THE HISTOLOGY OF HUMAN DRY BONE (A REVIEW)

Histología de huesos humanos secos (una revisión)

H. H. DE BOER* and G. J. R. MAAT*

ABSTRACT Despite archaeological preservation conditions, the histomorphology of human dry bone still contains useful information on the physiological and pathological status of deceased individuals. Histology can therefore be a helpful addition to routine archaeological methods. But practice has shown that, for many archaeologists, unfamiliarity with this technique shaped a pointless obstacle to add it to their tool box.

Thus, after having addressed the restrictions associated with histological analysis in general, we will show that the preparation of sections/slides does not need to be difficult, expensive or time-consuming. Then we will provide an introduction to the histological application of assessing age at death of the deceased. It's must be its theoretical basis, its value in comparison to other methods and its limits are discussed.

Finally, we will elaborate on the effectiveness of histology as an indicator of pathological processes, and explain that only a small number of disorders have distinct 'pathognonomic' microscopic features. In all other cases, the histological findings must be combined with gross anatomical and radiological findings from the same individual to come to a conclusive diagnosis or to a shortened list of differential (alternative) diagnoses.

Key words: Histology, Microscopy, Palaeopathology, Human dry bone, Age assessment, Diagnosis, Disease.

RESUMEN Independientemente de las condiciones de conservación arqueológica, la histomorfología de hueso seco humano conserva información útil sobre el estado fisiológico y patológico de las personas fallecidas. Por lo tanto la utilización del análisis histológico puede ser una adición útil a los métodos arqueológicos de rutina. No obstante, en la práctica se ha demostrado, que para muchos arqueólogos la falta de familiaridad con esta técnica constituye un obstáculo para su utilización. Por lo tanto, después de haber abordado las restricciones asociadas al análisis histológico en general, vamos a demostrar que la preparación de secciones / diapositivas no tiene por qué ser difícil, costoso o requerir mucho tiempo para su utilización. En este trabajo se expone una introducción a la aplicación de la evaluación histológica para poder determinar la edad de la persona fallecida. Se discute su base teórica, su valor en comparación con otros métodos y sus límites. Por último, vamos a tratar la eficacia de la histología como indicador de procesos patológicos y mostrar que sólo un pequeño número de trastornos tienen características microscópicas

* Barge's *Anthropologica* Dept. of Anatomy and Embryology, Leiden University Medical Center. Postzone S-01-PP.O. box 9600, 2300 RC Leiden The Netherlands. *h.h.deboer@amc.nl*

Fecha de recepción: 24-01-2013. Fecha de aceptación: 28-06-2013.

'patognonomicas' diferenciadas. En todos los demás casos los datos histológicos deben ser combinados con los resultados anatómicos macroscópicos y radiológicos del mismo individuo para obtener un diagnóstico concluyente o una lista abreviada de diferentes (alternativos) diagnósticos.

Palabras clave: Histología, Microscopía, Paleopatología, Hueso seco humano, Determinación de la edad, Diagnóstico, Enfermedad.

INTRODUCTION

On a light microscopic level, bone tissue contains useful physiological and pathological information. Investigation of bone by means of microscopy can therefore aid in the understanding of the effect that various phenomena such as ageing, mechanical strain, nutrition, genetics, general health and acquired diseases have on bone tissue (Frost, 1985; Vigorita, 1999). For archeologists this is of special interest, since over the past century it was shown that in dry bone tissue microarchitecture remains for a great deal intact, despite archaeological preservation conditions (Stout and Simmons, 1979).

To meet the specific demands of archaeological research, investigators extrapolated on routine histological methodology developed by the study of normal, physiological bone tissue or of the post-mortem alterations thereof in the first place. For instance, methods originally applied to study the bone remodeling process were further developed into methods to estimate the age at death of the deceased (e.g. Kerley, 1965; Stout and Simmons, 1979; Stout and Paine, 1992; Maat, 2006). Furthermore, microscopic studies on the biology of micro-organisms have proven to be of value to determine the extent and cause of taphonomical processes in excavated bone material (e.g. Hackett, 1981a; Bell, 1990; Hedges, 1995; Nielsen-Marsh, 2000a, 2000b; Jans, 2004). Also a long tradition of microscopic investigation of pathological human remains exists. 'Histopalaeopathologists' focused, much in line with their medical counterparts, on the microscopic diagnosis of diseases. All in all, light microscopy has become an important tool in the investigation of human remains from an archaeological context.

Despite the valuable contribution that light microscopy may have on archaeological research, many archaeologist are reluctant to use histological techniques. To some extent, this might be due to the understandable averseness toward invasive section-taking. However, it might also be due to unfamiliarity with histological techniques and the histomorphology of normal and pathological bone tissue. In this article we offer an introduction into the most basic techniques necessary to execute the histological analysis of human dry bone. In addition we provide a concise overview of histological analytical methods that are useful for osteoarchaeologists.

A discussion of the histological differentiation between human and animal bone or the histomorphology of taphonomical processes is beyond the scope of this article. For a recent review on the former see Hillier and Bell (2007). The latter is comprehensively discussed in various other publications (e.g. Hedges, 1995, 2002; Collins, 2002; Jans, 2002).

1. RESTRICTIONS WITH RESPECT TO THE HISTOLOGY OF HUMAN DRY BONE MATERIAL

Due to the specific nature of dry bone, its histology is considered challenging. By definition, dry bone remains are void of any remaining soft tissue and bone cells, leaving only the mineralized 'framework' for analysis. As medical pathologists use the soft-tissue components to come to an eventual diagnosis in the first place, absence of soft tissue severely hampers the interpretation of the remaining dry bone tissue.

Furthermore, post-mortem processes such as weathering, microbial destruction, protein degradation and mineral replacement (fossilization) can modify the original histomorphology. These 'taphonomical alterations' can subsequently lead to focal destruction, presence of included material, microfissures and loss of birefringence (Jans, 2002). As a result, taphonomical processes may lead to partial, or even total destruction of bone histomorphology. Histological analysis may therefore be of little value in badly preserved remains.

Finally, during life, bone tissue is a dynamic structure that is continuously adapting to a multitude of stimuli (Frost, 1985). Among others, growth, ageing, disease and nutrition all have their effect on bone (histo)morphology. As bone of the living can only react to a stimulus in a limited number of ways (see below), and as bone of the just deceased only represents a static end-point, and as taphonomic processes will even further alter that end-point status, reconstruction of past processes is indeed demanding.

The above mentioned dynamics make in-depth knowledge of the growth, ageing and pathology of bone tissue a prerequisite. Although most of this knowledge can be easily acquired from medical histology textbooks (Ross, 1995; Rosai, 2011), or from specialized orthopaedic pathology books such as Vigorita (1999), consultation of an (orthopaedic) pathologist is often wanted for.

2. THE PREPARATION OF SECTIONS/SLIDES FOR MICROSCOPY

The methods used for the preparation of sections for microscopy originate from those used in laboratories for anatomy and pathology. Still, the specific nature of dry bone does not allow for the direct application of these routinely used histological methods. For instance, the usual prior decalcification of the specimen is not an option, as this will destruct (chemically solve) it. Undecalcified tissue on the other hand, does not allow for sectioning by knife-microtome. The brittleness of the bone mineral 'framework' will cause the unembedded specimen to pulverize, or will at least create 'wash board' section surface deformities.

