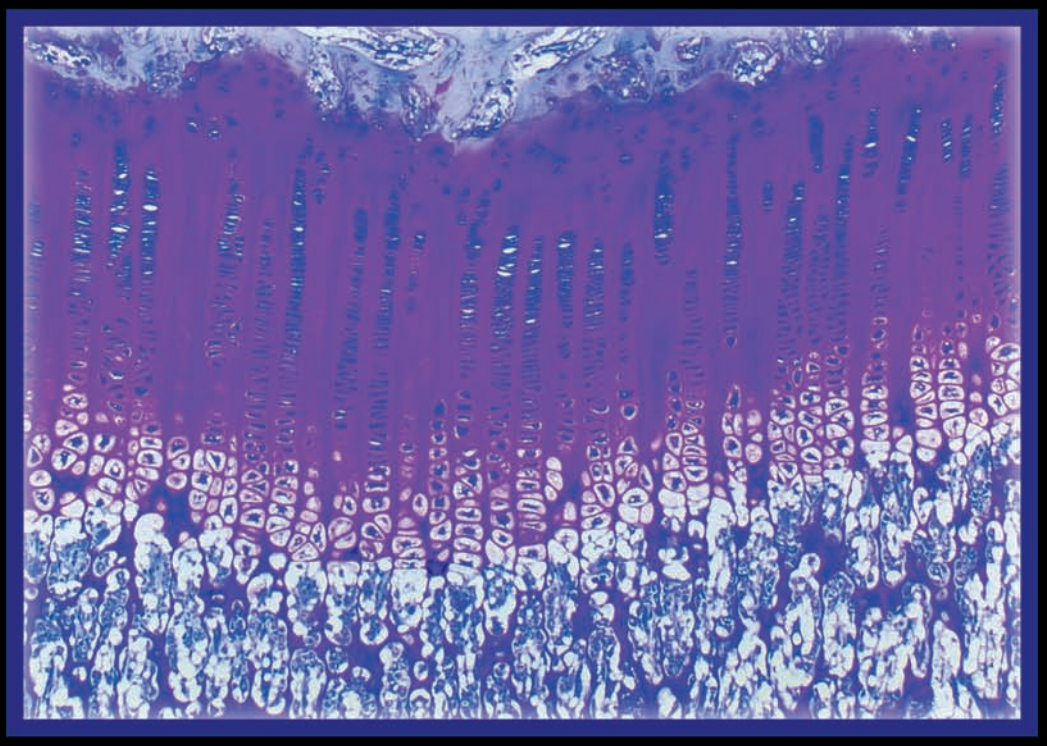
BONE HISTOLOGY



An Anthropological Perspective

Edited by

Christian Crowder and Sam Stout

CRC Press
Taylor & Francis Group

BONE HISTOLOGY

An Anthropological Perspective

This page intentionally left blank

BONE HISTOLOGY

An Anthropological Perspective

Edited by

Christian Crowder and Sam Stout

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20110708

International Standard Book Number-13: 978-1-4398-6702-0 (eBook - PDF)

Table of Contents

Preface

Bone Histology: An Anthropological Perspective is a comprehensive look into the histological examination of hard tissues (bones and teeth). Because of their nature as hard tissues of the body, bones and teeth are of considerable importance for anthropological research. With the exception of forensic anthropology and the relatively rare cases of mummification, the physical remains of humans available to bioarchaeologists, paleopathologists, and paleontologists are limited to skeletal material. Fortunately, the same characteristics of hard tissues that lead to their persistence after death make them a storehouse of information about biological processes experienced during the life of the individual. Unlike for the soft tissues of the body, the activity of cells involved in growth and development, tissue maintenance, and adaptation of hard tissues are encoded in their microstructure. Our ability to extract biologically important information from hard tissues has greatly improved with the understanding of bone provided over the last 5 decades by the work of researchers such as Amprino, Currey, Enlow, Frost, Johnson, and Marotti, and for teeth the work of Dean, Reid, Boyde, Beynon, Bromage, and Shellis. We now understand that although bone serves an important metabolic function relating to mineral homeostasis, its primary function is biomechanical. This edited volume arose from discussions by the editors and colleagues over the years and encouragement gained during a Forensic Histology Workshop organized by the Department of Medical Education of the Armed Forces Institute of Pathology in July 2009. Through discussions at this workshop the authors fully appreciated the need for a comprehensive volume covering theoretical and applied aspects in histological analysis of skeletal tissue.

Organization and Content

The first four chapters cover important aspects of the basic biology of hard tissues. Sam Stout and Christian Crowder provide an overview of bone remodeling and its importance for the interpretation of bone histomorphology. They discuss how bone microstructures should be considered in terms of how they relate to the bone remodeling process. In cortical bone, for example, the histomorphological features known as osteons or Haversian systems are products of the coordinated activity of bone resorbing and forming cells comprising basic multicellular units of bone remodeling. They focus attention on the relationship between histomorphology and the bone remodeling process, and emphasize that it is this relationship that underlies our ability to estimate age and infer bone remodeling activity from histomorphometry.

In Chapter 2, James Gosman explores our understanding of the biology underlying skeletal growth and development leading to adult skeletal morphology. His chapter presents an overview of the current knowledge of the fundamental process of longitudinal bone growth as it relates to histomorphology, regulatory systems, and ontogenetic changes in cortical and trabecular bone architecture. The chapter goes further by situating these concepts within a broader biocultural context, and discusses potential environmental and mechanobiological

influences on skeletal growth and development that are especially important for skeletal biology in general, and physical and forensic anthropology in particular. The chapter underscores that the study of skeletal developmental variation from the anthropological and forensic perspective must adopt an integrative stance, which considers the broader scope of interrelated components that contribute to normal and abnormal skeletal growth and morphology.

Related, but distinct in a number of ways, to bone remodeling is the bone modeling process. Chapter 3 by Corey Maggiano defines bone modeling, distinguishes it from remodeling, and discusses the function of modeling in growth and mechanical adaptation from a microstructural perspective. This well-illustrated and innovative chapter concludes with a discussion of current methods employed to investigate how bones change in morphology, and envisions possible future directions.

Like bone, teeth are hard tissues of the body. As such they also preserve a record of their growth in their histological structures and are a valuable resource for skeletal analyses. In Chapter 4, Debbie Guatelli-Steinberg and Michaela Huffman discuss histological features of dental hard tissues and their utility in biological anthropology. Dental hard tissues preserve a record of their own growth in their histological structures, including enamel and dentine, in which incremental growth is reflected in growth lines, and long-period lines of demonstrated periodicity, and cementum, in which incremental growth produces alternating layers, and provide a basis for estimating chronological age at death. Since disruptions to growth leave their mark in dental hard tissues, they also can provide a record of birth and of physiological stress occurring in childhood, making it possible to identify physiologically stressful events experienced during the early years of life.

For those challenged with extremely fragmented remains, the question of whether the bone is of human origin is often critical. In many cases the extent of fragmentation precludes the use of macroscopic methods to differentiate human bone from nonhuman bone when diagnostic landmarks are not present. Histological analysis is a potential means to differentiate human from nonhuman bone. In Chapter 5, Dawn Mulhern and Douglas Ubelaker discuss the similarities and differences between human and nonhuman mammalian bone on a microscopic level. Illustrations of the typical histomorphology of a number of taxa are described and illustrated. The authors point out that although common bone microstructure is found throughout mammals, differences have been identified. These differences are primarily in the organization or size of structures, rather than unique kinds of structures. It is often possible, therefore, to differentiate nonhuman bone fragments using the pattern or the size of histological structures. The use of discriminant function analysis may permit the identification of an unknown fragment of bone with a reported level of certainty, which is especially important for forensic applications.

In Chapter 6, Margaret Streeter addresses histological age estimation with special emphasis on a new method that is applicable to subadult ribs. Patterns of histomorphological features, such as drifting osteons, woven bone, and lamellar drift, that reflect bone remodeling and modeling activity are used to define four phases covering an age range of less than 5 years to adult. Besides age estimation, this method shows promise for the study of skeletal development rates among populations. Because several book chapters have been devoted to the physiological basis for and the detailed description of histological methods of adult age estimation, Streeter focuses on a few of the more widely used methods.

The "new bone biology" concept recognizes the importance of the biomechanical function of bone. Chapters 7 and 8 discuss two important issues relating to this. In Chapter 7, John Skedros addresses the biomechanics of cortical bone. In addition to reviewing basic

mechanical concepts, he describes how histomorphology can be used to interpret load history in the diaphyses of long bones when strain data are lacking or insufficient. Of particular interest to researchers and applied practitioners, such as forensic anthropologists, he introduces and discusses a worksheet/checklist of important considerations that facilitates the interpretation of load history in limb-bone diaphyses and ribs. Chapter 8, by Amanda Agnew and John Bolte, also deals with the biomechanics of bone but from the perspective of fracture risk. They note that, while risk of bone fracture has typically focused upon measures of bone quantity, the relationship between bone quality, and bone strength is important. Material properties of bone are discussed at several levels of organization, mineral and organic composition. The authors then discuss how type of bone, for example, woven, fibrolamellar, primary and secondary lamellar, and plexiform as well as the creation of fatigue damage in the form of microfractures produced during mechanical loading, affect bone quality. Findings from their ongoing research provide evidence that similar factors affect bone strength in human pediatric bone. Finally, of particular interest to anthropology and forensic science, they discuss the issue of bone fragility in past populations and how bone fracture patterns can help predict the loading context that caused a fracture.

When undertaking microstructural analysis of hard tissues in forensic, archaeological, paleontological, and paleopathological contexts the artifacts of taphonomic alteration must be recognized. Chapter 9 by Lynne Bell explores the subject of histotaphonomy, which is the analysis of taphonomy at the microstructural level. Although microbial alteration causes loss of information in archaeologically derived bone, the study of microbial bioerosion represents a tool for taphonomic reconstruction. She provides a brief history of histotaphonomy, reviews nomenclature associated with the field, and describes and provides examples of characteristic histotaphonomic changes to demonstrate how postmortem microstructural change can be used for taphonomic inquiry.

In Chapter 10, Michael Schultz discusses the application of light microscopy in paleopathology to classify pathological conditions. He explores the basis for reliable diagnosis, which consists of proper tissue selection and preparation to the interpretation of morphological structures in dry bone at the microlevel. He discusses a variety of pathological conditions with special emphasis on the diagnosis and differential diagnoses of a group of commonly seen inflammatory diseases in the archaeological record. Some principles of pathophysiology of the bony tissues are outlined and the morphological features seen at the microlevel characteristic for selected pathological conditions or even pathognomonic for special diseases are described and illustrated.

