous weight the feet have to bear. This feature was also described in cattle (Räder et al., 2004).

7.5 Signs of bad horn quality

7.5.1 Histopathological alterations in the different hoof segments

The most surprising fact in the histological examination was the enormous number of microscopic alterations in every segment of the "normal" hooves. This demonstrates that the horn quality of the foot taken as a whole was still rather poor, even in the macroscopically good-looking samples. Zenker (1991) declared that qualitatively bad horns first display symptoms of decline in very stressed areas. Therefore the question arises whether the elephants' hooves are too stressed in every segment and if this appears only in captive elephants. There have also been some histological examinations of a few feet of wild African elephants. They also show some microscopic alterations, but they do not approach the same bad quality as the samples from the captive elephants. The alterations of the wild animals'

samples affect the wall segment (micro cracks and vacuoles in the terminal horn), the sole segment (far distally reaching papillae in the horn part) and the pad segment (micro cracks up to a point near the papillae) in particular. Due to this occurrence in wild animals and for other reasons, these structures, together with the sole, are regarded as “loci of minor resistance” (see also chapter 7.4 and 7.6). The same segments have also other appearances (only occurring in captive elephants’ hooves) why they can be called “loci of minor resistance” nevertheless.

Fürst (1992) compiled the characteristics for the assessment of horn quality: the number of horn tubules per surface unit of measure, pigmentation, diameter of the horn tubules and proportion of cortex and marrow, delimitation of the horn tubules against the intertubular horn. He declared that the resistance of the horn is the greater and the wear the lesser the higher the number of horn tubules is, the more the horn is pigmented, the smaller the diameter of the horn tubules, the smaller the proportion of the marrow and cortex is and the more distinct the delimitation of the horn tubules against the intertubular horn. Fürst (1992) also described the features that indicate pathological alterations as being: intensified red staining of the cell membranes or the cell bodies of the horn cells with AB-PAS, dilated marrows in the horn tubules, loss of cortical cells, irregular shape and order of the intertubular horn, loss of staining of the horn cells whereby they develop a heterogeneous and frothy appearance, occurrence of micro cracks and vacuoles shortly after the cornifications. There are several already proven signs of bad horn quality, particularly an intensive staining of the horn cells by the AB-PAS-coloration, which indicates decay of the cells (Schmid, 1995; Koller, 1998). Dilated marrows in the horn tubules can just exist where the cortical cells begin to decay, which is indicated by the PAS positive reaction (Küng, 1991). Therefore, the dilated marrow is just a consequence of the disintegration of the cortical cell layers (Albarano, 1993). Albarano (1993), Leu (1987) and Zenker (1991) showed that histopathological changes, especially micro cracks, lead to diminished resilience and resistance of the horn. The horn that has micro cracks reaching near the papillae, which happened above all in the sole and pad horn, cannot perform its protection function and this is a typical sign of bad horn quality (Schmid, 1995).

Not many features of good horn quality were found in the samples of this study, contrary to a lot of pathological alterations in the examined slides. Instead, widely distributed signs of bad horn quality were detected in the hooves of captive elephants, even in neonate to juvenile elephants (see chapter 6.4.2.6 and 6.4.2.7).

The different segments are differently affected by the various kinds of alterations indicating bad horn quality that can be found in the respective segment. The alterations listed in chapter 6.4 can be taken as symptoms of bad horn quality for every segment. Vacuoles and micro cracks are a clear sign of diminished quality, but also fissures in the stratum spinosum

can be taken for this. This is an early indication of the process of cornification. Especially with the vacuoles, it was conspicuous that they were not as common in the African elephants' hooves as in the Asian elephants'. Some parts of the stratum spinosum and granulosum of some segments are thickened. This can be put down to disturbed processes of cornification caused by deficient supply and diseases, inflammations and delayed conversion of the stratum spinosum to corneum and therefore this leads to an increase of the thickness of the stratum spinosum (Fürst, 1992; Geyer and Schulze, 1994; Zenker et al., 1995).

The pad segment is possibly the segment with the most varied symptoms of reduced horn quality in the largest proportion of examined feet, although they are macroscopically not in bad shape. Geyer et al. (1984) showed a similar appearance of the pad segment in swines. They found a thin pad horn together with long papillae. Therefore, they argued that with strong mechanical influences or qualitatively bad horn in the pad segment, it is particularly easy for irritations of the corium, which is sensitive to pain and bleeding of the vessels in the papillae, to develop. Similar conclusions must be drawn for the elephants' pad segment.

It should not be forgotten that individual animals have varying abilities to build qualitatively better or poorer horn, even under the same environmental conditions (Zenker, 1991; Josseck et al., 1995; Schmitt, 1998). And it has to be taken into account that the horn in the weight bearing border is much more exposed to mechanical stress than proximally located horn (Zenker, 1991).