Osteoarchaeologists introduced various methods to tackle these problems. Unfortunately, the majority of these methods are time-consuming, costly and need specialized (automated) equipment. See for instance: Stout and Teitelbaum, 1976; Sturmer, 1979; Wolf, 1983; Schultz, 1988; Wallin, 1985. For archaeologists on a low budget and without access to a fully equipped histology laboratory, the manual methods as proposed by Maat (2001) and De Boer (2013) are good alternatives. These methods were widely tested (Beachesne and Saunders, 2006; Martiniakova, 2006; Van der Merwe, 2010; Turner-

Walker and Mays, 2008; Haas, 2011 pers. comm.) and proved to be suitable for cortical and even trabecular/fragile bone (Plate I). The methods are swift, cheap, and easy to learn and are widely applied by starters and experienced workers. Detailed manuals can be directly requested for free from the authors.

3. HISTOLOGICAL AGE-AT-DEATH ESTIMATION

Individual age at death assessment is one of the most basic analyses osteoarchaeologists have to deal with. Routinely used gross anatomical methods, such as dental eruption status, epiphyseal closure, cranial suture obliteration, or the alterations of the face of the pubic symphysis, the iliac auricular surface, the cancellous tissue in the proximal ends of long bones and of the fourth rib end, all share the precondition that they need gross skeletal anatomy for a great deal intact. In case of microscopic methods only a small, but essential portion of a bone is needed (the central shaft of the femur, of the clavicle, a rib, etc).

Kerley was the first to make an useful attempt to utilize microscopy for age-at-death assessment, when she published her seminal work on 'the microscopic determination of age in human bone' in 1965 (Kerley, 1965). The method was based on the principle that during life, cortical bone tissue of the shaft of a long bone undergoes gradual conversion/remodeling from circumferential lamellar bone (i.e., bone that was circumferentially deposited during the first anlage of the cortex, embedding pre-existing blood vessels, so-called non-Haversian canals) into new lamellar bone of Haversian systems (osteons with a central blood vessel). The 'older', the more mature, the bone, the more the original circumferential lamellar bone with its non-Haversian canals is replaced by Haversian systems. Replacement is executed by Basic Multicellular Units or BMUs. A BMU's consists of a 'cutting cone' at its head end and a 'closing cone' at its tail end that respectively 'drills' and replaces existing bone tissue. On a cellular level, the cutting is done by osteoclasts in the head end cone, 'drilling' a longitudinal channel. In the rear end cone, osteoblasts deposit new layers of lamellar bone, thereby 'closing' the channel (Plate II). The result, in a transverse microscopic slide, is a Haversian system (osteon) bordered by a jagged cement line that abruptly halts/cuts the original circumferential lamellae. This latter feature differentiates it from a non-Haversian canals, which borders are flowing in line with the original circumferential lamellar bone and that misses a cement line (Plate III). The rate of remodeled bone gives an estimation of the age at time of death. Kerley defined this in various bone specific regression formulas (Kerley, 1965, 1978). Initially, her method used diaphyseal cross sections of femoral, tibial and fibular cortices.

Several authors confirmed the correlation between histomorphology and age at these locations (Ahlqvist and Damsten, 1969; Stout, 1982; Thompson, 1979; Ericksen, 1991; Maat, 2006). Although the method also proved to be useful in manibulae (Singh and Gunberg, 1970), in ribs and clavicles (Stout and Paine, 1992) and in humeri and ulnae (Thompson, 1979), the femoral mid-shaft remained to be the most utilized (Maat, 2006).

In her original publication, Kerley used osteon counts (counts of intact Haversian systems), counts of fragmented/'older' osteons, the percentage of 'original' circumferential

lamellar bone and counts of non-Haversian canals for her regression formulas. However, the use of these features showed to cause definition and identification problems (e.g. Stout and Stanley, 1991), and thus to generate substantial inter-observer bias (Lynnerup, 2006). Various authors tried to tackle this problem by using other derivatives to define the developmental status of the remodeling process, e.g. by counting aggregations of osteons plus osteon fragments thus outlining the total amount of remodeled bone (Ahlqvist and Dahmsten, 1969; Thompson, 1979; Ericksen, 1991), or inversely, counting the percentage of bone surface occupied by original circumferential lamellar bone plus non-Haversian systems thus outlining the total amount of un-remodeled bone (Maat, 2006). In this way the definition problems could be circumvented to a large degree.

Kerley (1965) counted at four positions in a mid-shaft cross section of the femur (anterior, posterior, medial and lateral). Alhqvist and Damsten (1969) also used four fields, but positioned them between those of Kerley, thereby avoiding the linea aspera, the insertion site of thigh muscles. Mechanical strain by these muscles produces a histomorphology unrelated to age. To avoid muscle insertions and complete transsection of a femur, others used only the anterior parts of the shafts (Ericksen, 1991; Maat, 2006). Thompson (1979) proposed an even less invasive method, by using only a small core of bone tissue, at the expense of a higher statistical bias. Irrespective of which method is adopted, spatial variation in histomorphology within a single transverse slide is high (Saunders, 1987). Also, counting results between bones within a single individual differ (Stout and Stanley, 1991). Sampling and counting must therefore be done, exactly as prescribed in the original manuscript.

Although the overall results of histological age assessment showed to be acceptable, a few considerations need to be addressed. Bone remodeling is indeed defined by age to a great extent. Nevertheless, inter-individual variation in pace of ageing exists. Other factors have an effect too, for instance: disease, nutrition and mechanical stress (Frost, 1985). Correlation rates (r^2) between microscopic ageing features and true age at death of around 0.7-0.8 and standard errors of circa 10 years seem to reflect the highest possible accuracy (Maat, 2006). In spite of suggestions by some, difference between the sexes appears to be neglectable (Uberlaker, 2005; Maat, 2006)

The gradual increase in the amount of remodeled bone, or the decrease of the percentage of unremodeled bone tissue over age, is not a linear one, but a curvilinear process that comes asymptotically to an end when the last fragment of unremodeled bone tissue is remodeled (Kerley, 1965; Maat, 2006). Most regression-formulas did not take this into account and adopted an unnatural linear correlation.

The degree of inter-observer bias was addressed in several studies (Baccino, 1999; Lynnerup, 2006). Yet, in cases in which the method was deployed by experienced microscopists, inter-observer bias was shown to be low (Maat, 2006). Ideally, the histological approach should be used alongside other age assessment methods (Baccino, 1999).

4. THE HISTOLOGICAL DIAGNOSIS OF DISEASES IN GENERAL

On a gross anatomical level, the identification of pathological processes and their interpretation in fresh human skeletal remains is complicated. Their histology, histo-

palaeopathology, in dry bone tissue is even more difficult as bone can generally only react to a stimulus/disease in a very limited number of ways: resorption of bone tissue (an osteoclastic bone response), deposition of new bone tissue (an osteoblastic bone response) or a combination of the two (see e.g. Frost, 1985; Vigorita, 1999). This restricts the differential diagnostic power of microscopy. On a microscopic level, the reaction of bone tissue may be osteoclastic/resorptive, osteoblastic/depositional or a combination of both. Only a very few diseases present with a 'pathognomonic', specific, histomorphology. In all other cases, histomorphology alone is unable to produce a final diagnosis. More often, the combination of gross anatomical/radiological characteristics and distinct (but on itself not pathognomonic) histomorphology may provide a conclusive diagnosis. In most cases, histology must be regarded as a tool to reduce the list of differential diagnoses, or to support an established diagnosis. Below, the few disorders with pathognomonic microscopic features are discussed.