In Chapter 11, Susan Pfeiffer and Deborrah Pinto address the histological study of bone tissue of archaeological origin, focusing on research and methods based upon transmitted light microscopy. Whether determining if fragmentary remains are human, providing an age-at-death estimate, or evaluating skeletal health and disease, the authors demonstrate that the histological evaluation of bone provides useful information for bioarchaeological and paleopathological studies as well as modern forensic casework. They also note the important role that histological analysis can play in other types of analyses that require adequate preservation of bone tissue, such as assessing bone sample quality preliminary to ancient DNA or isotopic analyses. The authors make the important point that histological study of archaeologically derived material can contribute to research questions arising in forensic and biomedical fields. Given the potential for histological analysis in the broader scope of skeletal analysis, the authors propose that histology of normal cortical and cancellous human bone should be included in osteological training.

Access to collections of bone samples with known demographic information representing a diversity of populations is essential for hard tissue research, including the testing, improvement, and development of new methods. The next two chapters address this and describe two important resources. Chapter 12, authored by Brian Spatola, Franklin Damann, and Bruce Ragsdale, provide a history and description of the hard tissue collections maintained by the Anatomical Division of the National Museum of Health and Medicine (NMHM) at the Armed Forces Institute of Pathology. The NMHM's collection includes more than 10,000 glass slides of stained and undecalcified bone and joint specimens illustrating human growth and development, normal musculoskeletal anatomy, bone and joint pathology, tumor pathology, forensic bone histomorphometry, and comparative anatomy. This collection is available as part of the Anatomical Division's vast collection of anatomical and pathological specimens, which also includes more than 5600 dry bone skeletal specimens and 5500 formalin fixed soft tissue specimens dating back to the Civil War.

In Chapter 13, C. David Thomas and John Clement describe the Melbourne Femur Collection (MFC). This well-documented collection produced by researchers at the Melbourne Dental School (University of Melbourne, Australia) in collaboration with the Donor Tissue Bank of the Victorian Institute of Forensic Medicine currently contains approximately 500 samples of postmortem femoral bones as well as 90 surgical samples, such as femoral heads removed during hip replacements, making it one of the world's most complete and best documented archives of contemporary human bone tissue specimens. The history of the MFC and its future goals, including a discussion of ethical issues relating to obtaining biological samples are discussed. The chapter also includes examples of the range of potential research projects in which the MFC is applicable. The first describes the results of research into the relationship between histomorphological features of bone tissue from the femur midshaft, and the age at death. Their study is unique in terms of its methodology and sample size. It employed a semiautomated system that allowed the counting of Haversian canals from microradiographs made from sections of entire cross-sections of the femoral cortex. The second study illustrates the use of the collection as part of a biomedical study investigating a possible genetic marker of risk for developing osteoporosis in later life. These two chapters illustrate the importance of developing and maintaining hard tissue collections for scientific advancement.

The final two chapters of the book discuss technological aspects of hard tissue histology. Although forensic anthropologists employed in a medical examiner's office or crime laboratory typically have access to a standard histology department, other researchers and practitioners may not have access to these facilities. In Chapter 14, Helen Cho outlines practical issues and requirements for establishing a bone histology laboratory, including embedding, sectioning, mounting bone samples for histological analysis, and basic principles of light microscopy. Until relatively recently, methods of histomorphological analysis have provided only a two-dimensional perspective. Bone microstructure, however, exists in three dimensions. The final chapter, authored by David Cooper, David Thomas, and John Clement, provides an overview of conventional three-dimensional methodologies and describe recent developments in high-resolution imaging, such as the more powerful synchrotron x-ray sources, that offer the potential for greater detail for nondestructive histomorphological analyses in anthropological research.

This volume has assembled authors with extensive experience and expertise in various aspects of hard tissue histology. Its intended goal is to provide readers with an overview of the current state of research and potential applications in anthropology or any field that

employs a histological approach to study hard tissues. This volume illustrates the degree to which histological analysis of hard tissue (bone and teeth) can contribute to the comprehensive understanding of skeletal biology. It is our hope that the contents provide a useful resource for students, researchers, and practitioners of anthropology and other fields related to skeletal biology.

This page intentionally left blank

Editors

Christian M. Crowder, PhD, D-ABFA, received his BA from Texas A&M University, MA from the University of Texas at Arlington, and PhD from the University of Toronto. He is currently the Deputy Director of Forensic Anthropology for the Office of Chief Medical Examiner in New York City and a board certified forensic anthropologist. He is also an adjunct lecturer at Pace University, holds a faculty position at the New York University Medical Center and an affiliation with the NYU Anthropology Department. In his present position with the OCME, Crowder assists with anthropology casework in the five boroughs of New York City (Manhattan, Brooklyn, Queens, the Bronx, and Staten Island). He is also the site coordinator for the ongoing search and recovery of remains at the World Trade Center site. Prior to accepting the position in New York City, Crowder was a forensic anthropologist at the Joint POW/MIA Accounting Command–Central Identification Laboratory in Hawaii.

Sam D. Stout received his PhD in physical anthropology from Washington University in St. Louis in 1976, and is currently professor of anthropology in the Department of Anthropology at the Ohio State University, and professor emeritus of the University of Missouri Department of Anthropology. His research and teaching interests are in skeletal biology, particularly from a microscopic (histomorphological) perspective, and its applications in bioarchaeology and forensic anthropology. He is a fellow of the American Academy of Forensic Sciences.

This page intentionally left blank

Contributors

Amanda M. Agnew
Department of Anthropology
and Division of Anatomy
The Ohio State University
Columbus, Ohio

Lynne S. Bell
Centre for Forensic Research
School of Criminology
Simon Fraser University
Burnaby, British Columbia, Canada

John H. Bolte IV
Department of Biomedical Infomatics
and Injury Biomechanics Research Laboratory
The Ohio State University
Columbus, Ohio

Helen Cho
Department of Anthropology
Davidson College
Davidson, North Carolina

John G. Clement
University of Melbourne
Melbourne, Australia

David M. L. Cooper
Department of Anatomy and Cell Biology
University of Saskatchewan
Saskatoon, Saskatchewan, Canada

Franklin E. Damann
National Museum of Health and Medicine
Armed Forces Institute of Pathology
Washington, DC

James H. Gosman
Department of Anthropology
The Ohio State University
Columbus, Ohio

Debbie Guatelli-Steinberg
Department of Anthropology
Department of Evolutionary Ecology and
Organizational Biology
The Ohio State University
Columbus, Ohio

Michaela Huffman
Department of Anthropology
The Ohio State University
Columbus, Ohio

Corey M. Maggiano
Department of Anthropology
The Ohio State University
Columbus, Ohio

Dawn M. Mulhern
Department of Anthropology
Fort Lewis College
Durango, Colorado

Susan Pfeiffer
Department of Anthropology
University of Toronto
Toronto, Canada

Deborrah Pinto
Harris County Institute of Forensic Science
Houston, Texas

Bruce D. Ragsdale
Arizona State University
Tempe, Arizona
and Western Dermatopathology
San Luis Obispo, California

Michael Schultz
Department of Anatomy
University Medical School Göttingen
Göttingen, Germany

John G. Skedros
Bone and Joint Research Laboratory
Department of Veterans Affairs
SLC Health Care System
Salt Lake City, Utah

Department of Orthopaedics
University of Utah
Salt Lake City, Utah

Brian F. Spatola
National Museum of Health and Medicine
Armed Forces Institute of Pathology
Washington, DC

Margaret Streeter
Department of Anthropology
Boise State University
Boise, Idaho

C. David L. Thomas
University of Melbourne
Melbourne, Australia

Douglas H. Ubelaker
Department of Anthropology
National Museum of Natural History
Smithsonian Institution
Washington, DC

Bone Remodeling, Histomorphology, and Histomorphometry

1

SAM STOUT
CHRISTIAN CROWDER

Contents

1.1 Introduction

Skeletal analyses can be approached at several hierarchical levels, ranging from organ level macroscopic analyses of whole bones to the molecular level. Intermediate is the histological or tissue level, which Harold Frost (1986) described as the level of "skeletal intermediary organization," representing the collaborative activity of cells. Because of the mineralized structure of bone, the product of cellular activity is encrypted in its microscopic anatomy or histomorphology,* where it persists to be interpreted by the histomorphometrist. The first section of this chapter will discuss bone histomorphology as it relates to bone remodeling. Next, the chapter discusses aspects of histomorphometry and its role in evaluating bone at the histological level, followed by a discussion of methodological issues associated with applying histological techniques in anthropological research and forensic casework.

* The following three terms will be used throughout the chapter: Histology, the study of tissues, also called microscopal anatomy; histomorphology, the morphology of tissues; and histomorphometry, quantitative histology.

1.2 Bone Biology and Histomorphology

To interpret the biological information encrypted in the histomorphology of bone, a basic understanding of the biology underlying the creation of histomorphological structures is essential. Bone metabolism, whether for growth, adaptation, or homeostasis, involves two basic kinds of cells: (1) bone-forming osteoblasts and their derivatives, and (2) bone-resorbing osteoclasts. The normal activity of these cells is associated with two distinct physiological processes referred to as modeling and remodeling. Bone modeling is essentially restricted to the growing skeleton, where it works in concert with growth to adapt bones to their changing biomechanical environment by adjusting the amount and spatial distribution of bone tissue. Exceptions where modeling occurs in the adult skeleton are fracture repair and certain cases of mechanical loadings in pathological bone (Peck and Stout 2008). Bone remodeling is a lifelong process of bone turnover and is the predominant process in the adult skeleton responsible for its characteristic histomorphological features. Since this chapter will focus on adult bone histomorphology, with the exception of how modeling activity occurring in earlier life affects our interpretation of adult bone histomorphometry,* discussion will be limited to remodeling. For a more in-depth discussion regarding the biology of subadult bone and modeling, the reader is referred to chapters in this volume by James Gosman (Chapter 2), Corey Maggiano (Chapter 3), and Margaret Streeter (Chapter 6).

1.2.1 Bone Remodeling

Remodeling is the mechanism by which older bone is replaced by the coordinated (tethered) action of bone-resorbing osteoclasts and bone-forming osteoblasts, collectively referred to as a basic multicellular unit (BMU) of remodeling or simply bone remodeling units (BRU; Parfitt 1979). It is useful to describe the process in terms of three distinct phases: activation,† resorption, and formation. This sequence is sometimes denoted by the acronym ARF (Martin et al. 1998). The product of the ARF sequence is a basic structural unit (BSU) described histomorphologically as an osteon or Haversian system in cortical bone, or "hemi-osteon" in cancellous and endosteal bone. Histological age estimation in humans is possible because remodeling occurs throughout life and produces discrete, definable, and quantifiable microscopic features such as osteons‡ (see Streeter in this volume). Bone remodeling occurs in all animals, but the appearance of osteons in cortical bone is not universal and when it does occur often shows a very weak age association due to species differences in non-age-related factors such as locomotion (biomechanics), life span, and endocrine functions (Burr 1992; Mulhern and Ubelaker 2003; Paine and Godfrey 1997; Przybeck 1985; Schaffler and Burr 1984; Schock et al. 1975; Schock et al. 1972; Singh et al.

