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Chapter 10

SUMMARY AND GENERAL DISCUSSION

Physical anthropology, forensic anthropology and palaeopathology

Physical anthropology (also known as biological anthropology), studies mankind as a biological species. As such it encompasses many academic disciplines in which human evolution and human anatomical variation play a central role. The research in this thesis focused on two subspecialties of physical anthropology, namely forensic anthropology and palaeopathology.

Forensic anthropology uses physical anthropological knowledge in a forensic context. In the majority of cases, this concerns the identification of human remains when advanced decomposition or cremation makes the material unfit for standard identification methods. Forensic anthropologists are often consulted by forensic pathologists and Scene Of Crime Officers (SOCOs) to analyze decomposed remains related to an unnatural death, for instance in case of mechanical trauma.

Palaeopathology is the study of disease in an archaeological context. As such it provides insight into the (public) health status of individuals and gives information on the development of diseases. Palaeopathologists often deal with remains in an advanced state of decomposition such as skeletons, mummies or cremated remains. Even though palaeopathology may also encompass animal diseases, this thesis concentrates on human material.

Although forensic anthropologists and palaeopathologists work in different contexts, they share a common ground. Both combine anatomical features of age at death, stature, sex and ancestry with features of disease, trauma and cause of death. Often, both have to deal with a general lack of background information, like missing medical data. As a result, forensic anthropologists and palaeopathologists are confronted with the same methodological and diagnostic challenges.

This thesis focuses on one of these issues, namely on the value of microscopical/histological analysis of skeletonized human remains, that is of so-called 'dry bone'. To date, forensic anthropologists and palaeopathologists mainly use dry bone histology to distinguish human from animal material, to estimate the age at death or to evaluate the phase of decomposition. Apart from these applications, dry bone histology is not generally accepted as a reliable research tool. This thesis addresses the possibilities and impossibilities of dry bone histology.

Part 1: Technical aspects associated with dry bone histology

Chapter 1 introduces the technical difficulties that are associated with dry bone histology. After all, to make it suitable for analysis by light microscope, the material has to be processed into very thin, translucent sections. In case of 'fresh' material, the bone tissue is decalcified, embedded in paraffin or plastic and sectioned with a microtome. This approach is however unsuitable for dry bone material as it no longer contains sufficient bone protein, in particular collagen. To overcome this set-back, Maat introduced a simple, rapid and inexpensive production method for hand ground unstained sections (Maat *et al.*, 2001). Due to its robustness and reliability, this method was gradually applied by laboratories around the world (see for example Martiniakova *et al.*, 2006 or Turner-Walker and Mays, 2008).

Chapter 2 describes a valuable extension of the method of Maat, namely the application of histochemical staining. By means of a trial-and-error approach, we developed a protocol for staining with haematoxylin alone, and with haematoxylin and eosin. The use of these dyes improved the visibility of features that aid in the distinction between woven bone and lamellar bone. Also, it improved the visibility of the cement lines that line the Haversian systems. Furthermore, the visibility of the destruction of bone tissue by microbes was enhanced.

Although Maat's approach can also be used for the production of sections of fragile bone material, some consider it to be less suitable for this particular material (Beauchesne and Saunders, 2006). Prior to 2001, several researchers tried to circumvent this problem by immersing the bone tissue with resin (see e.g. Schultz, 1988). However, these methods proved to be time consuming, required expensive specialized equipment and could not be used in conjunction with histochemical staining.

A simple and inexpensive alternative is presented in **chapter 3**. In contrast to earlier approaches, the fragile bone tissue was not impregnated, but only surrounded/supported by resin. After curing, a slice with a thickness of approximately 1 mm was sawn off, which was then easily processed by hand into a section with a thickness of approximately 80 micrometres. In this way, the bone tissue at the surface of the slide was free of resin and thus remained suitable for histochemical staining. The slide could be examined before and after staining and the embedding did not limit the applicability of a polarizing filter. The bone tissue remained intact during the procedure, regardless of fragility and decomposition phase. The new method dramatically shortened the production process if compared with the existing methods, viz. from six weeks to two days for non-stained sections.

Part 2: The diagnostic value of dry bone histology

In 'fresh' bone material, a histological diagnosis is based on the cytonuclear, biochemical and architectural characteristics of bone fibres and surrounding soft tissues. By definition, these hallmarks are no longer available in dry bone. Consequently, the diagnosis of disorders in dry bone is problematic. As a result, the value of histological examination of dry bone remains a subject of ongoing discussion among palaeopathologists .

Chapter 4 adds depth to this discussion, as it presents an overview of all literature in which the diagnosis of bone disorders was at least partially based on dry bone histology. These diagnostic efforts were tested against the current knowledge on disease histomorphology. The results showed that only a limited number of disorders present a pathognomonic histomorphology in the remaining dry bone material. These disorders were osteoporosis, hyperparathyroidism, Paget's disease and possibly osteomalacia. In all other cases, such as tumours and infections, a definitive diagnosis could not be made on the histological analysis alone. In these cases the histological efforts only aided in the differential diagnostic process, for instance to reject or support a particular diagnosis.