4.1. Disorders with pathognomonic histomorphologic features

Paget's disease of bone is an excellent example of a disease with a pathognomonic histomorphology. In this disease, the normal physiological bone remodeling rate is elevated and chaotic. Microscopy shows alternating fields of excessive osteoclastic and osteoblastic activity (Ralston, 2008). Although the earliest phase of the disease demonstrates non-specific resorption, the osteoclastic-osteoblastic interplay produces a 'mosaic' pattern of woven and lamellar tissue, demarcated by numerous convoluted cement lines in trabecular and cortical bone tissue. Several palaeopathological cases of this 'Pagetic' histomorphology have been reported (Stout and Teitelbaum, 1976; Bell and Jones, 1991; Aaron, 1992; Roches, 2002).

Hyperparathyreoidy is caused by elevated blood serum levels of the hyperparathyroid hormone, an activator of widespread osteoclastic activity. Though osteoclastic resorption by itself is not a specific process, its pattern in case of hyperparathyreoidy is particularly outspoken in (sub)periosteal and in cancellous bone tissue (Vigorita, 1999; Fraser, 2011). In the latter, it causes the typical and pathognomonic 'dissecting osteitis' or 'tunneling resorption'. Hyperparathyreoidy is very rare and comes without any external gross anatomical bone changes. Still, various palaeopathological cases have been discovered in well-preserved dry bone skeletons (Weinstein, 1981; Cook, 1988; Zink, 2005).

The histomorphology of osteomalacia (the adult form of hypovitaminosis D) in dry bone tissue is dominated by an overall increased osteoclastic activity, as the living bone attempts to keep blood serum calcium levels within a normal range (Stout and Teitelbaum, 1976; Schamall, 2003; Brickley, 2007). The pathognomonic histomorpholoy of osteomalacia is not based on its specific osteoclastic pattern but on microscopic vestiges/remnants of calcification defects of osteoid. Osteoid is the 'to-be-mineralized' bone matrix substance. Brickley reported that these vestiges may show as so-called 'defect osteoid lines' in dry bone Haversian systems. They are caused by the post-mortem decomposition of osteoid (Brickley, 2007). It should be noted that this feature has not been reported by other palaeopathologists. Nevertheless, knowledge on these vestiges was supported by comparative fresh tissue specimens of known disease cases.

Osteoporosis is a symptom, not a disease/diagnosis and is defined as a decrease in total bone volume. As such, its histomorphology is pathognomonic by definition. Palaeopathologists used metric (Richmanna and Ortner, 1979; Martin and Armegalos, 1979 and 1985; Gonzales-Reimers and Arnay de-la-Rosa, 1992; Velasco-Vazques, 1999; Paine, 2006; Cho and Stout, 2011) and non-metric histomorphological methods to establish its existence (Roberts, 1992; Schultz, 1999). As osteoporosis is a disorder in terms of quantity, theoretically a metric diagnostic approach should be preferred.

Finally, accurate diagnoses can sometimes be made when 'dry bone', assumed to be composed of its mineral component only, still appears to contain remnants of soft tissue/cells, even if fossilized. In such cases, the combination of dry and soft tissue histology may provide a sound diagnosis. Examples are, for instance: a calcified myoma uteri (Strouhal, 1976), a sacral neurolemmoma (Strouhal, 2004) and sickle cell anaemia (Maat and Baig, 1990; Maat, 1991). Although such findings are rare, they illustrate that histology may yield unexpected results.

4.2. The complementary value of histology in the diagnosis of other diseases

The disorders in this category miss pathognomonic histomorphologic characteristics. Nonetheless, histological analysis may yield considerable corroborative information.

In scurvy, avitaminosis C, the increased tendency to develop subperiosteal hematomas may lead to their ossification if fresh supplies of vitamin C become available to the sufferer (Maat and Uytterschaut, 1984; Ortner, 2003; Maat, 2004). Gross anatomically, this pathologic change may look similar to that of infectious and tumorous lesions or to that of ossified hematomas from mechanical traumata or to hypertrophic (pulmonary) osteoarthropathic depositions. But in contrast to the latter two options, scurvy has a distinct/specific distribution pattern of depositions (Maat, 2004). And in contrast to the first two alternatives, ossified hematomas leave the periosteal surface intact (Schultz, 2001; Maat, 2004). This feature can only be properly visualized by microscope (Plate IV). Thus, ossified hematomas are not pathognomonic for scurvy. But if they occur symmetrically at anatomical predilection sites, i.e. especially at the metaphyses in the lower extremities, and come with other scorbutic characteristics, then the diagnosis becomes almost certain.

Like in case of osteoporosis, 'cribra orbitalia' and 'porotic hyperostosis' are non-specific disease features. Histology may help to differentiate between their potential causes: infection, taphonomy, chronic anaemia (hereditary or acquired haemolytic forms; Marscik, 1984; Maat and Baig, 1990; Maat, 1997; Schultz, 2001; Wapler, 2004; Walker, 2009). In the latter case, a radiating hypertrophy of hematopoietic bone marrow will expand the amount of cancellous bone at the cost of the external cortical lamina. In infections, the histomorphology is dominated irregular lytic changes and abundant fields of Howship's lacunae. Taphonomic causes demonstrate an absence of osteoclastic or —blastic processes and a disregard for microscopic anatomical dimensions/borders. Although none of these features are pathognomonic, the 'overall histological image' can be highly indicative for either one of the causes.

In primary bone tumours, histology can help to identify whether the growth process is benign or malignant. The former demonstrates a more regular/organized texture, grows non-invasive and is predominantly osteoblastic (Strouhal, 1996; Vyhanek, 1999; Hershkovitz, 1999; Eshed, 2002). Malignant lesions are invasive by definition, and present both osteoblastic and osteoclastic activity. In malignant lesions, the erosive areas feature numerous fields of Howship's lacunae, whereas the new appositional bone is an irregular combination of lamellar and woven bone. It shows little to no signs of regular physiologic remodeling (Suzuki, 1987; Schultz in: Strouhal, 1991; Strouhal, 1997). None of these features is pathognomonic, not even for tumours. Still, in combination with gross anatomical and radiological data, microscopy will cut back the list of differential diagnoses. Histology has proven to be especially useful if comparative fresh tissue samples of documented cases are available (Schamall, 1999; Eshed, 2002).

Secondary bone tumours (metastases) are malignant by definition. Their histomorphology is therefore similar to that of malignant primary bone tumours. In addition, the apposition of new bone in Haversian canals may suggest a hematogenous dissemination (Anderson, 1992; Wakely, 1995). In concordance to clinical practice, metastases are defined as either osteoblastic, osteoclastic or a combination thereof (examples of fresh tissue pathology references: Roodman, 2011; examples of palaeohistopathology references: Tkocz and Bierring, 1984; Campillo and Mari-Balcells, 1984; Grupe, 1988; Anderson, 1992; Schultz, 1993; Wakely, 1995; De la Rua, 1995; Sefcakova, 2001; Schultz, 2007; Molnar 2009). Histology is an apt instrument for such differentiation. Yet, the differentiation may be of little practical value for the diagnosis of the type of tumour, since there is no fixed relationship between the histology of the metastasis and the original/primary tumour. Clinical practice shows that a primary tumour may have both osteoblastic and osteoclastic metastases (Roodman, 2011).

