

THE HISTOLOGY OF HUMAN DRY BONE (A REVIEW)

Histología de huesos humanos secos (una revisión)

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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 'pathognomic' 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

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'patognomicas' 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 mandibulae (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). Ahlqvist 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 transection 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).

Hyperparathyroidism 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 hyperparathyroidism 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’. Hyperparathyroidism 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 histomorphology 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 treponematoses (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.

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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).

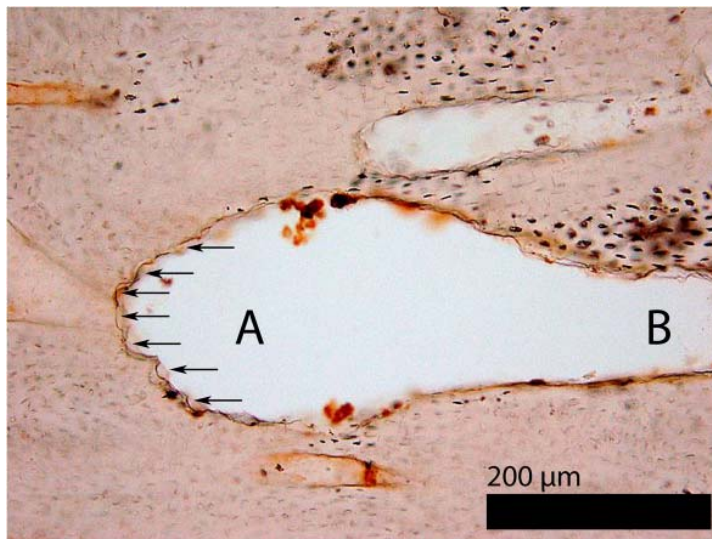


Plate II.—The 'cutting' end of a Basic Multicellular Unit (BMU) in a ground section of human dry bone. Longitudinal section of a tibia. Bright Field. Bar indicates scale. The head end of the BMU (A) is a 'drilling cone' characterized by numerous Howship's lacunae (arrows). The tail end, the 'closing 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.

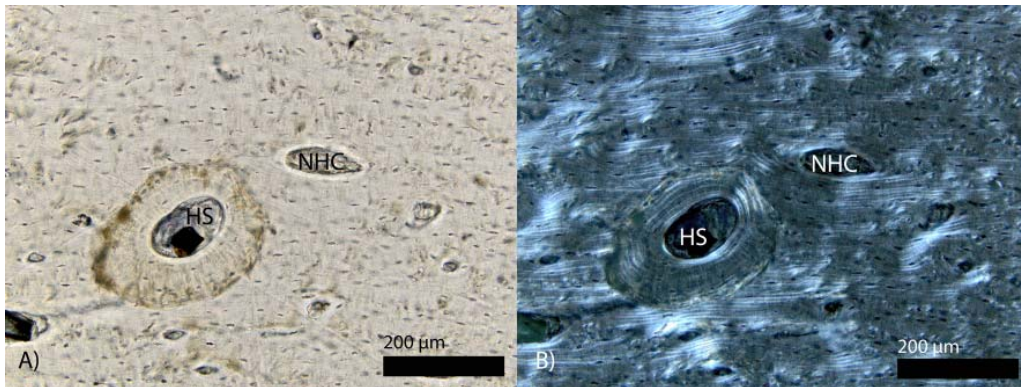


Plate III.—Section illustrating the distinction between a non-Haversian canal and a Haversian system (osteon) in a hand ground section. Transverse section of a human femur. Bar indicates scale. A) Section viewed with bright field. The circumference of the non-Haversian canal (NHC) is not delineated by 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).

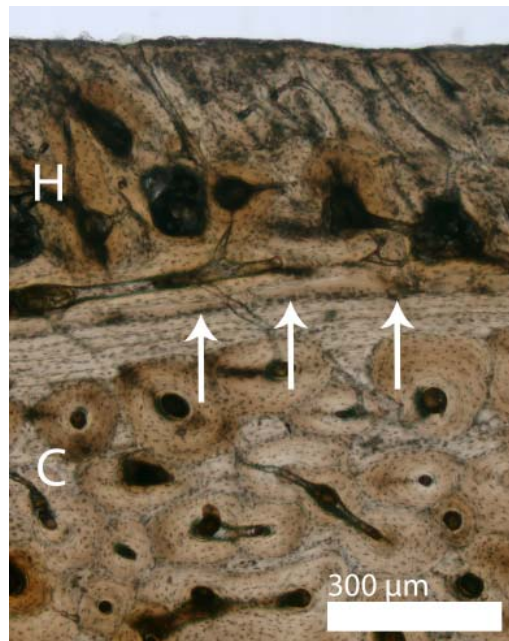


Plate IV.—Hand ground section of an ossified hematoma. Transverse section of the cortex of a human bone. Bright field. Bar indicates scale. The ossified hematoma (H) is seen as an apposition of bone tissue on top of the cortical surface (C). The original periosteal surface with its underlying external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

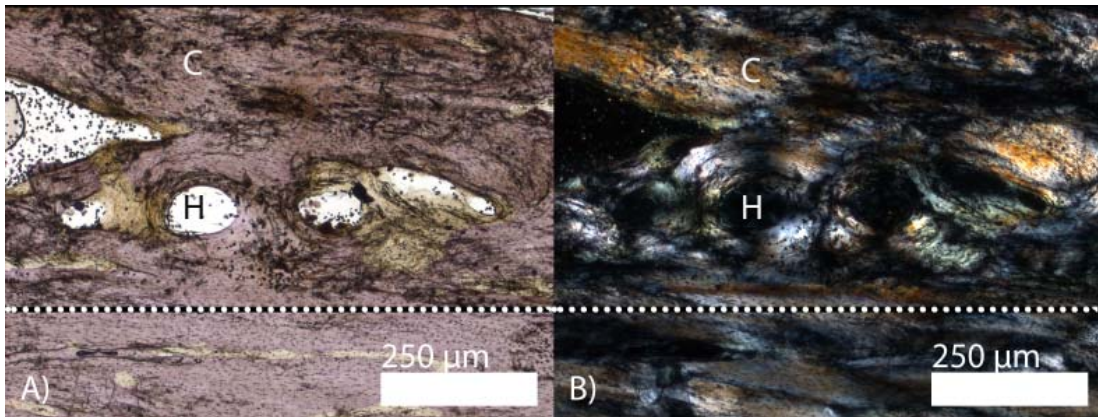


Plate V.—Hand ground section of the callus of a fractured human femur. Longitudinal section. Haematoxylin-stained. Bar indicates scale. A) Section viewed with bright field. The local histology displays proof that the minimum posttraumatic survival time has been six weeks, since time is needed to develop:

- 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.