For those who are unfamiliar with dry bone histology, **chapter 5** provides an accessible introduction in the histological aspects of the microscopic anatomy and dynamics of bone tissue. For a quick start, convenient methods for the production of sections of dry bone tissue are described. These methods are specifically suitable for those situations in which a fully equipped anatomy or pathology laboratory is not available. Furthermore it is shown how the continuous remodelling of bone tissue during life allows for a histological assessment of the age at death of an individual. The various parts of the skeleton on which this approach can be applied are introduced, together with the confounding influence of muscle activity during life and decomposition after death. Also, the necessary attention is given to the real, yet sometimes limited value of dry bone histology in the diagnosis of disorders.

How histology of dry bone material can be of use in an archaeological context is illustrated in **chapter 6**. Herein bone fragments from members of the famous and dramatic Franklin expedition of 1845 were analysed (Mays *et al*., 2013). The expedition, which aimed to find a northern passage from the Atlantic to the Pacific Ocean, disappeared a few months after departure in the polar ice of northern Canada. Reconstructions suggested that the demise of the crew was probably due to scurvy. Macroscopic examination of skeletal elements of several expedition members seemed to confirm this. However, histological examination showed no signs of active scurvy.

The combination of these findings with a revision of several historical sources, showed that scurvy probably played no major role in the end of the expedition.

Part 3: The use of histology for the detection of features of mechanical injury in dry bone

The last part of this thesis describes the use of dry bone histology in the investigation of mechanical traumas, i.e. fractures and amputations. Traditionally, lesions are considered to be either antemortem (having occurred before death), or postmortem (having occurred after death). If a lesion can not be attributed to one of these domains, a lesion is usually classified as perimortem (i.e. inflicted 'around' death). In antemortem lesions, a further specification of the time span between the traumatic event and eventual death is important, as this ‘posttraumatic survival time' can aid for instance to determine the relation between death and trauma. Since the healing processes of bone tissue follows a time-dependent and sequential pattern, determination of the healing phase can be used to estimate the length of the posttraumatic survival time.

In **chapter 7**, it was tested to what extent defined features of healing processes after bone traumas are still reliable detectable in dry bone material. For this, fractures and amputations from different healing phases were assessed microscopically and radiologically by three observers. The results showed sufficient consistency between the observations to justify the use of healing features to estimate posttraumatic survival time. This is an improvement to current practice, in which only a distinction is made between 'non-healing', 'healing' and 'healed' lesions. Determination of the posttraumatic survival time is of practical importance for forensic examinations, for instance in cases suspect of torture or child abuse.

Chapter 8 illustrates the usefulness of this approach when analysing fractures and amputations from an archaeological skeleton collection. It describes the examination of the remains of individuals exhumed from the Gladstone cemetery in Kimberley, South Africa (Van der Merwe *et al.*, 2010). At the end of 19th century, the related individuals worked in the diamond mines. The radiological and histological assessment of various posttraumatic survival times identified several interesting trends, such as the apparent medical importance of the first two weeks after trauma for the life expectancy of the wounded. In addition, earlier conclusions regarding medical care and causes of death, based on historical evidence only, could be confirmed. In one individual with multiple lesions, it was even possible to determine the order in which the traumas had occurred. The radiological and histological analysis also aided in the differentiation between ante- and perimortem injuries.

Chapter 9 describes the assessment of posttraumatic survival time in the deceased soldiers of Napoleon's army, specifically of those who took part in the Russian campaign of 1812. These individuals were exhumed in 2006 in Kaliningrad, formerly Koeningsberg, the terminus of the for Napoleon disastrous campaign (Buzhilova *et al.*, 2009). Due to the historico-cultural value of this collection, invasive histological examination was not allowed. This was reason to extend the plain radiological examination with computed tomography (CT) scanning. The analyses showed that the sole application of gross anatomical and radiological analysis still allowed for a reasonable estimation of the posttraumatic survival time, be it less accurate than with additional histology. The results corroborated that at least some adequate historically documented battlefield evacuation and provision of (on-the-spot) medical care was available throughout the military campaign. In addition they provided insight into medical decision making with respect to amputations.

Conclusion

This thesis presents an easy, rapid and inexpensive supplement to the well-known method of Maat *et al.* (2001). This new method allows for the histochemical staining of dry bone material, enhancing the visibility of important hallmarks of dry bone histomorphology. In addition, this thesis provides a new, easy, rapid and inexpensive method for the production of sections of fragile dry bone tissue, histochemically stained or not.

Furthermore we show that dry bone histology is a valuable tool in the diagnosis of bone disorders. Although the majority of bone disorders have no pathognomonic dry bone histomorphology, histology may still have a considerable value for the differential diagnostic process. Finally, when studying mechanical trauma, dry bone histology is a valuable tool for the estimation of posttraumatic survival time, especially when used in conjunction with gross anatomical and radiological analyses.

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