In all tumorous lesions (primary or secondary), the likely diagnosis depends on data such as age at death, sex of the individual, distribution pattern of the lesions and the gross anatomical/radiological appearance (e.g. Suzuki, 1987; Schultz: in Strouhal, 1991; Wakely, 1998).

In the same way, the histomorphology of infectious lesions is not pathognomonic. Generally, infectious lesions demonstrate abundant osteolytic/resorptive destruction of the original bone tissue architecture and the formation of reactive new bone tissue (Hackett, 1981; Blondiaux, 1994; Schultz, 2001; Schultz and Roberts, 2002; Wapler, 2004; Von Hunnius, 2006; Van der Merwe, 2010; Flohr, 2009a/2009b; Weston, 2009). As such, histology can be used to differentiate between infectious lesions and taphonomic alteration. Differentiation between infectious and tumours is however impossible with histology alone.

Whether specific infections, such as tuberculosis, lepra or treponematoses produce pathognomonic histomorphologies has been studied on several occasions (Hackett, 1981; Blondiaux, 1994; Schultz, 2001; Schultz and Roberts, 2002; Von Hunnius, 2006; Van der Merwe, 2009; Weston, 2009). Palaeopathologists nowadays generally agree that no such pathognomonic histomorphology exists (Weston, 2009; Van der Merwe, 2009; Schutskowski, 2010). The histomorphological features previously described as pathognomonic of for instance treptonematosis (see e.g. Schultz, 2001) must be regarded as indicative for a chronic, slowly developing infectious disease. The diagnosis of specific infectious

diseases should therefore be based on combination of gross anatomical, radiographic and sometimes histological analysis.

With respect to the diagnosis of mechanical traumas (ante- and postmortem fractures, amputations and ossified hematomas after long standing subperiosteal bleedings), practise shows that the diagnosis of fractures and amputations is seldom based on their histological analysis. Still, histology can be of use, since the microscopically assessed phase of their healing process can be linked to a certain minimum posttraumatic survival time, as illustrated in Plate V (Blondiaux, 2000; Maat, 2006; De Boer, 2012a, 2012b). Microscopy is also helpful to differentiate between ante- and postmortem traumas (Maat, 2008; De Boer, 2012a, 2012b). Finally, ossified subperiosteal hematomas show a very characteristic radiating architecture, that tends become solid over time by the 'filling in' of interradiate spaces, and finally by the regular remodelling process (Van der Merwe, 2010).

CONCLUSION

The nature of dry bone tissue excludes the possibility to apply microscopic section/slide preparation methods routinely used in (medical) histology laboratories. Nonetheless, sections can be made manually in a quick and cheap manner, by means of very basic tools, making histology accessible to all archaeologists.

Histological analysis of human remains may aid in the assessment of age-at-death and palaeopathological disorders. But only few disorders present specific, pathognomonic, features. In all other cases, histological findings must be combined with gross anatomical and radiological findings to come to a conclusive diagnosis or to a shortened list of differential (alternative) diagnoses.

LITERATURE

AARON, J. E., ROGERS, J., KANIS, J. A. (1992): "Paleohistology of Pagets-Disease in 2 Medieval Skeletons", *American Journal of Physical Anthropology* 89 (3), pp. 325-331.

AHLQVIST, J., DAMSTEN, O. (1969): "A modification of Kerley's method for the microscopic determination of age in human bones", *Journal of Forensic Science* 14, pp. 205-217.

ANDERSON, T., WAKELY, J., CARTER, A. (1992): "Medieval example of metastatic carcinoma: A dry bone, radiological, and SEM study", *American Journal of Physical Anthropology* 89, pp. 309-323.

BACCINO, E., UBELAKER, D. H., HAYEK, L-A. C., ZERILLI, A. (1999): "Evaluation of seven methods of estimating age at death from mature human skeletal remains", *Journal of Forensic Science* 44(5), pp. 931-936.

BEAUCHESNE, P. AND SAUNDERS, S. (2006): "A test of the revised Frost's 'rapid manual method' for the preparation of bone thin sections", *International Journal of Osteoarchaeology* 16, pp. 82-87.

BELL, L.S. (1990): "Palaeopathology and diagenesis: an SEM evaluation of structural changes using backscattered electron imaging", *Journal of Archaeological Science* 17 (1), pp. 85-102.

BELL, L. S., and JONES, S. J. (1991): "Macroscopic and microscopic evaluation of archaeological pathological bone: Backscattered electron imaging of putative pagetic bone", *International Journal of Osteoarchaeology* 1, pp. 179-184.

BLONDIAUX, J., DUVETTE, J. F., VATTEONI, S., EISENBERG, L. (1994): "Microradiographs of leprosy from an osteoarchaeological context",

International Journal of Osteoarchaeology 4 (1), pp. 13-20.

BLONDIAUX, J. (2000): "The ageing of fracture healing in paleopathology with light microscopic observation", *13th European Meeting of the palaeopathological association*, Chieti, Italy.

DE BOER, H. H., VAN DER MERWE A. E., HAMMER S., STEYN M., MAAT G. J. R. (2012a): "Assessing Posttraumatic Time Interval in Human Dry Bone" ,*International Journal of Osteoarchaeology* (in press) DOI: 10.1002/oa.2267.

DE BOER, H. H., VAN DER MERWE A. E., MAAT G. J. R. (2012b): "Survival time after fracture or amputation in a 19th century mining population at Kimberly, South Africa", *South African Archaeological Bulletin.* Goodwin edition (accepted for publication).

DE BOER, H. H., AARENTS, M. J., MAAT, G. J. R. (2013): "Manual for the Preparation and Staining of Embedded Natural Dry Bone Tissue Sections for Microscopy", *International Journal of Osteoarchaeology* 23, pp. 83-93.

BRICKLEY, M., MAYS, S., IVES, R. (2007): "An investigation of skeletal indicators of vitamin D deficiency in adults: Effective markers for interpreting past living conditions and pollution levels in 18th and 19th century Birmingham, England", *American Journal of Physical Anthropology* 132 (1), pp. 67-79.

CAMPILLO, D., and MARCÍ-BALCELLS V. J. (1984): "Microscopy of osteal tumors in palaeopathology", *Proceedings of the VIth European meeting of the PPA*, pp. 35-49.

CHO, H. and STOUT, SD. (2011): "Age-associated bone loss and intraskeletal variability in the Imperial Romans", *Journal of Archaeological Science* 89, pp. 109-25.

COLLINS, M. J., NIELSEN-MARSH, C. M., HILLER, J., SMITH, C. I., ROBERTS, J.P., PRIGODICH, R.V., WESS, T. J., CSAPÒ, J., MILLARD, A. R., TURNER-WALKER, G. (2002): "The survival of organic matter in bone: a review", *Archaeometry* 44, pp. 383-394.