The causes for this are rather difficult to identify and the problem cannot be solved by one single measure. However, bad horn quality and "loci of minor resistance" associated with the enormous weight pressures and the husbandry employed (e.g. hard floor, little moisture, hygiene, feeding, foot care) might promote foot problems of captive elephants (Buckley, 2001; Fowler, 2001; Clauss and Kiefer, 2003). A genetic factor cannot be excluded in connection with the existence of bad horn quality. Exercise and motion in captivity are essential to improve horn quality because they further a better blood supply (Stern, 2000). Possibly, foot disorders could be prevented by taking horn samples regularly to check horn quality.

The bad influence of different environmental substances, particularly of a faeces/urine-mix, on the horn quality and on foot health generally has already been demonstrated for domesticated animals (Geyer et al., 1984; Albarano, 1993; Monhart, 2002) and even in the wild (Keet et al., 1997). Monhart could show that qualitatively good and undamaged horn withstands the influences of faeces and urine better than already damaged horn. Albarano (1993) proved that husbandry, climate, hygiene, feeding, exercise and the care of claws influence the ability of the claw horn to withstand stress. This conclusion is certainly transferable to other species and in the case of the elephants it would affect the terminal, sole and pad horn.

7.5.2 Histological assessment of certain macroscopic alterations

A selection of common foot problems that have been examined microscopically showed that all the typical histopathological alterations were found in most macroscopic foot diseases.

The macroscopical alterations are therefore also connected with bad horn quality.

There are actually no signs of inflammation in any of the alterations, which can be explained by an intact basal membrane. The process of change is limited to the horn cells.

7.5.2.1 Overgrowth of the periople

This is an alteration that often happens in Asian elephants. Already Fowler (1980) described a macroscopically similar alteration, but he called it parakeratosis. The histological view of the described change in this study showed different signs of decay of the horn cells and of bad horn quality in the periopic segment. A compare to the parakeratosis stands also to reason due to this microscopic description because a parakeratosis caused by a deficiency of zinc leads to an insufficient cornification and a premature decay of horn cells and therefore to the building of crusts of the skin visible on extremities, tail, ears and snout of the swine (Kessler et al., 1996; Cameron, 1999). This has also been proved in cattle (Stöber, 2002). Therefore, a zinc deficiency could also be taken as a possible cause for this manifestation. This alteration usually found together with overgrowth of sole or nail occurs as a roughened, split area of skin proximal to the nail (Rajankutty, 2004) and is actually an extensive cornification of the periople.

7.5.2.2 Crack in the horn wall

Vertical cracks in the horn wall are one of the most common foot disorders in both elephant species according to Mikota et al. (1994). They can also be found in a lot of other species, such as pigs (Geyer and Tagwerker, 1986), cattle (Fessler, 1992), horses (Geyer and Schulze, 1994), wild hoofed animals in zoological gardens (Göltenboth and Klös, 1995) and rhinoceros (von Houwald, 2001). In these examinations, even several young elephants have been observed to have cracks in the horn wall (the youngest was 11 months old).

No histological description of the cracks has been made so far, although this might contradict or confirm what are only assumptions at present. Seilkopf (1959) characterized the cracks as exclusively superficial longitudinal fissures. As the microscopic examination demonstrated, the cracks can reach very deep into the nail tissue, even to near the corium and the corial leaflets. But in every investigated sample, the corium was protected by a thin horn layer. It can be imagined that this thin cornification can burst through further spreading, opening the corium to bacteria. This is actually a confirmation of Fowler's finding (1980).

A granulation tissue, made out of horn, is built. This protects the deeper tissues against the environment. Around and in the closer area of the crack, there are a lot of alterations to be seen, which represent poor horn quality in the nail (and even the sole, if it is also affected). A special feature is the fact that the coronary horn beside the cracks disappears. The cracks

are just surrounded by terminal horn. This could be caused by the expansion of the crack by pushing away the neighbouring structures, but also by early symptoms of decline of the cornification in the coronary segment, triggered by the beginning of the crack.

So, it seems that the crack is not caused by an outside happening alone and that it is not a limited alteration, but that also the surrounding structures are involved. So, the poor horn quality may enable the building of cracks or may be a consequence thereof. This is confirmed by Seilkopf (1959), Ruthe and Seilkopf (1962) and Fowler (1980), who named poor horn quality as a main factor for cracks, beside other elements (such as insufficient wear, injuries by foreign bodies, feeding, genetic and environmental factors). Animals showing cracks are therefore possible instances of diminished horn quality. This can be a singular case in an individual animal, or it may be a general problem in a zoo. If several animals of the same zoo are affected and the cracks recur consistently, then it is a sign of a husbandry problem or of another cause that has an effect on the horn quality of all animals. The recurrence of the problem in the same nail or even in the same elephant is confirmed by the interviewed keepers and vets.