* Histomorphometry refers to quantitative histology where histomophological features are described in terms of number per unit area or size (area) analogous to osteometry.

† Because the histological observation of a BMU in a cross-section of bone does not necessarily represent the actual birth of that BMU but rather the point at which the plane of section crosses a resorptive bay or cutting cone (Martin 1994; Parfitt 2005), the terms *initiation* (Peck and Woods 1988) and *origination* (Parfitt 2005) have been suggested.

‡ Application to other animals is possible but problematic because of the highly variable expression of cortical remodeling in other species due to biomechanical and physiological differences (Schaffler and Burr 1984).

1974; Van Wagenen 1970). Discussion in this chapter is restricted to humans. For discussion of nonhuman bone histomorphology, see the chapter by Mulhern and Ubelaker in this volume.

Bone remodeling occurs on all bone envelopes (surfaces), including the endosteum and trabeculae. The histomorphological evidence for remodeling is most readily observed in cortical bone as the secondary osteon. For cancellous and endosteal bone surfaces, remodeling is associated with a sinus, referred to as a bone remodeling compartment (BRC), which is lined on its marrow side by flattened cells and on its osseous side by the bone surface (Hauge et al. 2001). Because BSUs in cortical bone (osteons) are relatively well defined in bone cross-sections, most of the histomorphometric methods typically employed in anthropological analyses are based upon intracortical remodeling.* Our discussion, therefore, will focus on cortical bone histomorphology and histomorphometry, particularly as it relates to the bone remodeling process and age estimation, which is the most common use of bone histology in anthropology. The accurate identification of histomorphological features that are traces of earlier remodeling events is crucial for histological age estimation. This requires an understanding of the remodeling process itself.

The bone remodeling process begins with the appearance of osteoclasts at the site of BMU initiation (activation) in response to systemic and biomechanical factors perceived by another cell of the osteoblast lineage, the osteocyte. These bone resorbing cells derive from hematopoietic stem cells within bone marrow that, under the influence of the cytokine colony stimulating factor (CSF)-1, proliferate and differentiate into osteoclast precursors that then enter the peripheral blood and arrive at the site of BMU initiation (Pettit et al. 2008). Teams of osteoclasts remove bone to create a cavity that is observable histomorphologically as a resorptive bay or cutting cone in cortical bone or a "hemicone" in cancellous bone (Parfitt 2005). In cortical bone the osteoclasts of the resorptive phase follow a trajectory related to the directions of mechanical strains, which for typical long bones this is longitudinal (van Oers 2008). Circulating osteoclast precursors express the receptor activator of NFκB, also called RANK. The binding of RANK ligand to this receptor is an important osteoclastogenic signal. Our understanding of how the production of osteoclasts and rate of bone resorption are regulated is described as a RANKL/RANK/OPG system wherein the production of osteoclasts is inhibited by the presence of another cytokine osteoprotegrin (OPG). It is interesting that all three of these regulators of remodeling cell activity are produced by cells of the osteoblast lineage, including stromal cells and bone lining cells (BLC). In cortical bone the cutting cone excavated by osteoclasts typically reaches a diameter of approximately 150 to 350 μm, which determines the cross-sectional size of the histomorphological feature (BSU or osteon) created by the BMU (van Oers et al. 2008). Reported rates at which cortical bone BMUs tunnel through bone is about 20 μm per day (Martin et al. 1998).

Following closely behind the osteoclasts is a growing capillary that nourishes and provides new osteoclast precursors to the advancing BMU. During a short reversal phase that occurs between the resorptive and formation phases a group of mononuclear cells line the resorptive bay. The exact function of these cells remains unclear. Evidence suggests that they smooth off the scalloped periphery of the resorptive bay in preparation for the deposition of a thin, wavy, mineral-deficient, sulfur-rich layer of matrix called a reversal line that

* For an illustration of the potential use of new technology for three-dimensional analysis of cortical bone, see the chapter by Cooper, Thomas, and Clement in this volume.

separates a BSU from surrounding interstitial lamellae (Everts et al. 2002; Robling et al. 2006). Recent evidence suggests that the bone lining cells, discussed later, play important roles in the transition between resorption and formation and the coupling of these processes during bone remodeling (Everts et al. 2002; Tang et al. 2009), and that the coupling of these two processes during bone remodeling results from the release of transforming growth factor (TGF)-beta1 during bone resorption. This polypeptide cytokine transforming growth factor causes the migration of osteoblast precursor cells, stromal, or mesenchymal stem cells, to the resorptive site of the forming BMU (Tang et al. 2009). Differentiation of these cells involves a 2- to 3-day multistage process (Martin et al. 1998).

Subsequent to the reversal phase of the remodeling BMU, which lasts approximately 9 days (Ott 2002), the osteoblasts begin to secrete the organic matrix of bone referred to as osteoid, which is composed of type I collagen and other noncollagenous proteins, proteoglycans, and water (Marks and Odgren 2002; Martin et al. 1998). Osteoid is mineralized through the deposition of calcium phosphate crystals. Because there is a lag time of about 10 days between osteoid deposition and mineralization (Martin et al. 1998), a layer of osteoid is found between the matrix depositing osteoblasts and mineralized bone. The area where mineralization begins is referred to as the mineralization front and it is at this location that in vivo labels of bone formation, such as tetracycline, are incorporated. The thickness of the intervening osteoid seam is normally about 7 μm (Pirok et al. 1966). Increased osteoid seam widths are observed in pathological conditions, such as osteomalacia, that are characterized by mineralization defects (Monier-Faugere et al. 1998). During mineralization crystals of calcium phosphate produced by osteoblasts are deposited within the osteoid matrix. The mineralization process is regulated by inorganic pyrophosphate, which inhibits abnormal calcification, and the levels of which are regulated by several other molecules, nucleotide pyrophosphatase phosphodiesterase 1 (NPP1) and ankylosis protein (ANK), which increase pyrophosphate levels, and nonspecific alkaline phosphatase (TNAP), which breaks down pyrophosphate. Another possible regulator of the bone mineralization process is a protein called osteopontin, levels of which are highly correlated with pyrophosphate levels (Johnson et al. 2006; NIAMS 2006).

Whereas osteoclasts undergo apoptosis after completing their resorptive task, osteoblasts have three potential fates. While some osteoblasts undergo apoptosis, some become trapped in the matrix that they are secreting to become the major bone cell, the osteocytes. Still others persist as the flat bone lining cells (BLC) that line bone surfaces. Both osteocytes and BLCs play important roles in bone metabolism in general and bone remodeling in particular.

After becoming embedded in bone matrix, osteocytes generate cytoplasmic processes that are contained within small spaces called canaliculi within the mineralized bone surrounding the cell that allow osteocytes to retain communication with surrounding cells, such as other osteocytes, osteoblasts, and bone lining cells. Osteocytes play a crucial role in bone maintenance. While they play a role in mineral homeostasis (Nijweide et al. 2002), it is now believed that their major role is biomechanical, sensing mechanical stains and initiation of microfracture repair resulting from mechanical loading (Parfitt 2005). Osteocytes continuously send signals that inhibit the activation of new remodeling BMUs, and it is the disruption of this signal after osteocyte apoptosis or microfracture that initiates activation of a new remodeling cycle (Martin 2000a).

Osteoblasts that do not become entombed in bone matrix to become osteocytes or undergo apoptosis remain on the surface of completed BMUs where they form a lining of

flat cells called bone lining cells (BLCs; Martin et al. 1998). Evidence suggests that these "retired" osteoblasts are responsible for the activation of new BMUs in response to signals from osteocytes or hormones (Burr 2002; Martin 2000a).

Reported lengths for typical BMUs range from 4000 μm (Parfitt 2005) to 300 μm (Martin et al. 1998), the discrepancy probably being due to the difficulty in defining the longitudinal boundaries for individual BMUs. In cortical bone BMUs are observable histomorphologically as osteons (Figure 1.1). The terms *osteon* and *Haversian system* are synonymous; however, *secondary osteon* is commonly used to refer to these structures resulting from intracortical remodeling activity, distinguishing them from primary osteons that are similar in appearance. Primary osteons are not produced by remodeling but rather result from centripetal formation of bone lamellae around a central canal without preceding resorption of the ARF sequence that defines remodeling. Primary osteons usually occur near the endosteal surface where voids or spaces are filled in by osteoblasts during endosteal drift or trabecular compaction, although Carter and Beaupré (2001) also define primary osteons as structures produced when osteoblasts fill in large vascular canals formed in woven bone in rapidly growing animals. In the literature, non-Haversian canals are sometimes referred to by the term *primary osteon*, but these merely represent blood vessels that have become incorporated into the compact bone during radial bone growth (Figure 1.2).

Through the processes of bone remodeling it is possible for several types of secondary osteons to develop, such as type I osteons, type II (embedded) osteons, double-zonal osteons, and drifting osteons. Type I osteons are common secondary osteons and are typically the focus of most histological age estimation methods because of their accumulation with age. A less common secondary osteon is the type II or embedded osteon, which appears as a separate smaller osteon with its own reversal line set within a larger secondary osteon (Ericksen 1991). Their relationship to internal or external factors is unknown. Double-zonal osteons, another less common secondary osteon, demonstrate a hypercalcified ring

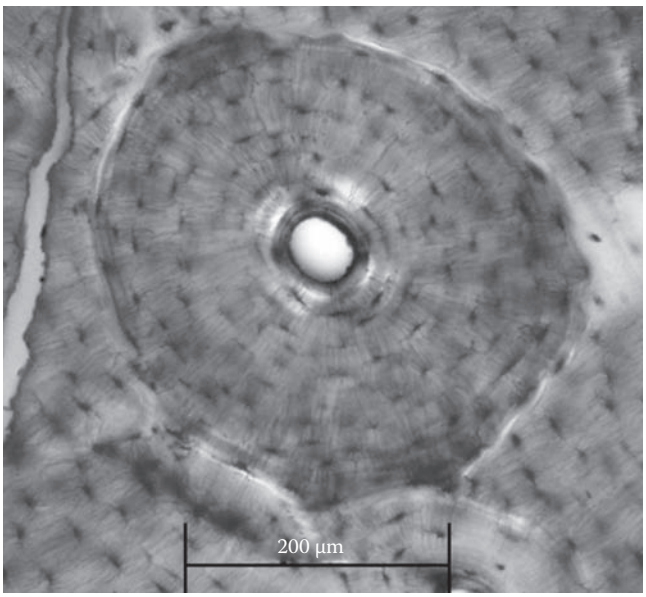


Figure 1.1 Example of a secondary osteon or Haversian system.