COOK, M., MOLTO, E., ANDERSON, C. (1988): "Possible case of hyperparathyroidism in a roman period skeleton from the Dakhleh Oasis, Egypt, diagnosed using bone histomorphometry", *American Journal of Physical Anthropology* 75 (1), pp. 23-30.

DE LA RUA, C., BARAYBAR, J. P., ETXEBERRIA, F. (1995): "Neolithic case of metastasizing carcinoma: Multiple approaches to differential diagnosis", *International Journal of Osteoarchaeology* 5 (3), pp. 254-264.

ERICKSEN, M. F. (1991): "Histologic estimation of age at death using the anterior cortex of the femur", *American Journal of Physical Anthropology* 84, pp. 171-179.

ESHED, V., LATIMER, B., GREENWALD, C. M., JELLEMA, L. M., ROTHSCHILD, B. M., WISH-BARATZ, S., HERSHKOVITZ, I. (2002): "Button osteoma: Its etiology and pathophysiology", *American Journal of Physical Anthropology* 118, pp. 217-230.

FLOHR, S. and SCHULTZ, M. (2009): "Mastoiditis-Paleopathological evidence of a rarely reported disease", *American Journal of Physical Anthropology* 138, pp. 266-273.

FLOHR, S. and SCHULTZ, M. (2009): "Osseous changes due to mastoiditis in human skeletal remains", *International Journal of Osteoarchaeology* 19, pp. 99-106.

FRASER, WD. (2011): "Hyperparathyroidism", *The Lancet* 374 (9684), pp. 145-158.

FROST, H. D. (1985): "The new bone: some anthropological potentials", *American Journal of Physical Anthropology* 28 (S6), pp. 211-226.

GONZALEZ-REIMERS, E. and ARNAY-DE-LA-ROSA, M. (1992): "Ancient skeletal remains of the Canary Islands: bone histology and chemical analysis", *Anthropol. Anz.* 50 (3), pp. 201-215.

GRUPE, G. (1988): "Metastasizing carcinoma in a medieval skeleton: Differential diagnosis and etiology", *American Journal of Physical Anthropology* 75, pp. 369-374.

HACKETT, C. J. (1981a): "Microscopical focal destruction (tunnels) in exhumed human bones", *Medicine, science, and the law* 21 (4), pp. 243-265.

HACKETT, C. J. (1981b): "Development of caries sicca in a dry calvaria", *Virchows Archiv* 391 (1), pp. 53-79.

HEDGES, R. E. M., MILLARD, A. R., PIKE, A. W. G. (1995): "Measurements and Relationships of Diagenetic Alteration of Bone from 3 Archaeological Sites", *Journal of Archaeological Science* 22 (2), pp. 201-209.

HERSHKOVITZ, I., GREENWALD, C., ROTHSCHILD, B. M., LATIMER, B., DUTOUR, O., JELLEMA, L. M., WISH-BARATZ, S. (1999): "Hyperostosis frontalis interna: An anthropological perspective", *American Journal of Physical Anthropology* 109, pp. 303-325.

VON HUNNIUS, T. E., ROBERTS, C. A., BOYLSTON, A., SAUNDERS, S. R. (2006): "Histological identification of syphilis in pre-Columbian England", *American Journal of Physical Anthropology* 129 (4), pp. 559-566.

HILLIER, M. L. and BELL, L. S. (2007): "Differentiating Human Bone from Animal Bone: A Review of Histological Methods", *Journal of Forensic Science* 52, pp. 249-263.

JANS, M. M. E., KARS, H., NIELSEN-MARSH, C. M., SMITH, C. I., NORD, A. G., ARTHUR, P., EARL, N. (2002): "*In situ* preservation of archaeological bone: a histological study within a multidisciplinary approach", *Archaeometry* 44, pp. 343-352.

JANS, M. M. E., NIELSEN-MARSH, C. M., SMITH, C. I., COLLINS, M. J., KARS, H. (2004): "Characterisation of microbial attack on archaeological bone", *Journal of Archaeological Science* 31 (1), pp. 87-95.

KERLEY, E. R. (1965): "The microscopic determination of age in human bone", *American Journal of Physical Anthropology* 23 (2), pp. 149-163.

KERLEY, E. R. and UBELAKER, D. H. (1978): "Revisions in the microscopic method of estimating age at death in human cortical bone", *American Journal of Physical Anthropology* 49 (4), pp. 545-6.

LYNNERUP, N, FROHLICH, B., THOMSEN, J. L. (2006): "Assessment of age at death by microscopy: unbiased quantification of secondary osteons in femoral cross sections", *Forensic Science International* 15, p. 159.

MAAT, G. J. R. and UYTTERSCHAUT, H. T. (1984): "Microscopical observations on scurvy in Dutch Whalers buried at Spitsbergen", *Proceedings of the VIth European meeting of the PPA*, pp. 211-218.

MAAT, G. J. R. and BAIG, M. S. (1990): "Microscopy electron scanning of fossilized sickle-cells", *International Journal of Anthropology* 5(3), pp. 271-275.

MAAT, G. J. R. (1991): "Ultrastructure of normal and pathological fossilized red blood cells compared with pseudopathological biological structures", *International Journal of Osteoarchaeology* 1, pp. 209-214.

MAAT, G. J. R. and BAIG, M. S. (1997): "A survey of the analyses of human remains for Ikaros", *Medical Principles and Practice* 6, pp. 142-151.

MAAT, G. J. R., VAN DEN BOS, R. P., AARENTS, M. J. (2001): "Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of Frost's «rapid manual method»'', *International Journal of Osteoarchaeology* 11 (5), pp. 366-374.

MAAT, G. J. R. (2004): "Scurvy in adults en ayoungsters: the Dutch experience. A review of the history and pathology of a disregarded disease", *International Journal of Osteoarchaeology* 14, pp. 77-81.

MAAT, G. J. R, MAES, A., AARENTS, M. J., NAGELKERKE, N. J. D. (2006): "Histological Age Prediction from the Femur in a Contemporary Dutch Sample*", *Journal of Forensic Sciences* 51 (2), pp. 230-237.

MAAT, G. J. R. (2006): "Dating of fractures in human dry bone tissue. The Berisha case", *Skeletal trauma: Identification of injuries resulting from human rights abuse and armed conflict* (Kimmerle, E. H. and Baraybar, J. P., eds.), CRC Press (Taylor and Francis Group), Boca Raton: 245-254.

MAAT, G. J. R. (2008): "Histological fracture dating in fresh and dry bone tissue", *Forensic aspects of pediatric fractures. Differentiating accidental traumas from child abuse* (Bilo, R. A. C., Robben, S. G. F., Van Rijn, R. R., eds.), Heidelberg, Springer-Verlag.

MARCSIK, A., KOSA, F., KURUCS, E. (1984): "Scanning electron-microscopical investigation into palaeophatological findings (porotic hyperostosis)", *Proceedings of the VIth European meeting of the PPA,* pp. 219-233.

MARTIN, D. L. and ARMELAGOS, G. J. (1979): "Morphometrics of compact bone: An example from Sudanese Nubia", *American Journal of Physical Anthropology* 51 (4), pp. 571-577.

MARTIN, D. L. and ARMELAGOS, G. J. (1985): "Skeletal remodeling and mineralization as indicators of health: an example from prehistoric Sudanese Nubia", *Journal of Human Evolution* 14 (5), pp. 527-537.