According to the histological anatomy of the horn wall, it appears logical that the healing of cracks can only occur in healthy horn from the proximal direction. If the horn is of good quality, the chance is high that the alteration won't recur.

In Sri Lanka, but also in Ruthe and Seilkopf (1962) and Fowler (1993), cracks are explained as the result of a wrong feed management (e.g. deficiency of minerals) and therefore with diminished horn quality.

7.5.2.3 "Horn rings" in the horn wall

Surprisingly, "horn rings" in the horn wall turned out to be one of the most common alterations in this study, although there were no reports to be found on this occurrence in the returned questionnaires. It seems that this is taken as a normal symptom in the elephants' horn wall. Nevertheless, the look of this feature is similar to the "horn rings" in horses and cattle.

The histological examinations of the "horn rings" show that the same changes can be observed as in the "horn rings" of cattle (Fürst, 1992). Several signs of poor horn quality and an undulatory surface of the stratum spinosum are the most remarkable features. This is in connection with a thickening of the stratum spinosum, which can be explained by a delayed process of differentiation from the stratum spinosum to the stratum corneum. It seems that the "horn rings" come from interim bad horn quality creating an undulatory horn surface on the nails. Probably, this is an effect of bad blood circulation in the corium.

"Horn rings" in horses may be associated with poor horn quality (laminitis), but can also be affected by the seasons (poorer quality horn is produced in winter time). The "grass rings" of spring time are a known phenomenon caused by the new green feed resulting in a bit more

or less horn being produced. This horn is often of poor quality due to some problems such as diarrhoea. "Horn rings" are not inevitably linked to diminished horn quality. They can also be a sign of differing growth intensity. The development of "horn rings" can also be found after a general illness, during gestation and due to deficient blood supply (Fürst, 1992; Geyer, 2004).

The question remains whether the "horn rings" in elephants could also be a consequence of the above mentioned causes or a state of chronic laminitis. This illness still leaves a lot of question marks in connection with elephants, although Clauss and Kiefer (2003) claim that a lot of foot problems in zoo animals are indicated by chronic laminitis, also in elephants. It has to be considered that "horn rings" have also been seen in wild animals. This could support all of the above considerations.

7.5.2.4 Crack in the sole

As mentioned in chapter 4.6.2.1, cracks in the sole are frequently associated with cracks in the horn wall, but also with fistules and abscesses in the sole. As with the nail cracks, the sole cracks can be just superficial, but they may also reach into the living part of the hoof.

Histologically, first horn tubules are conspicuously ordered around the papillae in the same region, which could mean that horn is already more built up proximally to protect the corium. Otherwise, similar alterations as in the nail crack can be observed. This is a sign of poor horn quality. It can be assumed for the sole cracks too that the diminished horn quality promotes the creation of cracks. In sole ulcers, which can also be caused by cracks, the inflammatory process can involve the deeper layers and penetrate as far as the supporting structures.

7.5.2.5 Pododermatitis chronica suppurativa et proliferativa (or foot rot)

Very little of the original structures are recognizable, but this is probably a histological feature of the pododermatitis. Other signs of very poor horn quality are also visible. This is more a consequence of the illness than a cause. A lot of horn material is just decayed. Similar histological alterations as seen in this kind of pododermatitis, were found by Abgottspon (2001) in cattle with foot rot. Even in the wild, ulcerative pododermatitis was seen and was responsible for a high incidence of lameness (Keet et al., 1997). The histopathology of the examined feet showed ulceration with accompanied necrosis and purulent exudation as well as an extensive layer of granulation tissue. Bacteria of different kinds were found in the crusts and edges. The causes for this in the wild were stated to be "drought together with constant elephant browsing, [which] resulted in sharp, woody projections or stubble". Contamination of the surroundings of rare water sources with faeces and urine also promotes the occurrence of this pododermatitis.

7.6 Thickness of the horn at defined locations

These data should help to get an idea of the thickness of the horn in the horn wall, sole and pad. It could be useful for any hoof trimming, for any therapies or simply for having an indication of the dimensions involved.

It is rather difficult to measure at exactly the same point in each hoof. Therefore, the figures are guidelines and the variations are given in the form of descriptive statistics. The reason for these differences can also diverge quite a lot, e.g. horn quality, horn growth rate, use of feet, age and state of health.

The horn portions that are involved in the different measured sections are only differentiable by histological investigations. The same is valid for the assessment of the exact beginning of the horn part. Because of the shape of the horn wall, it is clear that the nail is thin proximally and gets constantly thicker in the distal direction. As already mentioned above, in the distal section of the horn wall, the coronary horn is sometimes abraded. Therefore, it is probably narrower in some cases than it would usually be.