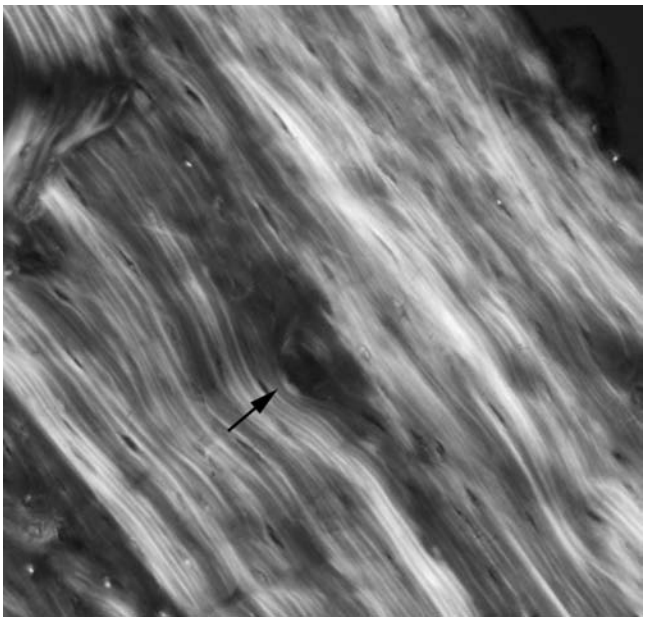


Figure 1.2 Example of a primary vascular canal (arrow) in the periosteal cortex of a human femur. Primary vascular canals are vessels trapped in the lamellar bone during apposition. The lamellae appear to flow around the canal.

within their concentric lamellae (Robling and Stout 2000). It is believed that this represents an arrest line due to a disruption during the remodeling process, possibly caused by some type of stress (e.g., disease, nutritional). Drifting or waltzing osteons are defined as "Haversian systems in which there is continuous resorption on one side and continuous formation on the other" (Robling and Stout 1999:193). The resulting effect, when viewing a bone cross-section, is an osteon exhibiting a tail or whirlwind pattern (Figure 1.3). The tail indicates the direction of drift as it moved through the cortex. They are frequently seen in bones of children, especially during the first decade of life. Other researchers have noted that drifting osteons may be present at any decade of life (Crowder 2005; Epker and Frost 1965; Robling and Stout 1999).

The remodeling process is one of constant adjustment to retain bone integrity and homeostasis within the matrix. Bone formation takes roughly 10 times longer than bone resorption. This suggests that bone formation–resorption must be in perfect balance to retain bone integrity. A theoretical equilibrium ratio for bone remodeling proposed by Ortner and Putschar (1985) estimates that there would be 10 osteons in the formation stage for each resorption space (10:1) in normal bone. Deviations from this ratio may indicate possible pathological or nutritional conditions. Diet is an extrinsic variable that is believed to have a direct effect on the synthesis of the bone matrix (Garrow et al. 2000; Ortner 1975). A diet concentrated on one food type and lacking another may alter the equilibrium ratio. Early studies indicate that Eskimo populations demonstrated rapid bone loss in adulthood when compared to U.S. whites (Ericksen 1973; Thompson and Cowen 1984; Thompson and Gunness-Hey 1981). The disparities in the resorption rates were attributed to dietary differences between populations. Stout and Lueck (1995) compared cortical bone remodeling rates from three archaeological populations with those of a modern sample and determined that the earlier populations reached skeletal maturity at an older age. However, in a

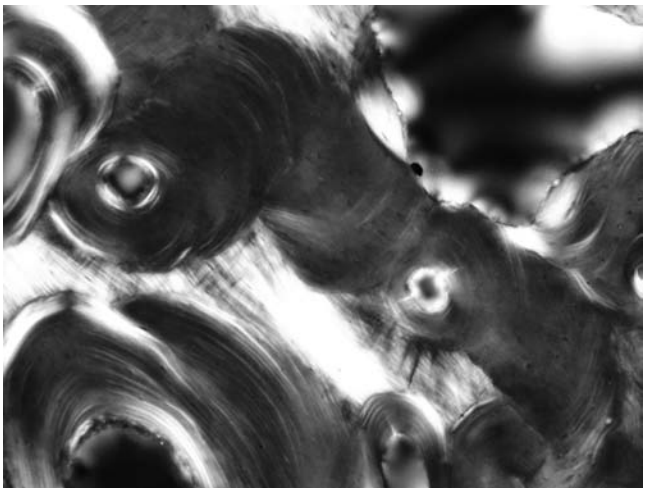


Figure 1.3 Image depicting drifting osteons from a rib cross-section.

study by Burr and colleagues (1990), there were no significant differences in bone loss with age between Pecos Indian archaeological remains and modern white populations. Pfeiffer and Lazenby (1994) stated that the discrepancies in age-related bone loss might be due to the fact that earlier (archaeological) populations died at younger ages or that "their diet or lifestyle facilitated effective bone maintenance" (p. 35). Abbott and colleagues (1996) report bone remodeling rates for Middle and Late Pleistocene human lower limb bones (Shanidar, Tabun) to be slower than rates reported for a more recent archeological sample from Pecos Pueblo, New Mexico, by Burr et al. (1990). However, recalculation of the data reported in Abbott and colleagues (1996) by Streeter et al. (2010) produced remodeling rates for the Pleistocene group that are similar to the values obtained in the more recent comparative sample. Whether bone remodeling rates differ significantly among populations and over time remains open to question. Insight into this question may be gained through an understanding of the theoretical aspects of bone remodeling.

1.2.2 Theory and the Origins of the "New Bone Biology"

Prior to the 19th century, bone architecture was often attributed to divine design or magic (Frost 1998a, 1998b). Researchers as far back as Galileo contemplated bone structure in the context of its mechanical environment (Martin et al. 1998). However, the idea was not popularized until Julius Wolff introduced a theory, known as Wolff's law, in 1892. Wolff's law states that bone's mechanical environment determines its final mass and trabecular architecture. While this theory is accepted as the foundation for functional adaptation of bone, Wolff's theory is based on a static mathematical relationship (Forwood and Turner 1995). Furthermore, Wolff never attempted to formulate this mathematical theory. Prior to Wolff, but within the same century, Bernard and Roux introduced two concepts of bone adaptation: the idea of physiologic homeostasis and the principle of bone functional adaptation based on the dynamic interaction between bone cells and the mechanical environment (Martin 2003a; Martin et al. 1998). The paradigm leading into the next century states that bone structure optimizes strength and that changes in structure are performed through bone cells in response to mechanical strain.

1.2.2.1 The Mechanostat

In the 1980s, orthopedic surgeon Harold M. Frost proposed a concept to explain the mechanism that controls bone mass during longitudinal bone growth, modeling, and BMU-based remodeling (Frost 1983, 1987a). Frost explained that a mechanical feedback system dubbed the mechanostat, which is based on the magnitude of strain resulting from an applied load, is responsible for controlling bone mass. Using a house thermostat as an analogy, the mechanism controlling bone mass would turn on in response to an error and off in its absence (Frost 1987a). Strain thresholds or set points called minimum effective strains (MES) activate or depress bone modeling and remodeling. The threshold strain ranges determine if, when, where, and how long biologic activity turns on or off (Frost 1998b). According to Frost, the MES set points may be altered by hormones and biochemical agents, hence fooling the mechanostat (Turner 1999). For more detailed discussion on biomechanical adaptation of bone see chapters by Maggiano, Agnew and Bolte, and Skedros in this volume.

During remodeling, strains above the threshold of the minimum effective strain for remodeling (MESr) will keep remodeling in conservation mode, retaining bone. When strains fall below the threshold, remodeling goes into disuse mode, removing bone permanently from the endosteal envelope (Frost 1997, 1998a). This can lead to osteopenia and, in severe cases, disuse osteoporosis. Mechanical loading can also affect remodeling (BMU activation rates) when strains are within the conservative mode. Repeated straining of bone will eventually create fatigue damage, producing microfractures in the bone (Frost 2000a). By mechanisms not fully understood, bone signals the BMU to remodel microdamaged areas (Frost 1997). Frost (1960) proposed that strain causes microcracks that disrupt the canalicular connection between bone cells within the matrix. This disruption provides the cells with the stimulus to initiate remodeling. If the strain increases to a level where microfractures are accumulating at a rate that the BMU cannot repair, gross bone failure will occur, resulting in fatigue fractures. For more on microfractures and fracture risk see Chapter 9 by Agnew and Bolte in this volume. The biological determinants of bone strength include baseline conditions that are found at birth (Frost 1999a). This indicates that genes may predetermine a skeletal element's baseline condition, while the mechanostat adds the functional adaptation.

1.2.2.2 The Utah Paradigm

In the mid-1990s, a series of Hard Tissue Workshops held at the University of Utah discussed the increasing discordance between the subfields of skeletal biology and lack of multidisciplinary approaches to skeletal research. From these workshops a new paradigm in skeletal physiology, dubbed the Utah paradigm, evolved (Frost 1998a). The Utah paradigm proposed a shift from the 1960 paradigm of skeletal physiology, which suggested that the bone effector cells determined bone health and disease under the control of nonmechanical agents to meet homeostatic needs (Frost 1998b, 2000b). The new paradigm explored the load-bearing skeletal elements at the organ, tissue, and cellular level focusing on the mechanical competence of bone (Frost 1999a, 1999b, 2000b). The mechanostat is the leading hypothesis in the Utah paradigm, in that it explains how load-bearing skeletal organs attain mechanical competence. This hypothesis does not explain the forces that control other skeletal elements, such as the cranial bones, which do not carry large biomechanical loads, implying that there are other factors involved. The Utah paradigm indicated that the driving force of load-bearing skeletal architecture and strength are mechanical

factors, and that nonmechanical factors, such as hormones, vitamins, minerals, sex, and age, either hinder or help the process but do not replace it (Frost 1999b, 2000b). Growth hormones have the greatest effect on the mechanostat because they are directly associated with increasing muscle strength, body weight, and longitudinal bone growth (Frost 1998b). According the Utah paradigm of skeletal physiology, these nonmechanical agents do not dominate control of bone strength. Instead, control depends on the mechanical loads (Schoenau and Frost 2002).

In 2003, Frost published an update of the mechanostat hypothesis. It focused on postnatal bone mass and strength, how these variables adapt over time to the mechanical forces placed on them, and the application of the mechanostat to nonskeletal tissues. Although the mechanostat remains the leading theory describing factors controlling bone mass and strength, empirical data establishing a direct cause and effect relationship between muscle force and bone strength has not been established. Results of clinical experiments designed to test the mechanostat hypothesis indicate that bone development is driven by muscle development; however, it is not clear how much genetic factors, rather than mechanical factors, account for the correlation between bone and muscle mass (Parfitt 2004; Rauch et al. 2004).

1.2.2.3 Osteocyte Inhibitor Theory

Burr (2002) states that bone remodeling fulfills three goals: (1) maintenance of mineral homeostasis, (2) adaptation to bone's mechanical environment, and (3) repair of microdamage to insure mechanical integrity. Studies have shown that targeted remodeling occurs at sites associated with microcracks, indicating a cause-and-effect relationship (Burr and Martin 1993; Martin 2003a, 2003b). Frost's (1960) original proposal stated that microcracks disrupt canalicular connections between osteocytes, thus providing the stimulus to initiate remodeling. Burr (2002) describes a slightly different view, in which the osteocyte–canalicular system acts as an inhibitor of the osteoclastic activity.