MARTINIAKOVA, M., VONDRAKOVA, M., RADOSLAV, O. (2006): "Manual Preparation of Thin Sections from Historical Human Skeletal Material", *Timisoara Medical Journal* 56(1), pp. 15-17.

VAN DER MERWE, A. E., MAAT, G. J. R., STEYN, M. (2010): "Ossified haematomas and infectious bone changes on the anterior tibia: histomorphological features as an aid for accurate diagnosis", *International Journal of Osteoarchaeology* 20 (2), pp. 227-239

MOLNAR, E., MARSCIK, A., BERECZKI, Z., SCHMIDT-SCHULTZ, T. H., SCHULTZ, M., PALFI, G. (2009): "Malignant tumor in

osteoarchaeological samples for Hungary", *Acta Biologica Szegediensis* 53(2), pp. 117-124.

NIELSEN-MARSH, C. M. and HEDGES, R. E. M. (2000a): "Patterns of diagenesis in bone I: The effects of site environments", *Journal of Archaeological Science* 27 (12), pp. 1139-1150.

NIELSEN-MARSH, C. M. (2000b): "Patterns of diagenesis in bone II: Effects of acetic acid treatment and the removal of diagenetic CO32", *Journal of Archaeological Science* 27 (12), pp. 1151-1159.

ORTNER, D. J. (2003): *Identification of pathological conditions in human skeletal remains*, San Diego: Academic Press.

PAINE, R. R. and BRENTON, B. P. (2006): "Dietary health does affect histological age assessment: an evaluation of the Stout and Paine (1992) Age estimation equation using secondary osteons from the rib", *Journal of Forensic Sciences* 51 (3), pp. 489-492.

RALSTON, S. H., LANGSTON, A. L., REID, I. R. (2008): "Pathogenesis and management of Paget's disease of bone", *The Lancet* 372 (9633), pp. 155-163.

RICHMAN, R. J. and ORTNER, D. J. (1979): "Differences in intracortical bone remodeling in three aboriginal American populations: Possible dietary factors", *Calcified Tissue International* 28 (1), pp. 209-214

ROBERTS, C. and WAKELY, J. (1992): "Microscopical findings associated with the diagnosis of osteoporosis in palaeopathology", *International Journal of Osteoarchaeology* 2, pp. 23-30.

ROCHES, E., BLONDIAUX, J., COTTON, A., CHASTANET, P., FLIPO, R. M. (2002): "Microscopic evidence for Paget's Disease in two osteoarchaeological samples from early northern France", *International Journal of Osteoarchaeology* 12 (4), pp. 229-234.

ROODMAN, G. D. (2011): "Mechanism of bone metastasis", *UptoDate,* ed. DS Barlow Walthan, MA: UpToDate.

ROSAI, J. and ACKERMANS, L. V. (2011): *Surgical Pathology*, 10th edition, Elsevier, New York.

ROSS, M. H., KAY, G. I., PAWLINA, W. (1995): *Histology, a text and atlas,* 4th edition, Lippincot, Williams and Williams, Baltimore.

SAUNDERS, S. R. (1987): "Growth remodeling of the human femur", *Canadian Review of Physical Anthropology* 6, pp. 20-30.

SCHAMALL, D., TESCHLER-NICOLA, M., HÜBSCH, P., KNEISSEL, M., PLENK, H. (1999): "Differential diagnosis on ancient skeletal remains: conventional methods and novel application of the BSE-mode in SEM on a skull tumor of the early Bronze Age", *Collegium Antropologicum* 23(2), pp. 483-94.

SCHAMALL, D., TESCHLER-NICOLA, M., KAINBERGER, F., TANGL, S. T., BRANDSTATTER, F., PATZAK, B., MUHSIL, J., PLENK, H. (2003): "Changes in trabecular bone structure in rickets and osteomalacia: the potential of a medico-historical collection", *International Journal of Osteoarchaeology* 13, pp. 283-288.

SCHULTZ, M. (1988): "Methoden der Licht— und Elektronenmikroskopie", *Handbuch der vergleichenden Biologie des Menschen* (Knussmann, R., ed.), Gustav Fischer Verlag, Stuttgart, pp. 698-730.

SCHULTZ, M. (1991): "Microscopic investigation on bone samples of individual I3, appendix to Strouhal, 1991, a case of primary carcinoma", *Journal of Paleopathology* 3, pp. 162-165.

SCHULTZ, M. (1993): "Microscopic investigatjon on tumorous lesions from Christian Sayala (Egyptian Nubia)", *Anthropoligscher Anzeiger* 51, pp. 117-121.

SCHULTZ, M. (1999): "Microscopic investigation in fossil hominoidea: A clue to taxonomy, functional anatomy, and the history of diseases", *Anatomical Record* 257 (6), pp. 225-232.

SCHULTZ, M. (2001): "Paleohistopathology of bone: A new approach to the study of ancient diseases", *Yearbook of Physical Anthropology* 44, pp. 106-147.

SCHULTZ, M. and ROBERTS, C. A. (2002): "Diagnosis of leprosy in skeletons from an English later Medieval hospital using histological analysis", *The past and present of leprosy: archaeological, historical, palaeopathological and clinical approaches*, Archaeopress Oxford, pp. 89-104.

SCHULTZ, M., PARZINGER, H., POSDNJAKOV, D. V., CHIKISHEVA, T. A., SCHMIDT-SCHULTZ, T. H. (2007): "Oldest known case of metastasizing prostate carcinoma diagnosed in the skeleton of a 2,700-year-old Scythian king from Arzhan (Siberia, Russia)", *International Journal of Cancer.* 121(12), pp. 2591-5.

SCHUTKOWSKI H. and FERNANDEZ-GIL, M. (2010): "Histological manifestation of mycobacterial bone lesions", Paper presented at *18th European meeting of the paleopathology association*.

ŠEFČÁKOVÁ, A., STROUHAL, E., NĚMEČKOVÁ, A., THURZO, M., STAŠŠÍKOVÁ-ŠTUKOVSKÁ, D. (2001): "Case of metastatic carcinoma from end of the 8th-Early 9th century Slovakia", *American Journal of Physical Anthropology* 116, pp. 216-229.

SINGH, I. J. and GUNBERG, D. L. (1970): Estimation of age at death in human males from quantitative histology of bone fragments, *American Journal of Physical Anthropology* 33(3), pp. 373-81.

STOUT, S. D. and TEITELBAUM, S. L. (1976): "Histological analysis of undecalcified thin-Sections of archeological bone", *American Journal of Physical Anthropology* 44 (2), pp. 263-269.

STOUT, S. D. and SIMMONS, D. J. (1979): "Use of histology in ancient bone research", *Yearbook of Physical Anthropology* 22, pp. 228-249.

STOUT, S. D. and GEHLERT, S. J. (1982): "Effects of field size when using Kerley's histological method for determination of age at death", *American Journal of Physical Anthropology*. 58, pp. 123-125.

STOUT, S. D. and STANLEY, S. C. (1991): "Percent osteonal bone vs. osteon counts: the variable of choice for estimating age at death", *American Journal of Physical Anthropology* 86, pp. 515-520.

STOUT, S. D. and PAINE, R. R. (1992): "Histological age estimation using rib and clavicle", *American Journal of Physical Anthropology* 87, pp. 111-115.