Generally, the captive animals of both species do not differ very much in the thickness of the horn at almost every measured location. The dimension of the horn after the fold increases rather quickly, especially distally in the area of the weight bearing border. The most conspicuous element of the measurements is the fact that, despite the enormous weight of the animal, the thickness of the weight bearing surface reaches only slightly more than 10 mm at different locations in the captive elephants of both species. Remarkable too are the horn layers in the pad, which are very thin in places (e.g. 1.4 mm, 2.9 mm) and the big differences in thickness between different feet at different locations. The thin horn of the weight bearing surface of the captive elephants was considered as one of the most important findings and is confirmed by the small relation of the papillae to the horn part. Due to its abnormal occurrence, the thin weight bearing surface (terminal, sole and pad horn) is regarded as a "locus of minor resistance". Therefore, the statement of Ramsay and Henry (2001), who gave a thickness of 4 to 12 cms for the pad horn layer as a normal measure of thickness was very surprising. The meaning and consequences of these features were discussed in chapter 7.2.4, especially in connection with the at least double thickness of the horn layer of the wild animals. This confirms the questions about the quality and correctness of current husbandry techniques (e.g. foot care, kind of floor).

The measured wild African relatives showed other thicknesses of the horn layer. They are thicker at every location than those of the captive African animals, but the horn wall of the captive Asian elephants is partly thicker than that of the wild African elephants. As mentioned in 6.5.2, the horn wall was not abraded in the histologically examined wild African elephants and, therefore, their horn wall presents a much better constant. In a comparison between the

young wild and captive animals having almost the same thickness, the assumption is made that the young elephant, even in captivity, show a natural thickness.

It would be interesting to compare these data with data from purely wild animals (see also chapter 7.2.4).

Finally, it has to be mentioned that the pad in particular has physiological variations in thickness due to the furrows.

Some questions arise out of this: is this relatively thin horn layer enough to withstand the enormous pressures of weight and was this thickness of the horn layer created by the husbandry in Europe or any husbandry at all (see chapter 7.2.4)? Are these thin horn layers to be found in countries outside Europe, too?

7.7 Biotin blood concentration and its relevance

Biotin or Vitamin H occurs naturally in certain feed stuffs such as e.g. yeast/brewer's yeast (plenty), green feed (moderate) and cereals (very little and bad bioavailability). Deficiencies are seldom in any animal species (Friedrich, 1988). The biochemical effect of biotin in epidermal cells is proved (Fritsche et al., 1991). The importance of biotin for elephants has not been examined much to date (Sadler, 2001).

With the intention of improving hoof horn quality with biotin supplementation, as mentioned for other species (Geyer et al., 1984; Geyer and Schulze, 1994; Josseck et al., 1995; Schmid, 1995; Zenker et al., 1995; Schmitt, 1998; Lischer et al., 2002), an attempt was made to obtain basis values for biotin plasma concentration in non-supplemented and supplemented animals.

As the measured blood concentrations showed, the reabsorption of biotin seems to be good in elephants. How the reabsorption in elephants takes place has not been clarified so far. It is assumed that it works as in horses, because the elephants have a similar digestive tract (Ullrey et al., 1988). As Ullrey (pers. comm., 2004) stated with respect to the elevated blood concentrations of biotin after supplementation, "it has still not been established that foot health would benefit. Considering the apparently poor biotin absorption and retention, I am very sceptical." So it has not been proved so far that biotin reaches the intended domain, despite a high blood concentration. This has not been verified in other species either. It has been demonstrated that biotin with supplementation achieves higher blood concentrations than without. However, the high blood concentration alone is not evidence enough. But, as mentioned above, the effect of improving hoof horn quality has been well demonstrated for pigs (see Geyer and Tagwerker, 1986), horses (e.g. Josseck et al., 1995; Zenker et al., 1995) and cattle (e.g. Schmid, 1995; Lischer et al., 2002). Some observations in elephants also point to improved conditions of the hoof horn, including the pad, after biotin supplementation (Hurtienne, 1985; Geyer, 2004).