According to the mechanostat, disuse activates remodeling and inhibits modeling, while overload inhibits remodeling and activates modeling. Martin (2000a, 2000b) states that the mechanostat limits itself to effects of strain on modeling and remodeling, and does not consider the removal of microdamage, caused by high strains, part of bone's mechanical adaptation. Martin (2000b, 2003a, 2003b) indicates that observations of bone in disuse and overload coincide with the idea that the osteocytes inhibit rather than stimulate remodeling.

Strain levels are necessary to nourish the osteocytes through interstitial fluid flow and lack of nutrition causes a disruption in the osteocyte network, producing a signal to switch bone into disuse mode (Martin 2003a, 2003b). When the osteocyte network is disrupted by osteocyte apoptosis, the constraining mechanism is released. This stimulates resorption activity. Osteocyte apoptosis has been correlated with regions of microdamage and new remodeling events (Burr 2002; Verborgt et al. 2000). Burr (2002) states that 70 percent of bone remodeling is nontargeted, leaving 30 percent that is damage initiated. The nontargeted or stochastic remodeling may be associated with metabolic purposes (Martin 2002). Unlike Frost's theory, the osteocyte inhibitor theory currently has little supporting evidence, so the current understanding of the relationship between stimulus and bone remodeling remains controversial because the relationship between causative and stochastic bone remodeling is not fully understood. It is likely that the osteocytes of osteons, like most cells, have a life span; and once the cell begins to fail in its normal functions or natural cell death occurs, remodeling is initiated. More recent studies have linked osteocyte apoptosis to bone resorption; however, the spatial and temporal relationships are still not

fully characterized. Emerton et al. (2010) report that osteocyte apoptosis is necessary to initiate endocortical remodeling in the response to estrogen withdrawal in adult female mice. They observed that osteoclastic activity did not occur in the absence of osteocyte apoptosis. According to Bonewald and Johnson (2008), the study of osteocyte biology is becoming an intense area of research interest.

1.2.2.4 The Principle of Cellular Accommodation Theory

The mechanostat theory remained relatively unchallenged until 1999 when Charles Turner presented a mathematical description of bone biology in which he coined the principle of cellular accommodation. Turner (1999) draws attention to the fact that the mechanostat theory does not explain why nonweight-bearing bones do not resorb away due to disuse remodeling. Furthermore, he states that the mechanostat theory does not conform to experimental observation. Although Frost postulates that the MES set points can be fooled by hormones, he does not explain how or why skeletal sites are regulated differently. The principle of cellular accommodation theory states that bone cells learn from their physical and biological environment and that they accommodate to the new environment. Therefore, the set points vary from site to site depending on the local strain. The set point in weight-bearing bones will be high, while the set point in nonweight-bearing bones will be much lower. Turner claims that Frost places biomechanics at the center of bone biology ignoring the fact that bone is "insensitive to mechanical loading in the absence of either parathyroid or growth hormones" (2000:186). This indicates that endocrine and paracrine agents do not have a secondary role but a primary role in bone formation. Further research indicates that bone cells not only learn from and accommodate to their environment, but they may retain a cellular memory of their previous loading environment (Turner et al. 2002). Turner's ideas and research may be a step toward a unified theory of bone remodeling considering loading history, cellular memory, and the role of hormonal/anabolic agents.

1.2.3 Bone Remodeling and Histological Age Estimation

When performing histological age estimation analyses, recall that they are based upon the accumulated evidence of bone remodeling. Discrete evidence of remodeling events in the past (osteons) is easily distinguished in the unstained section.* The key to accurately distinguishing secondary osteons (cortical BSUs) from other similar structures, such as primary osteons and vascular canals, is the presence of a well-defined reversal line, indicative of the resorptive and reversal phases of BMU creation. Osteons that lack an intact Haversian canal because of their partial resorption by subsequent remodeling activity can also be distinguished from interstitial primary lamellar bone by the presence of a reversal line, and the concentric nature of their lamellae and osteocytic lacunae. In practice, because of the effects of depth of field in light microscopy, reversal lines and lamellar patterns are more easily and reliably identified if the microscopist uses the fine focus to focus up and down when viewing sections (Figure 1.4). This procedure is well known by microscopists and provides some measure of dimensionality to microscopic structures. The lack of depth of

* The material nature of bone makes the use of histochemistry (staining) usually unnecessary for histomorphological and histomorphometric analyses of the bone samples available for anthropological age estimation.

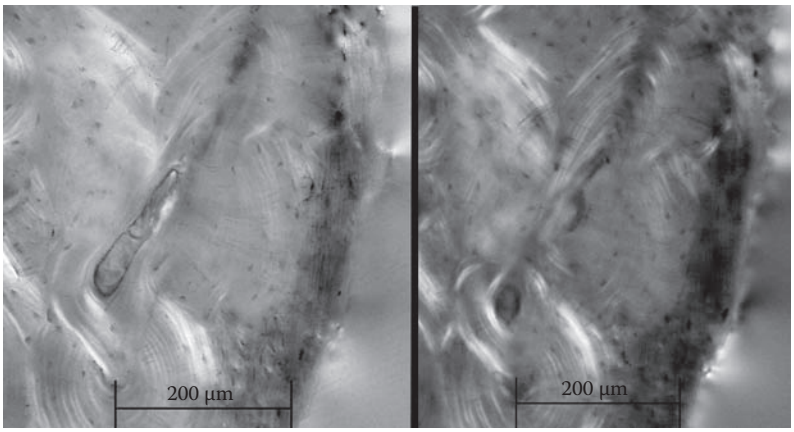


Figure 1.4 Images taken at two focal planes. The left image shows either a Haversian canal branching event or an obliquely sectioned osteon producing an eccentric Haversian canal. The right image is slightly out of focus but shows that the microstructures in the previous focal plane represent a branching event. The area of interest now shows an intact osteon with a Haversian canal.

field for digitally captured images, therefore, can make the accurate differentiation of histomorphological features, such as reversal lines, difficult. This is why we recommend that analyses be performed directly through the microscope as well as from digital images.

Three important issues that should be considered when undertaking histological age estimations are mean tissue age (MTA), effective age of adult compacta, and osteon population density (OPD) asymptote. Cortical osteons and osteon fragments observed in a bone cross-section do not represent BMUs that have accumulated since birth but rather a later age characteristic of each bone called the effective age of adult compacta. Cortical drifts during growth remove some of the visible evidence of earlier BMU creations, osteons and their fragments, and the chronological age of an individual is usually greater than the actual age of a given area of bone tissue. Based upon data from the use of in vivo tissue-time labeling, the MTA and effective age for the birth of adult compacta for the middle third of the rib is estimated to be approximately 12.5 years (Wu et al. 1970). The years over which the BSUs observed in the cortex of the rib have accumulated can be estimated by chronological age minus 12.5 years. It is not appropriate, therefore, to apply most currently available histological age estimation formulas derived primarily from adult skeletons to subadults.

At the other end of the age scale, there is a problem as well. Some existing BSUs are partially removed by the creating of new BMUs to produce what are referred to as osteon fragments. Osteons and their fragments are the visible evidence of past remodeling activity and these are the major predicting variables used in most histological age estimation methods. However, the creation of a BMU can also completely remove an existing BSU. Although this is not a significant problem for bones from younger individuals that include significant amounts of unremodeled primary lamellar bone tissue, in older individuals the relative number of missing osteons (BSUs) increases eventually reaching an OPD asymptote. The OPD at which an asymptote is attained varies for different bones, depending upon factors such as remodeling activation rates, cortical diameter, and osteon size. Using data from Kerley (1965), Frost (1987b, 1987c) estimates an OPD asymptote for the midshaft femur to be about 50/mm^2. Asymptote for the rib is reported to be reached at an OPD of about 30/mm^2 (Stout and Paine 1994). Once asymptote is reached for OPD, visible evidence

for BMU activation does not increase with age. This is a major problem for histological age estimation for elderly individuals. For example, OPD asymptote can occur as early as 50 years for the rib. Therefore, a histological age estimate of 50 years based upon an OPD of about 30/mm^2 can be only reported as an age 50 years or older. Taking advantage of other histomorphological features known to vary with age in addition to OPD may allow age estimation for elderly individuals. Stout and Streeter (2004, 2006) used osteon size and relative cortical area of the rib from a Mayan ruler to support a ninth decade of life age based upon inscriptions. Certain bones, such as the femur, are probably better for estimating age for older individuals because the OPD asymptotes occur at relatively older ages owing to their larger cortical areas. New age estimating methods that are more applicable to older individuals should be considered that employ combinations of bones and variables other than OPD should be explored. In order to quantify the amount, size, and shape of histological features, histomorphometric techniques are applied.

1.3 Bone Histomorphometry

Increasingly, skeletal biologists are employing histomorphometry in the analysis of both modern and ancient bone (Abbott et al. 1996; Ericksen 1976; Frost 1987b, 1987c; Kerley and Ubelaker 1978; Kimura 1992; Martin and Armelagos 1985; Mulhern and van Gerven 1997; Stout and Lueck 1995; Streeter et al. 2010). In short, histomorphometry is the quantification of microstructures in the skeletal tissue or their characteristics. Histomorphometric analysis of bone provides quantitative information, such as bone turnover (remodeling) and microarchitecture, which cannot be obtained from other approaches. Age-at-death estimation and the determination of human from nonhuman bone, in particular, have been widely used by anthropologists. As previously mentioned, histological age estimation is based on the observation of age-dependent change in the bone microstructure, most frequently the evidence of bone remodeling activity, the amount of unremodeled lamellar bone, and mean osteon size. Several book chapters have been devoted to the physiological basis for and the detailed description of histological age estimation methods (Crowder 2009; Robling and Stout 2008; Stout 1989; Stout and Paine 1992) and the determination of human versus nonhuman bone (Mulhern 2009). Therefore, the following will be limited to a general discussion of bone histomorphometry as it relates to anthropological analyses. Several chapters within this volume address quantitative methods and report data collected from numerous studies (see Streeter; Mulhern and Ubelaker; Cooper, Thomas, and Clement; and Guatelli-Steinberg and Huffman).

The quantification of bone turnover, microarchitecture, and static and dynamic cell activity is performed through measuring and counting of structures to characterize changes in bone histomorphology. Anthropology, as a discipline, is a relative newcomer in the quantification of bone structure and organization and, as result, analyses are often performed without full consideration of the underlying processes governing bone biology. To reliably evaluate bone at the histological level the analyst must interpret histomorphometric results in the context of bone biology. Anthropologists should also follow the standardization of nomenclature, symbols, and unit released by the American Society for Bone and Mineral Research (ASBMR) to assist in a universal understanding of bone histomorphometry and eliminate ambiguity in the reporting of data (Parfitt et al. 1987).