STROUHAL, E. (1976): "Tumors in the remains of ancient Egyptians", *American Journal of Physical Anthropology* 45 (3), pp. 613-20.

STROUHAL, E., VHYNÁNEK, I., HORÁČKOVÁ, I., BENEŠOVÁ, L. NĚMEČKOVÁ, A. (1996): "Two Unusual Benign Tumours in Skulls from the Ossuary at Křtiny (Czech Republic)", *International Journal of Osteoarchaeology* 6, pp. 289-299.

STROUHAL E., VYHNÁNEK L, HORÁČKOVÁ L, BENEŠOVÁ L NĚMECKOVÁ A. (1997): "A Case of Osteosarcoma in a Late Medieval-Early Modern Skull from Kyjov (Czech Republic)", *International Journal of Osteoarchaeology* 7, pp. 82-90.

STROUHAL, E. and NĚMEČKOVÁ, A. (2004): "Paleopathological find of a sacral neurilemmoma from ancient Egypt", *American Journal of Physical Anthropology* 125, pp. 320-328.

STURMER, K. M. (1979): "Automatic Grinding of Undecalcified Bone Sections with Exact Adjustment of Thickness", *Acta Anatomica* 103, pp. 100-108.

SUZUKI, T. (1987): "Paleopathological study on a case of osteosarcoma", *American Journal of Physical Anthropology* 74, pp. 309-318.

THOMPSON, D. D. (1979) "The core technique in the *determination* of *age* at death of skeletons", *Journal of Forensic Sciences* 24, pp. 902-15.

TKOCZ, I. and BIERRING, F. (1984): "A medieval case of metastasizing carcinoma with multiple osteosclerotic bone lesions", *American Journal of Physical Anthropology* 65, pp. 373-380.

TURNER-WALKER, G. and MAYS, S. (2008): "Histological studies on ancient bone", *Advances in human palaeopathology* (Pinhasi, R. and Mays, S., eds.), John Wiley and Sons, Sussex.

UBERLAKER, D. H. (2005): "Chapter 5: Estimating age at death", Forensic Medicine of the Lower Extremity (Rich J. and Dean D. E., eds.), Humana Press.

VELASCO-VAZQUEZ, J., GONZALEZ-REIMERS, E., ARNAY-DE-LA-ROSA, M., BARROS-LOPEZ, N., MARTIN-RODRIGUEZ, E., SANTOLARIA-FERNANDEZ, F. (1999): "Bone histology of prehistoric inhabitants of the Canary Islands: comparison between El Hierro and Gran Canaria", *American Journal of Physical Anthropology* 110 (2), pp. 201-213.

VIGORITA, V. J. (1999): *Orthopaedic Pathology*, Philadelphia, Lippincott Williams & Wilkins.

VYHNÁNEK, L., STROUHAL, E. NĚMEČKOVÁ, A. (1999): "«Kissing» osteochondroma: A case from Ancient Egypt", *International Journal of Osteoarchaeology* 9, pp. 361-368.

WAKELY, J., ANDERSON, T., CARTER, A. A. (1995) "Multidisciplinary case study of prostatic(?) carcinoma from medieval Canterbury", *Journal of Archaeological Science 22,* pp. 469-77.

WAKELY, J., STROUHAL, E., VYHNÁNEK, L., NĚMEČKOVÁ, A. (1998) "Case of a Malignant Tumour from Abingdon, Oxfordshire, England", *Journal of Archaeological Science* 25(10), pp. 949-955.

WALKER, P. L. (2009): "The causes of porotic hyperostosis and cribra orbitalia: a reappraisal of the iron-deficiency-anemia hypothesis", *American Journal of Physical Anthropology* 139, p. 109.

WAPLER, U., CRUBZY, E., SCHULTZ, M. (2004): "Is cribra orbitalia synonymous with anemia? Analysis and interpretation of cranial pathology in Sudan", *American Journal of Physical Anthropology* 123 (4), pp. 333-339.

WALLIN, J. A. TKOCZ, I., LEVINSEN, J. (1985): "A simplified procedure for preparation of undecalcified human bone sections", *Biotechnic and Histochemistry* 60, pp. 331-336.

WEINSTEIN, R. S., SIMMONS, D. J., LOVEJOY, C. O. (1981): "Ancient bone disease in a Peruvian mummy revealed by quantitative skeletal histomorphometry", *American Journal of Physical Anthropology* 54 (3), pp. 321-326.

WESTON, D. A. (2009): "Brief Communication: Paleohistopathological Analysis of Pathology Museum Specimens: Can Periosteal Reaction Microstructure Explain Lesion Etiology?", *American Journal of Physical Anthropology* 140 (1), pp. 186-193.

WOLF, E., ALBERT, E., POMPE, B. (1983): "Rational Preparation of Thin Bone Sections by Mechanical Grinding. I. Method", *Zentralblatt fur allgemeine Pathologie und pathologische Anatomie* 127, pp. 355-365.

ZINK, A. R., PANZER, S., FESQ-MARTIN, M., BURGER-HEINRICH, E., WAHL, J., NERLICH, A. G. (2005): "Evidence for a 7000-Year-Old Case of Primary Hyperparathyroidism", *JAMA: The Journal of the American Medical Association* 293 (1), pp. 40-42.

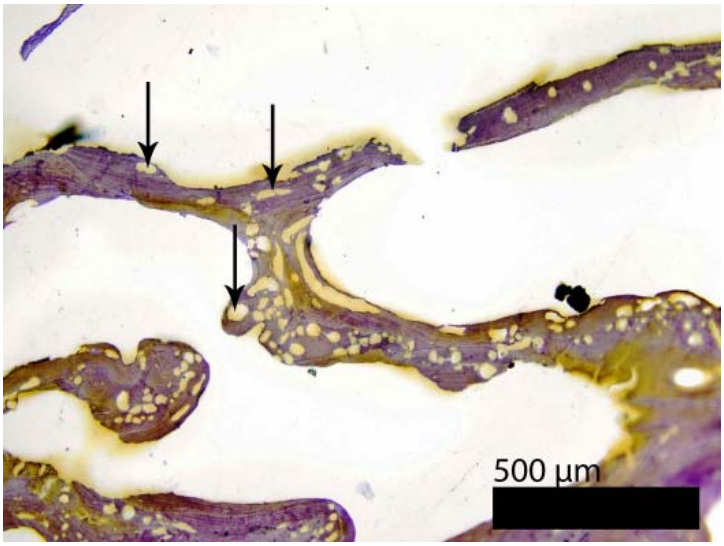

Plate I.—Hand-ground section of human dry bone tissue. Transverse section of a human sternum. Bright field. Bar indicates scale. The section was haematoxylin-stained according to De Boer (2013). The macroscopic and microscopic architecture remained intact throughout slide production. The surface staining with haematoxylin enhances the lamellar structure of the fragile trabecular bone and aids in the identification of taphonomically altered bone (arrows).