Plasma biotin levels provide a very helpful instrument for control if animals are supplemented or not supplemented with biotin. The few results in biotin treated and untreated elephants vary quite a lot. But a border between treated and untreated elephants can be drawn in the region of 500 ng biotin/l plasma. Most of the not-supplemented animals had a blood concentration of less than 500 ng/l and most of the supplemented animals (75 – 100 mg per elephant and day for zoo A and 35 – 50 mg per elephant and day for zoo B) had a blood concentration higher than 500 ng/l. Two measured concentrations (7800 and 1760 ng/l) were over the 500 ng/l. With the first zoo (A), it was generally not clear when the blood samples were taken, but it seems that the very high concentrations were taken a few hours after the biotin feeding. This assumption is based on the fact that Ullrey (1988) measured a peak concentration of 4500 ng/l two hours after feeding a single dose of 200 mg biotin. The concentrations of less than 500 ng/l in the supplemented animals may have one of two explanations. Either, the blood samples were taken before and too long after the biotin feeding as, according to Ullrey (1988), the baseline of biotin concentration was reached again 16 hours after feeding. Secondly, the biotin may not have been given every day nor constantly, or it was given to the wrong animal or the wrong animal ate the biotin supplementation, as was reported in other studies (Geyer and Schulze, 1994; Josseck et al., 1995; Zenker et al., 1995). This could also explain the result of 660 ng/l in an actually not-supplemented animal. Therefore, it is very important that the biotin is given in a reliable manner. The feeder has to be sure that every animal gets the planned amount every day. Zoo (B) reported that it always collected its blood samples 5 to 10 minutes after the biotin feeding. The results were all over 500 ng/l, in comparison to the not-supplemented animals. The basis concentration of biotin in blood was measured by Ullrey (1988) to be 1600 ng/l. In this study, this high basis value could not be established; the unsupplemented blood concentration rather ranged from 280 till 660 ng/l. It could be that in the study of Ullrey (1998) the animals received another feeding, which also contained biotin (e.g. green plants, brewer's yeast, molasses).

In comparison, the normal blood concentration of biotin in horses achieves 200 – 500 ng/l (Josseck, 1991), that of swines 400 – 1100 ng/l (Geyer et al., 1984) and that of cattle 400 – 550 ng/l (Schmid, 1995). Plasma levels in biotin supplemented horses were each over 1000 ng/l (Josseck et al., 1995) and in supplemented cattle 3000 – 8000 ng/l (Schmid, 1995). The concentrations in elephants seem to be much smaller. To prove this, an experiment over a longer period of time, taking blood samples several hours after the biotin feeding, would be needed. Then, the actual peak of the concentrations would be found as in horse and cattle as mentioned above.

It would be better to give biotin twice a day in the same amount as the once daily dose. It is recommended to use biotin supplementation in the form of pellets to ensure that the right elephants get the necessary amount.

For exact demonstrations of biotin effects in foot health, the elephant hooves should be controlled in intervals of about 3 months over 1 to 2 years and the macroscopic foot condition has to be assessed regularly.

7.8 Conclusions

The study describes the anatomy of the normal structures of an elephant's hoof at a macroscopic and microscopic level. The histological view of the hooves in particular confirms their similarity to those of cattle and horses and could prove, but also contradict, some long held assumptions. Besides, the common occurrence of poor horn quality could be detected in the different segments of the hooves, together with the discovery of some "loci of minor resistance", which are found partially only in captive elephants' hooves and partially in captive and wild animals' hooves. This knowledge allows the consideration that poor horn quality and "loci of minor resistance", associated with the weight of these animals and the husbandry so far from their countries of origin, have the effect of promoting foot problems of captive elephants. Even the "loci of minor resistance", which occur in captive and wild animals would cause problems in husbandry, depending on the surface of the enclosure. The "loci of minor resistance", which also occur in wild animals probably happen partially through the normal abrasion provoked by the ground. The microscopic descriptions of some macroscopically pathological alterations are also given. This gives a possibility for further investigations to assess whether the healed areas with the newly built horn can withstand the load so that no recurrence happens.

An important conclusion is the recognition that the differences between Asian and African elephants are very slight microscopically. Therefore, there were no significant indications for the differences in the occurrence of foot problems as Ramsay and Henry (2001) claimed. Anyway, it has to be accepted that African elephants show roughly the same foot problems as the Asian species, but in this study the African elephants have not been sufficiently well represented to make a definite statement. Differences found macroscopically and microscopically between the wild and captive elephants are another explanation for foot disorders of the animals in captivity and for demonstrating that the confined elephants have involuntarily changed a part of their foot anatomy. Equally, the measured horn growth rate in the horn wall can support the assertion of a prognosis for the time needed for any pathological alteration in the horn wall to grow out.

This study demonstrated that the floor in most zoos might be made of a substrate that is too hard. In addition, the thin sole and pad horn encourages foot disorders and is caused by a

floor that is too abrasive and foot care that is too frequent. Anyway, foot care should be done in all 4 feet at the same time due to the risk of an artificial pressure distribution. Another cause for foot problems and poor horn quality might be the feeding, which has not been thought the case up to now. To clarify all these considerations, more investigations are needed.

The husbandry situation of captive Asian and African elephants can be compared to a certain degree with that of the Indian and African rhinoceros. They are also both kept under the same conditions, although the Indian rhinoceros needs a soft and non-abrasive floor material to match its natural habitat. It has also been shown that the foot problems of the rhinoceros have arisen from faulty husbandry (von Houwald, 2001). Equally, it stands to reason that the same will be valid for both elephant species. The differences of the habitats of the Asian and African elephants should be considered when choosing the type of floor when planning a new enclosure. This is confirmed by the few macroscopically anatomical differences in the feet of the two species.