To ensure the proper application of any method utilizing histomorphometric techniques it is important to consult the methods from the original publication. The proper magnification, sampling area, and other specified procedures must be followed as prescribed for each method. Evaluating histological structures is a slow process and requires constant manipulation of the microscope's fine focus and light. Both polarized and nonpolarized light should be used to provide full resolution of structures during assessment. The application of automated techniques may be beneficial and reduce evaluation time; however, current technology may not allow for the complete dismissal of direct viewing through the microscope. This is illustrated in the study discussed in Chapter 13 by Thomas and Clement where they describe the results of research evaluating the relationship between histomorphological features of bone tissue and the age at death. Their study is unique in terms of its methodology and sample size. It employed a semiautomated system that allowed the counting of Haversian canals from microradiographs made from sections of entire cross-sections of the femoral cortex. While the results were less than promising, finding a poor association between Haversian canals and chronological age, the study provided valuable information relating to methodology, including sampling, sample size, and choice of variables.

1.3.1 Static Histomorphometry

Static histomorphometry allows for the quantification of histological structures being evaluated in a bone at a particular point in time. In most anthropological research and practical applications of bone histology methods (i.e., age estimation, human vs. nonhuman determination), static indices of bone microarchitecture are evaluated and calculated. Histological structures are manually or automatically measured through a variety of measurement or counting techniques. Typical variables (measurements) recorded include, but are not limited to, volume, length, perimeter, diameter, area, circularity, and osteon population density (osteon counts). As previously mentioned, the proper nomenclature should be followed when recording and reporting histological data. For example, the following is a list of commonly measured histological features for age estimation:

1. Total area of bone sampled (Sa.Ar.)—Amount of cortical bone. Sa.Ar. is expressed in square millimeters (mm^2), and can be determined either directly under the microscope using an appropriate eyepiece counting reticule or from digital images using image analysis software.
2. Number of intact osteons (N.On.)—Secondary osteons with at least 90 percent of their Haversian canal perimeters intact or unremodeled. Refer to Robling and Stout (2008) for an in-depth description of intact secondary osteons.
3. Number of fragmentary osteons (N.On.Fg.)—Secondary osteons in which 10 percent or more of the perimeters of their Haversian canals, if present, have been remodeled by subsequent generations of osteons. This variable also includes remnants of preexisting secondary osteons that no longer contain a Haversian canal. Refer to Robling and Stout (2008) for a more in-depth description of intact secondary osteons.
4. Osteon population density in #/mm2 (OPD)—This variable is the sum of N.On. and N.On.Fg. divided by Sa.Ar.
5. Relative cortical area (Ct.Ar./Tt.Ar.)—The relative amount of cortical bone in cross-sectional area of bone, or the ratio of cortical bone area (Ct.Ar.) to total area (Tt.Ar.) of a rib cross-section.

6. Mean osteonal cross-sectional area in mm^2 (On.Ar.)—The average area of bone contained within the cement lines of structurally complete osteons for each rib specimen. Osteons are considered structurally complete if their reversal lines were intact. Complete osteons with Haversian canals that deviated significantly from circular structures should be excluded. Mean area is calculated as the average cross-sectional area of 25 to 50 complete osteons per cross-section.

Several authors have used these variables and others to create algorithms to derive static indices of bone remodeling to be used for various purposes. Research by Stout and colleagues (1986, 1992, 1996) and Cho et al. (2002) have focused on developing equations to evaluate and predict age-related bone turnover in the rib. Frost (1987b,c) developed an algorithm to estimate the number of missing osteons that correspond to observed osteon populations densities (Stout and Paine 1994). The algorithm appeared promising to allow for more accurate age estimations when evaluating remodeling rates through static histomorphometry. Because new osteon creations eventually begin to remove all evidence of older turnover events, the algorithm allows for the estimation of the total number of osteon creations from the observed osteon population density of a cross-section. Frost provided a caveat, in that the lack of systematic studies to accurately determine the values for the variables within the algorithm, such as mean tissue age (MTA) and the fragment packing factor (k), limits its overall utility. Advances in dynamic histomorphometry will likely lead to a better understanding in how factors, such as biomechanics, affect the variables proposed by Frost. Dynamic histomorphometry, however, does not allow for the measure of bone remodeling rates averaged over the lifetime of an individual (Stout and Paine 1994) suggesting that a combination of static and dynamic research methods are needed to make advances in the field.

Basic static histomorphometry is typically performed through the preparation of undecalcified bone. Undecalcified* or decalcified bone samples may require special stains depending on the structures or processes that are being quantified. Traditionally used trichrome stains for paraffin-embedded tissue are not compatible with plastic-embedded bone (Villanueva and Mehr 1977); however, a number of stains are available for the histochemistry of plastic-embedded undecalcified bone (Anderson 1982). Various stains and staining techniques may be used to differentiate calcified bone from osteoid to allow the analyst to appreciate and measure the osteoid seam to quantify the amount of bone formation and resorption. Mineralized and unmineralized components of bone (i.e., osteoblasts, osteoclasts, and other cells) can easily be differentiated with the appropriate staining technique.† While evaluation of bone for age estimation or the determination of human versus nonhuman may not require any staining protocol, the analysis of, for example, a fracture callus using specific stains to estimate the timing of the injury will assist in quantifying the area of the mineralized callus, area of cartilaginous tissue within the callus, and the area or proportion of fibrotic tissue within the callus (Figures 1.5 and 1.6).

* Because the standard in most histology laboratories is to decalcify bone, the term undecalcified is often used rather than calcified bone.

† Histochemical staining is essentially limited to relatively fresh specimens, such as that acquired through biopsy or at autopsy. Bone cells and osteoid tissue is not preserved in archaeological or unfixed skeletal remains, making the use of histological staining unnecessary except for enhancing certain histomorphological features such as cement lines.

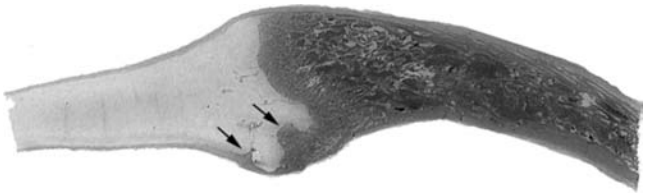


Figure 1.5 Longitudinal section of the costochondral junction from an infant rib (hematoxylin and eosin [H&E] stain). The arrows denote fibrotic tissue and new bone development along the posterior aspect of the rib as the result of an antemortem costochondral fracture.

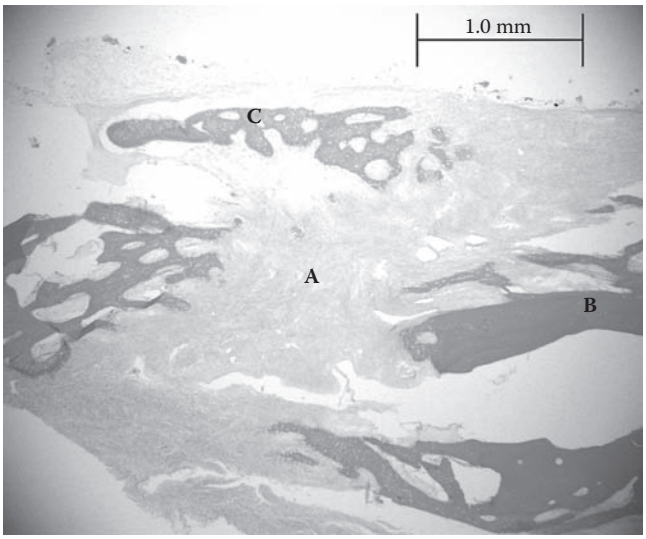


Figure 1.6 Cross-section of an antemortem cranial fracture in an infant (H&E stain). (A) Fibrous tissue within the fracture gap, (B) primary lamellar bone, (C) new woven bone.

1.3.2 Dynamic Histomorphometry

Dynamic histomorphometry allows for the quantification of changes in cellular activity over time. To observe specific cellular responses, studies are performed in vitro or in vivo and the subject is exposed to various labeling techniques. One such procedure is to double-label bone with a fluorochrome, such as Tetracyclines, and measure histomorphometric parameters involved in bone turnover and bone mineralization over a specific period of time (Bassett et al. 1990; Frost 1969; Maggiano et al. 2006). The information obtained from static and dynamic histomorphometry provides useful information of bone turnover and other cellular responses in bone (Cho and Stout 2003; Cho et al. 2006; Pirok et al. 1966; Stout and Lueck 1995; Wu et al. 1970). Whereas dynamic histomorphometry reveals bone-remodeling rates occurring at the time that the tissue label was administered, static histomorphometry provides a measure of bone remodeling rates averaged over the age (effective age of adult compacta). Because static histomorphometry does not require the administration of an in vivo tissue time marker, it is most appropriate for anthropological research and can be used to estimate bone remodeling rates for unlabeled skeletal remains (Stout and Lueck 1995; Stout and Paine 1994; Wu et al. 1970). Although skeletal remains typically studied by anthropologists, including bioarchaeologists, forensic anthropologists,

and paleontologists, are unlikely to contain intravital tissue labels, fortuitous evidence for labeling has been reported. For example, labeling was identified in skull fragments and used to support identification of the individual to whom they belonged (Stout and Ross 1991). Evidence of in vivo labeling of archaeological bone thought to have occurred through the consumption of stored grains infected with tetracycline-producing Streptomycetes has been reported (Bassett et al. 1990; Maggiano et al. 2003; Megan et al. 1989).

1.4 Reliability of Histological Analyses

Method reliability and validity is strongly emphasized in forensic anthropological research primarily because methods used in the forensic context must meet specific demands owing to the evidentiary nature of analytical results (Christensen and Crowder 2009; Crowder 2009). In the United States, the legal requirements for the evaluation of scientific evidence are set forth in *Daubert v. Merrell Dow Pharmaceuticals, Inc.*, 113 S.Ct. 2786 (1993). According to the *Daubert* criteria, a trial judge must determine if a method is adequately tested, subjected to peer review in a published journal, provided with potential error rates, and enjoys general acceptance within the relevant scientific community. Currently, methods of histological examination may not meet these standards. Very few large validation studies have been performed; thus error rates are unclear. Because of the guidelines established by the decisions in *Daubert v. Merrell Dow Pharmaceuticals, Inc.* (1993), *General Electric Co. v. Joiner* (1997), and *Kumho Tire Co. v. Carmichael* (1999), and in the wake of the National Academy of Sciences (NAS) report on the current state of the forensic sciences, methods used by forensic specialists may be more frequently challenged by the courts. Following the 1993 *Daubert* ruling and subsequent court rulings expanding the scope of the *Daubert* criteria, many forensic disciplines have determined that there is a need to critically reevaluate some of the techniques and methods used in their examinations, as well as the validity of the underlying scientific theories (Christensen and Crowder 2009). Methods with vague descriptions of samples, procedures, variables, or potential error rates should not be considered for use in the forensic setting. Thus, new methods or clarification of current methods are needed to improve scientific standards within the field. As previously mentioned, the ASBMR standardized the nomenclature for hard-tissue histologists (Parfitt et al. 1987) to relieve confusion and to reduce the semantic barriers that they must cross within the field; however, few researchers in anthropology have adhered to the protocol.