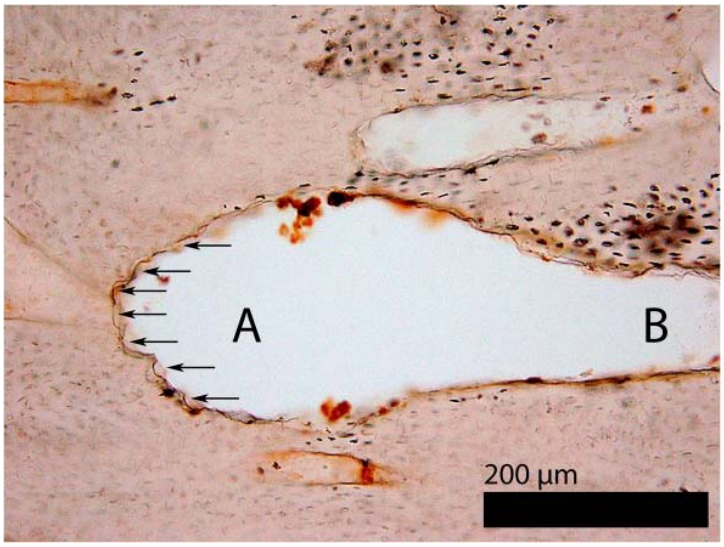

Plate II.—The 'cutting' end of a Basic Multicellular Unit (BMU) in a ground section of human dry bone. Longitudinal section of a tibia. Bright Field. Bar indicates scale. The head end of the BMU (A) is a 'drilling cone' characterized by numerous Howship's lacunae (arrows). The tail end, the 'closing cone' of the BMU, is not visible in this photograph. Nevertheless, the narrowing towards its end can be appreciated (B). The latter is caused by ongoing apposition of bone tissue by osteoblasts inside the BMU. Photograph on courtesy of G.J.R. Maat.

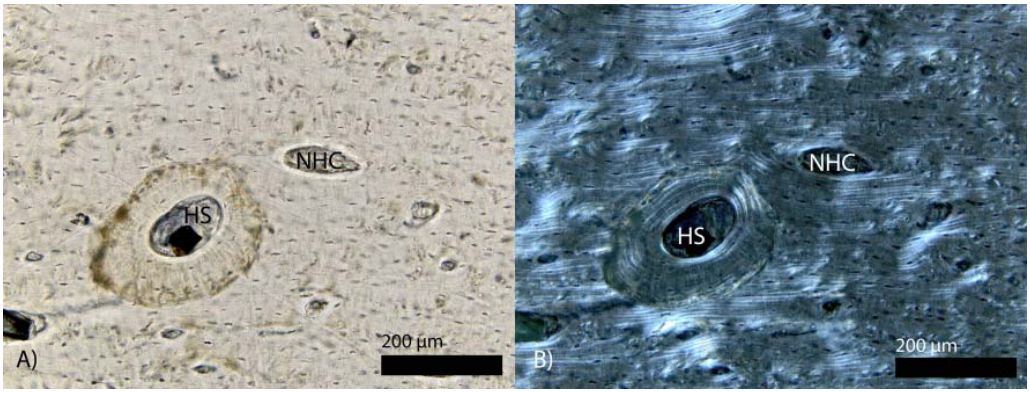

Plate III.—Section illustrating the distinction between a non-Haversian canal and a Haversian system (osteon) in a hand ground section. Transverse section of a human femur. Bar indicates scale. A) Section viewed with bright field. The circumference of the non-Haversian canal (NHC) is not delineated by a cement line, and its 'passing' circumferential lamellae flow in line with its border. In contrast, a Haversian system (HS) is delineated by a cement line, marking the extent of the osteoclastic resorption by the BMU. After resorption, the remaining 'cavity'was internally filled in by osteoblastic apposition of the same BMU. As a result, a Haversian system abruptly 'halts' / cuts off the 'approaching' circumferential lamellae. B) Same section as A, now viewed with polarized light. The polarization enhances the visibility of the differences between the non-Haversian canal (NHC) and the Haversian system (HS).

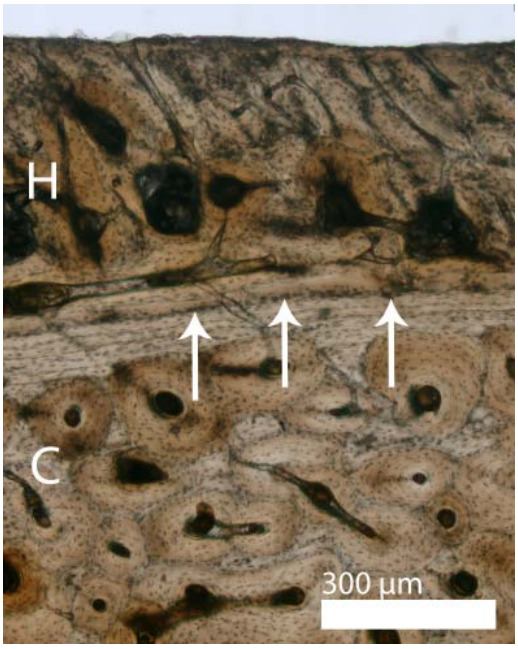

Plate IV.—Hand ground section of an ossified hematoma. Transverse section of the cortex of a human bone. Bright field. Bar indicates scale. The ossified hematoma (H) is seen as an apposition of bone tissue on top of the cortical surface (C). The original periosteal surface with its underlying external circumferential lamella has stayed intact (arrows). Photo on courtesy of dr. A.E. van der Merwe.

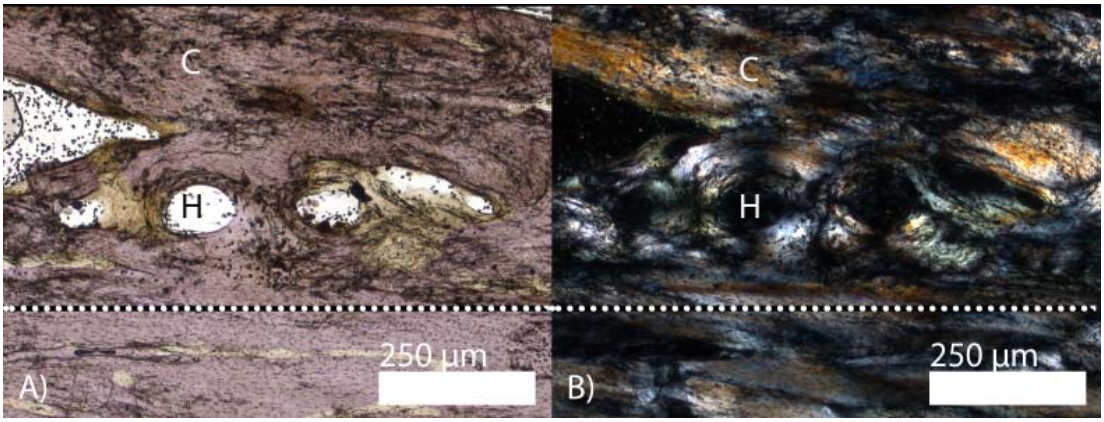

Plate V.—Hand ground section of the callus of a fractured human femur. Longitudinal section. Haematoxylin-stained. Bar indicates scale. A) Section viewed with bright field. The local histology displays proof that the minimum posttraumatic survival time has been six weeks, since time is needed to develop:
— the external callus (C) (at least seven days),
— the appearance of Haversian systems (H) (at least two weeks),
— the 'firm' attachment of the callus on the periosteal surface (dotted line) (at least six weeks).
B) Same section as A, viewed with polarized light. The polarization enhances the visibility of the time indicators as seen in A.