After the discussion of different studies, it can also be taken as a fact that the blood supply within the foot is of prime importance. Therefore, exercise and motion in captivity is not just essential for abrasion of the horn, but also for a better blood supply and therefore a better horn growth rate and horn quality.

Features for the evaluation of horn quality are given and they can be taken as a reference for assessing horn samples. If the horn quality is recognized early as diminished in a single animal or in a group, before any macroscopic alterations occur, protracted foot disorders can possibly be prevented.

Because even neonate to juvenile elephants showed histological alterations and signs of bad horn quality, the conclusion can be drawn that this is an occurrence not just limited to individuals (certainly with exceptions), but a husbandry problem in general consisting of different factors. A genetic factor might also be involved.

For a definitive and exact judgement of horn quality, further investigations are required such as checking the tensile strength, the hardness and content of dry matter and realizing tests with the horn placed in different environmental substances (e.g. faeces, urine). Equally, further detailed studies of the digital cushion would be recommended due to the fact that this anatomical structure is the most important part for cushioning the enormous weight of this animal.

As for swines (Geyer et al., 1984), so for elephants: the great strain on the hooves and the resultant susceptibility to injuries make it necessary to improve the hoof quality by adapting the enclosure facilities, the quality of care and the nutrition of the animals.

Guidelines for the thickness of the horn layer at different locations could be stated, which helps when carrying out treatment or foot care. Here there was a big difference between wild and captive elephants.

Further questions arise, for example what do the histological structures of the wild-living elephants look like and are histological symptoms of laminitis found in horn samples in the hooves of elephants (Marks and Budras, 1987; Clauss and Kiefer, 2003). It becomes clear that a lot of questions are still unanswered and they should all be clarified.

The causes of many foot problems remain speculative, due to the lack of possibilities for direct experimentation on elephants. Therefore, the opinions about them vary widely among elephant specialists. Nutrition, the type of flooring, body weight, foot care, hygiene, access to moisture, climate or exercise certainly appear to influence the health of the feet of the elephants. Therefore, all these factors have to be taken into account when evaluating feet problems. Buckley (2001) summarized some considerations of the causes of foot problems and introduced some recommendations for adapting the husbandry systems, too.

The study also presents an approximate borderline for the recognition of biotin supplementation, which lies around 500 ng/l biotin in the plasma of supplemented or not-supplemented animals. Biotin can be given as a prophylaxis in a therapeutic dosage and not only after the occurrence of foot disorders because if the alterations are already present, the blood supply, a precondition for the effect of biotin, might be disturbed and the effect of biotin is then diminished. This could also be a cause for the ineffectness of biotin in many cases. Therefore, treatment with biotin can only be effective as long as the triggered factors are not eliminated. Due to the fact that biotin does not increase the horn growth rate, the time of replacement of the horn has to elapse before any results of the biotin supplementation will be seen. In a case of no therapeutic efficacy during biotin supplementation, it is possible that the horn damage is triggered by causes that are not susceptible to biotin, such as mechanical or environmental influences (e.g. floor, microclimate, feeding). Of course, further investigations would be necessary for evaluating the effect of biotin over a longer period of time observing several elephants under the same conditions in a double-blind-study that would include a microscopic control of the macroscopically visible improvements and of the improvement of the horn quality provoked by biotin. Additionally, a more detailed study into the development of the blood concentration of biotin after biotin supplementation with several elephants over a longer period of time should clarify the absorption and retention rates of biotin.

The aim in favour of the elephants should be to encourage collaboration between keepers, vets and zoologists as well as among the zoos themselves for finding solutions to permanently improve husbandry and particularly elephant foot health.

8 Appendix

CASE HISTORY

Name of the institution: _____

Signalement:

Name of the elephant: _____ Date: _____

Species: _____ Sex: _____ Age: _____ Weight: _____

Husbandry:

- | | | |
|--|--|---|
| <input type="checkbox"/> Inside enclosure | <input type="checkbox"/> Outdoor enclosure | <input type="checkbox"/> Chained during feeding |
| <input type="checkbox"/> Chained during night | <input type="checkbox"/> Free-ranging | <input type="checkbox"/> Pasture |
| <input type="checkbox"/> Area of diverse terrain | <input type="checkbox"/> Natural substrates | <input type="checkbox"/> Elephant riding |
| <input type="checkbox"/> Exercise | <input type="checkbox"/> Walking | <input type="checkbox"/> Digging |
| <input type="checkbox"/> Daily washing | <input type="checkbox"/> Mud bath area | <input type="checkbox"/> Pool |
| <input type="checkbox"/> Moving tree stumps | <input type="checkbox"/> Dusting | <input type="checkbox"/> Demonstrations |
| <input type="checkbox"/> Training | <input type="checkbox"/> Several feeding station | <input type="checkbox"/> Feeding at different times |