Crowder (2005) identified specific methodological issues in histological analyses and suggested protocols to improve histological methods (for the estimation of age at death) so that they may become a conventional tool for anthropological analysis and address the evidentiary standards. Recognizing and quantifying potential biases in histological analyses will support the development of best practices for histological analysis. Although various histological methods have been revised in the literature, the fundamental issues concerning the reliability and repeatability of these methods have not been fully addressed. The application of qualitative and quantitative bone histology as a preferred method in anthropological analyses has been hindered by researchers' uncertainty regarding the accuracy and reliability of the methods, as well as, an incomplete understanding in the literature of the biological processes behind intracortical bone remodeling. For example, while microscopic age changes are considered to be universal, the inconsistencies in reported accuracy of the methods when they are applied

to individuals outside of the reference samples suggest that intrinsic and extrinsic biological factors, such as genetics and a wide range of suggested behaviors, have varying effects on bone microstructure. These issues can only be resolved through research designs that consider the dynamic nature of bone and evaluate results in the scope of skeletal biology.

1.5 Conclusions

The anthropological analyses of skeletal tissue should ideally employ a multilevel (e.g., gross [macroscopic] and microscopic) and multimodality (e.g., radiographs, CTs) approach. The inclusion of microscopic methods, especially histomorphometry can strengthen other analytical results and most important provide information that cannot be obtained from other methods. Most notably, in addition to age-at-death estimation, histomorphometry can provide insights into bone remodeling history that can be used to estimate remodeling rates creating what can be called "paleophysiology."

References

Abbot S, Trinkaus E, Burr D (1996) Dynamic bone remodeling in later Pleistocene fossil hominids. Am J Phys Anthropol 99:585–601.

Anderson C (1982) Manual for the Examination of Bone. Boca Raton, FL: CRC Press, p. 116.

Bassett EM, Keith M, Armelagos G (1990) Tetracycline-labeled human bone from prehistoric Sudanese Nubia (A.D. 350). Science 209:1532–1534.

Bonewald LF, Johnson ML (2008). Osteocytes, mechanosensing and Wnt signaling. Bone 42(4):606–615.

Burr DB (1992) Estimated intracortical bone turnover in the femur of growing macaques: Implications for their use as models in skeletal pathology. Anat Rec 232:180–189.

Burr DB (2002) Targeted and nontargeted remodeling. Bone 30(1):2–4.

Burr DB, Ruff CB, Thompson DD (1990) Patterns of skeletal histologic change through time: Comparison of and archaic native American population with modern populations. Anat Rec 226:307–313.

Burr DB, Martin RB (1993) Calculating probability that microcracks initiate resorption spaces. J Biomech 26:613–616.

Carter DR, Beaupré G (2001) Skeletal Function and Form. Cambridge: Cambridge University Press.

Cho H, Stout SD, Madsen RW, Streeter MA (2002) Population-specific histological age-estimating method: A model for known African-American and European-American skeletal remains. J Forensic Sci 47(1):12–18.

Cho H, Stout S (2003) Bone remodeling and age-associated bone loss in the past: An histomorphometric analysis of the imperial Roman skeletal population of Isola Sacra. In: Agarwal SC, and Stout S, editors. Bone Loss and Osteoporosis: An Anthropological Perspective. New York: Kluwer Academic/Plenum, pp. 91–101.

Cho H, Stout S, Bishop T (2006) Cortical bone remodeling rates in a sample of African American and European American descent groups from the American Midwest: Comparisons of age and sex in ribs. Am J Phys Anthropol 130(2):214–226.

Christensen A, Crowder C (2009) Evidentiary standards for forensic anthropology. J Forensic Sci 54(6):1211–1216.

Crowder C (2005) Evaluating the use of quantitative bone histology to estimate adult age at death. Doctoral dissertation, University of Toronto, Ontario, Canada.

Crowder C (2009) Histological age estimation. In: Ubelaker DH, Blau S, editors. Handbook of Forensic Archaeology and Anthropology. Walnut Creek, CA: Left Coast Press, pp. 222–235.

Daubert v. Merrell Dow Pharmaceuticals, Inc., 509 U.S. 579 (1993).

Emerton KB, Hu B, Woo AA, Sinofsky A, Hernandez C, Majeska RJ, Jepsen KJ, Schaffler MB (2010) Osteocyte apoptosis and control of bone resorption following ovariectomy in mice. Bone 46(3):577–583.

Epker BN, Frost HM (1965) The direction of transverse drift of activity forming osteons in the human rib cortex. J Bone Joint Surg 47a:1211–1215.

Ericksen MF (1973) Age-related bone remodeling in three aboriginal American populations. PhD dissertation, George Washington University, Washington DC.

Ericksen MF (1976) Cortical bone loss in three native American populations. Am J Phys Anthropol 45:443–452.

Ericksen MF (1991) Histological estimation of age at death using the anterior cortex of the femur. Am J Phys Anthropol 84:171–179.

Everts V, Delaissé JM, Korper W, Jansen DC, Tigchelaar-Gutter W, Saftig P, Beertsen W (2002) The bone lining cell: Its role in cleaning Howship's lacunae and initiating bone formation. J Bone Miner Res 17(1):77–90.

Forwood MR, Turner CH (1995) Skeletal adaptations to mechanical usage: Results from tibial loading studies in rats. Bone 17(4) Suppl:1975–2005.

Frost HM (1960) Presence of microscopic cracks in vivo in bone. Henry Ford Hosp Med Bull 8:25–35.

Frost HM (1969) Tetracycline based histological analysis of bone remodeling. Calcif Tissue Res 3:211–237.

Frost HM (1983) The skeletal intermediary organization. Metab Bone Dis Rel Res 4:281–290.

Frost HM (1986) Intermediary organization of the skeleton. Boca Raton, FL: CRC Press.

Frost HM (1987a) Bone "mass" and the "mechanostat": A proposal. Anat Rec 219:1–9.

Frost HM (1987b) Secondary osteon population densities: An algorithm for estimating missing osteons. Yrbk Phys Anthropol 30:239–254.

Frost HM (1987c) Secondary osteon populations: An algorithm for determining mean bone tissue age. Yrbk Phys Anthropol 30:221–238.

Frost HM (1997) Why do marathon runners have less bone than weight lifters? A vital-biomechanical view and explanation. Bone 20(3):183–189.

Frost HM (1998a) Changing concepts in skeletal physiology: Wolff's law, the mechanostat, and the "Utah paradigm." Am J Hum Biol 10:599–605.

Frost HM (1998b) From Wolff's law to the mechanostat: A new "face" of physiology. J Orthop Sci 3:282–286.

Frost HM (1999a) An approach to estimating bone and joint loads and muscle strength in living subjects and skeletal remains. Am J Hum Biol 11:437–455.

Frost HM (1999b) Why do bone strength and "mass" in aging adults become unresponsive to vigorous exercise? Insights of the Utah paradigm. J Bone Miner Metab 17:90–97.

Frost HM (2000a) Does bone design intend to minimize fatigue failures? A case for the affirmative. J Bone Miner Metab 18:278–282.

Frost HM (2000b) The Utah paradigm of skeletal physiology: An overview of its insights for bone, cartilage and collagenous tissue organs. J Bone Miner Metab 18:305–316.

Frost HM (2003) Bone's mechanostat: A 2003 update. Anat Rec Part A 275A:1081–1101.

Garrow JS, James WPT, Ralph A (2000) Human Nutrition and Dietetics, 10th edition. Edinburgh: Churchill Livingstone.

General Electric Co. v. Joiner, 522 US 136 (1997).

Hauge EM, Qvesel D, Eriksen EF, Mosekilde L, Melsen F (2001) Cancellous bone remodeling occurs in specialized compartments lined by cells expressing osteoblastic markers. J Bone Miner Res 16(9):1575–1582.

Johnson K, Goding J, Van Etten D, Sali A, Hu S-I, Farley D, Krug H, Hessle L, Millán JL, Terkeltaub R (2006) Linked deficiencies in extracellular PPi and Osteopontin mediate pathologic calcification associated with defective PC-1 and ANK expression. J Bone Miner Res 18(6):994–1004.

Kerley ER (1965) The microscopic determination of age in human bone. Am J Phys Anthropol 23:149–164.

Kerley ER, Ubelaker DH (1978) Revisions in the microscopic method of estimating age at death in human cortical bone. Am J Phys Anthropol 49:545–546.

Kimura K (1992) Estimation of age at death from second metacarpals. Z Morph Anthropol 79:169–181.

Kumho Tire Co. v. Carmichael, 526 U.S. 137 (1999).

Maggiano C, Dupras T, Schultz M, Biggerstaff J (2006) Spectral and photobleaching analysis using confocal laser scanning microscopy: A comparison of modern and archaeological bone fluorescence. Mol Cell Probes 20(3–4):154–162.

Maggiano C, Dupras TL, Biggerstaff J (2003) Ancient antibiotics: evidence for tetracycline in human and animal bone from kellis. In: Mills AJ, and Cope CA, editors. The Dakhleh Oasis Monograph. Oxford: Oxbow Books, pp. 331–344.

Marks SC, Odgren PR (2002) Structure and development of the skeleton. In: Bilezikian JP, Raisz LG, and Rodan GA, editors. Principles of Bone Biology. San Diego: Academic Press, pp. 3–16.

Martin DL, Armelagos GJ (1985) Skeletal remodeling and mineralization as indicators of health: An example from prehistoric Sudanese Nubia. J Hum Evol 14:527–537.

Martin RB (1994) On the histological measurement of osteonal BMU activation frequency. Bone 15:547–549.

Martin RB (2000a) Does osteocytes formation cause the nonlinear refilling of osteons? Bone 26(1):71–78.

Martin RB (2000b) Toward a unifying theory of bone remodeling. Bone 26(1):1–6.

Martin RB (2002) Is all cortical bone remodeling initiated by microdamage? Bone 30(1):8–13.

Martin RB (2003a) Functional adaptation and fragility of the skeleton. In Agarwald, SC and Stout, SD, editors. Bone Loss and Osteoporosis: An Anthropological Perspective. New York: Kluwer Academic/Plenum, pp. 121–136.

Martin RB (2003b) Fatigue damage, remodeling, and the minimization of skeletal weight. J Theor Biol 220:271–276.

Martin RB, Burr DB, Sharkey NA (1998) Skeletal Tissue Mechanics. New York: Springer-Verlag.

Megan C, El M, Anderson C (1989) Fluorochrome labelling in Roman period skeletons from Dakhleh Oasis, Egypt. Am J Phys Anthropol 80(2):137–143.