Stream through the enclosure Others: _____

Feeding: _____

Changing of the feeding? If yes, what kind of changing? _____

Type of floor in the inside enclosure: _____

in the outside enclosure: _____

Information to the animal's health:

For females: Pregnancy: _____ Number of birth: _____

Former diseases: _____

Latest diseases: _____

Foot disease:

Foot care: How often/year: _____ The last time: _____ Done by who: _____

Case history of the foot disease / pretreatment / therapeutic efficacy

EXAMINATION FORM FOR LAMENESS

Name of the elephant: _____ Date: _____

Clinical examination: _____

Position of the extremities:

Front legs: _____ Hind legs: _____

Visual examination when resting:

Front legs: _____ Hind legs: _____

Evaluation of the gait:

normal cautious stiff

Difference between soft/hard surface: _____

Kind and degree of lameness:

Definition of the degree: 0 = none, 1 = slight, 2 = moderately, 3 = high-grade

Left front leg: _____

Right front leg: _____

Left hind leg: _____

Right hind leg: _____









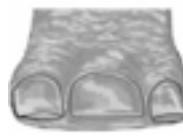



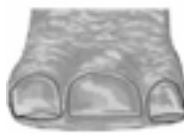



Kind of symptoms in the feet (lf: left front; rf: right front; lh: left hind; rh: right hind):

Pain: lf, rf, lh, rh Heat: lf, rf, lh, rh Swelling: lf, rf, lh, rh

Others: _____

EXAMINATION FORM FOR ASIAN ELEPHANT HOOVES

Name of the elephant: _____ Date: _____

Horn quality Hardness soft hard very hard Consistency crumbly compact Humidity humid dry rough	Left front foot  apical  palmar  lateral  medial	Right front foot  apical  palmar  lateral  medial
Nail		
W. B. B.		
Sole		
Pad		
Horn quality Hardness soft hard very hard Consistency crumbly compact Humidity humid dry rough	Left hind foot  apical  plantar  lateral  medial	Right hind foot  apical  plantar  lateral  medial
Nail		
W. B. B.		
Sole		
Pad		

The different alterations are subdivided into degree 0 to 3: 0 = none, 1 = slight, 2 = moderately, 3 = highgrade. They can be drawn into the figures and the number of digit has to be named where the alteration is:

Nail: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, exungulation, grooves, other alterations, angle of the nail against the ground


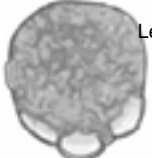






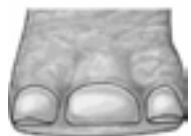



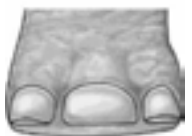



Weight bearing border (W. B. B.): abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, other alterations

Sole: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, other alterations

Pad: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, foreign body, infection, type of surface (1 = circle-shaped horn pieces, 2 = less furrowed, 3 = smooth), thickness of the horn part (1 = thin, 2 = normal, 3 = thick)

EXAMINATION FORM FOR AFRICAN ELEPHANT HOOVES

Name of the elephant: _____ Date: _____

Horn quality Hardness soft hard very hard Consistency crumbly compact Humidity humid dry rough	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  apical </div> <div style="text-align: center;">  palmar </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;">  lateral </div> <div style="text-align: center;">  medial </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  apical </div> <div style="text-align: center;">  palmar </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;">  lateral </div> <div style="text-align: center;">  medial </div> </div>
Nail		
W. B. B.		
Sole		
Pad		
Horn quality Hardness soft hard very hard Consistency crumbly compact Humidity humid dry rough	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  apical </div> <div style="text-align: center;">  plantar </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;">  lateral </div> <div style="text-align: center;">  medial </div> </div>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  apical </div> <div style="text-align: center;">  plantar </div> </div> <div style="display: flex; justify-content: space-around; margin-top: 10px;"> <div style="text-align: center;">  lateral </div> <div style="text-align: center;">  medial </div> </div>
Nail		
W. B. B.		
Sole		
Pad		

The different alterations are subdivided into degree 0 to 3: 0 = none, 1 = slight, 2 = moderately, 3 = highgrade. They can be drawn into the figures and the number of digit has to be named where the alteration is:

Nail: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, exungulation, grooves, other alterations, angle of the nail against the ground

Weight bearing border (W. B. B.): abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, other alterations

Sole: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, infection, other alterations

Pad: abscess, crack (vertical and horizontal), fistule, fissure, overgrown, foreign body, infection, type of surface (1 = furrowed, 2 = less furrowed, 3 = smooth), thickness of the horn part (1 = thin, 2 = normal, 3 = thick)

HEALING PROCESS

Name of the elephant: _____ Date: _____

Day	Nail	W. b. b. ¹	Sole	Pad	Comments

Note: ¹ W.b.b. = Weight bearing border

Recurrence of the different problems or the same disease in different elephants or the same animal?

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10 Acknowledgement

This thesis was only possible due to the intensive support and help of many people. I am particular grateful to the following:

Prof. Dr. H. Geyer for providing the topic, for the excellent scientific collaboration and for his financial and moral support. He was always convinced that everything was going well. His friendliness and helpfulness at all times has provided a great example for me.

Prof. Dr. K. Eulenberger for taking on the role of co-referee and for the detailed correction of the manuscript as well as for his support during the study.

Dr. T.B. Hildebrandt and Dr. G. Fritsch of the Institute for Zoo and Wildlife Research/Berlin, Dr. G. Weissengruber of the Anatomy Department of the University of Veterinary Medicine/Vienna, Dr. R. Asher and Dr. P. Giere of the "Naturkundemuseum"/Berlin, Mr. D. Oppliger of the "Naturhistorisches Museum" in Basel, Zurich Zoo and Diergaarde Blijdorp Rotterdam for donating some of their collected elephants' feet or samples of elephants' feet for the histological part of the study.

Dr. F. von Houwald of Basel Zoo, Dr. W. Schaftenaar of Diergaarde Blijdorp/Rotterdam, Dr. W. Rietschel of "Zoologisch-Botanischer Garten Wilhelma"/Stuttgart, Dr. A. Ochs of Berlin Zoo, Prof. Dr. J.-M. Hatt of the University of Zurich and Dr. R. Zingg of Zurich Zoo for supporting me and my thesis in many different ways.

Dr. W. Zenker of "Schönbrunner Tiergarten"/Vienna for his particular support, efforts and help during the study. He has a very friendly and supportive manner and is always open to any kind of questions.

Dr. J. Deepanij for leading me through different elephant institutions in beautiful Sri Lanka and becoming a good friend.

All the elephant keepers and vets of the zoos that participated in this study for their cooperation and interest in the topic and all the elephants in the visited zoos who had to stand with raised feet for some time or to stand on a funny plastic foil. Not all of them always appreciated it.

Dr. M. Hofmeyer and Dr. I. Whyte from Kruger National Park/South Africa, as well Dr. P. Martelli from Singapore Zoological Gardens and Dr. H. Schwammer from “Schönbrunner Tiergarten”/Vienna for sending several figures of wild and captive elephants and for supporting me with information.

Mrs. J. Peter for doing the enormous job of inserting the graphics that adorn this thesis. She was always very helpful and enthusiastic as well as very interested in the topic.

Dr. M. Hässig for advice on the statistical analysis.

Mrs. J. Leclerc, Mrs. V. Horvat and Mrs. E. Bohrer-Siegenthaler for their helping hands during the laboratory work.

All members of the Institute of Veterinary-Anatomy for their help in every possible way and for ensuring I had a good time.

Roche Vitamine GmbH, Grenzach-Wyhlen/Germany for providing Rovimix[®] H-2 (biotin).

A special thank you to Dr. G. Weber, DSM Nutritional Products/R&D, Human/Nutrition and Health – Analytics (VFHA), Kaiseraugst/Switzerland for determining biotin in the blood samples and sponsoring the printing of the thesis, which is realised by R. Affentranger, RoNexus Services AG, Basel/Switzerland.

Kristin Munzinger, Christa Blessing, Nicola Benz for proof-reading the manuscript and August Benz for helping editing and organizing the text.

Finally, my family and Stephanie Blessing for their support and encouragement in many different ways and for standing through hard times with me. I am deeply indebted to all of you. Last but not least, a big thank you to Carmen who kept me company during all the time in front of the microscope and computer.

Dear friends, I am back again!

Curriculum Vitae

Name	Andreas Benz
Date of Birth	8th of July 1974
Nationality	Swiss
Place of Origin	Schübelbach SZ
1989 – 1994	Christ-King College Nuolen Matura Type B
1995 – 2001	Study of Veterinary Medicine at the Veterinary Medicine Faculty of Zurich University
2001	Final exams at the Veterinary Medicine Faculty of Zurich University
2002 – 2003	Assistant in the clinic for large animals of Dr. Bernhard Benninger, Mettau
2002	Start of doctoral thesis at the Veterinary- Anatomy Institute of Zurich University
2003 - 2004	Zoo-vet and responsible person for the gorillas at Schmiding Zoo in Krenglbach, Austria
Since 2005	Assistant in the clinic for small animals “Kleintierklinik am See” of Dr. Markus Fehr, Rorschach

29 September 2005