Monier-Faugere M, Langub MC, Malluche HH (1998) Bone biopsies: A modern approach. In: Avioli LV, Krane SM, editors. Metabolic Bone Diseases and Clinically Related Disorders, 3rd edition. San Diego: Academic Press, pp. 237–273.

Mulhern D (2009) Differentiating human from nonhuman skeletal remains. In: Ubelaker DH, Blau S, editors. Handbook of Forensic Archaeology and Anthropology. Walnut Creek, CA: Left Coast Press, pp. 222–235.

Mulhern DM, Van Gerven DP (1997) Patterns of femoral bone remodeling dynamics in a medieval Nubian population. Am J Phys Anthropol 104:133–146.

Mulhern D, Ubelaker DH (2003) Histologic examination of bone development in juvenile chimpanzees. Am J Phys Anthropol 122:127–133.

NIAMS (2006) Scientists Gain New Clues to Bone Mineralization. News and Events.

Nijweide PJ, Burger EH, Klein-Nulend J (2002) The osteocyte. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of Bone Biology, 2nd edition. San Diego: Academic Press, pp. 93–107.

Ortner DJ, Putschar WG (1985) Identification of Pathological Conditions in Human Skeletal Remains. Smithsonian Institution Press: Washington, DC.

Ortner DJ (1975) Aging effects on osteon remodeling. Calcif Tissue Res 18:27–36.

Ott SM (2002) Histomorphometric analysis of bone remodeling. In: Bilezikian JP, Raisz LG, Rodan GA, editors. Principles of Bone Biology. San Diego: Academic Press, pp. 303–319.

Paine R, Godfrey L (1997) The scaling of skeletal microanatomy in non-human primates. J Zool, Lond 241:803–821.

Parfitt A (1979) Quantum concept of bone remodeling and turnover: Implications for the pathogenesis of osteoporosis. Calcif Tissue Int 28:1–5.

Parfitt AM (2004) The attainment of peak bone mass: What is the relationship between muscle growth and bone growth? Bone 34:767–770.

Parfitt AM (2005) Targeted and nontargeted remodeling: Relationship to basic multicellular unit organization and progression. Bone 30(1):5–7.

Parfitt AM, Drezner MK, Glorieux FH, Kanis JH, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone histomorphometry: Standardization of nomenclature, symbols, and units. J Bone Miner Res 2:595–610.

Peck J, Stout S (2008) The effects of total hip arthroplasty on the structural and biomechanical properties of adult bone. Am J Phys Anthropol 138(2):221–230.

Peck W, Woods W (1988) The cells of bone. In: Riggs B, Melton LI, editors. Osteoporosis: Etiology, Diagnosis, and Management. New York: Raven, pp. 1–44.

Pettit AR, Chang MK, Hume DA (2008) Osteal macrophages: A new twist on coupling during bone dynamics. Bone 43:976–982.

Pfeiffer S, Lazenby R (1994) Low bone mass in past and present aboriginal populations. In: Draper HH, editor. Advances in Nutritional Research, Vol. 9. New York: Plenum Press, pp. 35–51.

Pirok DJ, Ramser JR, Takahashi H, Villanueva AR, Frost H (1966) Normal histological, tetracycline and dynamic parameters in human, mineralized bone sections. Henry Ford Hospital Medical Bulletin 14:195–218.

Przybeck T (1985) Histomorphology of the rib: Bone mass and cortical remodeling. In: Davies R, Leathers C, editors. Behavior and Pathology of Aging in Rhesus Monkeys. New York: Alan R. Liss, pp. 303–326.

Rauch F, Baily DA, Baxter-Jones A, Mirwald R, Faulkner R (2004) The "muscle-bone unit" during the pubertal growth spurt. Bone 34:771–775.

Robling AG, Castillo AB, Turner CH (2006) Biomechanical and molecular regulation of bone remodeling. Annu Rev Biomed Eng 8(1):455–498.

Robling AG, Stout SD (2008). Histomorphometry of human cortical bone: Applications to age estimation. In Katzenberg S, Saunders S, editors. Biological Anthropology of the Human Skeleton, 2nd edition. New York: Wiley-Liss.

Robling AR, Stout SD (1999) Morphology of the drifting osteon. Cells Tissue Organs 164:192–204.

Schaffler MB, Burr DB (1984) Primate cortical bone microstructure: Relationship to locomotion. Am J Phys Anthropol 65(2):191–197.

Schock C, Noyes F, Crouch M, Matthews C (1975) The effects of immobility on long bone remodelling in the rhesus monkey. Henry Ford Hosp Med J 23:107–115.

Schock C, Noyes F, Villaneuva A (1972) Measurement of Haversian bone remodelling by means of tetracycline labelling in the rib of rhesus monkeys. Henry Ford Hospital Medical Bulletin 20:131–144.

Schoenau E, Frost HM (2002) The "muscle-bone unit" in children and adolescents. Calcif Tissue Int 70:405–407.

Singh I, Tonna E, Grandel C (1974) A comparative histological study of mammalian bone. J Morphol 144:421–438.

Stout SD (1986) The use of bone histomorphometry in skeletal identification: The case of Francisco Pizarro. J Forensic Sci 31(1):296–300.

Stout SD (1989) Histomorphometric analysis of human skeletal remains. In Işcan MY, Kennedy KA, editors. Reconstruction of Life from the Skeleton. New York: Wiley-Liss, pp. 41–52.

Stout S, Streeter M (2006) Histomorphometric analysis of the cortical bone of the rib of Hanaab-Pakal. In: Tiesler V, and Cucina A, editors. Studying Janaab'Pakal and Recreating Maya Dynastic History. Tucson, AZ: University of Arizona Press.

Stout S, Streeter M (2004) Un Analisis Histomorfologico del Hueso Cortical de la Costilla de Janaab'Pakal. In: Tiesler V, Cucina A, editors. Janaab'Pakal De Palenque: Vida Y Muerte De Un Gobernanante Maya. Universidad National Automoma de Mexico, pp. 123–136.

Stout SD, Marcello AP, Perotti B (1996) Brief communication: A test and correction of the clavicle method of Stout and Paine for histological age estimation of skeletal remains. Am J Phys Anthropol 100:139–142.

Stout SD, Lueck R (1995) Bone remodeling rates and maturation in three archaeological skeletal populations. Am J Phys Anthropol 98:161–171.

Stout S, Paine R (1994) Bone remodeling rates: A test on an algorithm for estimating missing osteons. Am J Phys Anthropol 93:123–129.

Stout SD, Paine RR (1992) Brief communication: Histological age estimation using rib and clavicle. Am J Phys Anthropol 87:111–115.

Stout SD, Ross LM (1991) Bone fragments a body can make. J Forensic Sci 36(3):953–957.

Streeter M, Stout S, Trinkaus E, Burr D (2010) Brief communication: Bone remodeling rates in Pleistocene humans are not slower than the rates observed in modern populations: A reexamination of Abbott et al. (1996). Am J Phys Anthropol 141(2):315–318.

Tang Y, Wu X, Lei W, Pang L, Wan C, Shi Z, Zhao L, Nagy TR, Peng X, Hu J et al. (2009) TGF-beta1-induced migration of bone mesenchymal stem cells couples bone resorption with formation. Nat Med 15(7):757–765.

Thompson DD, Gunness-Hey M (1981) Bone mineral-osteon analysis of Yupik-Inupiaq skeletons. Am J Phys Anthropol 55:1–7.

Thompson DD, Cowen KS (1984) Age at death and bone biology of the Barrow mummies. Arctic Anthropol 21:83–88.

Turner CH (1999) Toward a mathematical description of bone biology: The principle of cellular accommodation. Calcif Tissue Int 65:466–481.

Turner CH (2000) Toward a mathematical description of bone biology: The principle of cellular accommodation. Calcif Tissue Int 67:185–187.

Turner CH, Robling AG, Duncan, RL, Burr, DB (2002) Do bone cells behave like a neuronal network? Calcif Tissue Int 70:435–442.

van Oers R (2008) A unified theory for osteonal and hemi-osteonal remodeling. Bone 42(2):250–259.

van Oers R, Ruimerman R, van Reitbergen B, Hilbers A, Huiskes R (2008) Relating osteon diameter to strain. Bone 43:476–482.

Van Wagenen G (1970) Menopause in a subhuman primate. Anat Rec 166:392.

Verborgt O, Gibson GJ, Schaffler MB (2000) Loss of osteocyte integrity in association with microdamage and bone remodeling after fatigue in vivo. J Bone Miner Res 15:60–67.

Villanueva AR, Mehr LA (1977) Modifications of the Goldner and Gomori one-step trichrome stains for plastic-embedded thin sections of bone. Am J Med Technol 43(6):536–538.

Wu K, Schubeck K, Frost H, Villanueva A (1970) Haversian bone formation rates determined by a new method in a mastadon and in human diabetes mellitus and osteoporosis. Calcif Tissue Res 6:204–219.

This page intentionally left blank

Growth and Development
Morphology, Mechanisms, and Abnormalities

2

JAMES H. GOSMAN

Contents

2.1 Introduction

Forensic scientists analyze skeletal remains to make inferences about the individuals when living. These include assessments of age at death, cause and manner of death, ancestry, pathological conditions, health, trauma, skeletal biomechanics, and other aspects of behavioral interpretation. Essential to all these endeavors is an understanding of the biology underlying skeletal growth and development, and the journey to adult skeletal morphology. This pathway is characterized by complex, interactive, hierarchical biological systems, which are the subjects of intense research interest in many areas of concern: genetic influences, diet/nutrition, linear growth/stature, and bone functional adaptation. This chapter does not provide an exhaustive review of this vast topic nor embark into the familiar aging framework but rather presents a selective overview of the current knowledge of the fundamental process of longitudinal bone growth focusing on histomorphology, regulatory systems, and ontogenetic changes in cortical and trabecular bone architecture. These concepts are placed within a broader biocultural context, discussing important environmental

and mechanobiological influences on skeletal growth and development, and their implications for forensic anthropologists, researchers, and scientists.

2.2 Endochondral Ossification

2.2.1 General Process

Endochondral ossification is the foundation of linear growth of human long bones and is the procedure by which the skeletal cartilage anlagen are replaced by bone (Olsen et al. 2000). This process involves the formation of a cartilage primordium and growth plate, where chondrocytes initially undergo proliferation and a series of differentiation steps secreting a cartilage template that is eventually replaced by bone (Lai and Mitchell 2005). The anlagen elongate and expand in width by proliferation of chondrocytes and deposition of cartilage matrix. Chondrocytes undergo further maturation to hypertrophic chondrocytes (HC) and synthesize an extracellular matrix. Angiogenic factors secreted by hypertrophic chondrocytes induce angiogenesis from the perichondrium; osteoblasts, osteoclasts, and hematopoietic cells come with the blood vessels. The primary ossification center is thus formed.

Within the ossification center, the hypertrophic chondrocytic matrix is degraded, the hypertrophic chondrocytes undergo apoptosis, and osteoblasts replace the disappearing cartilage with trabecular bone. Bone marrow is also formed during this process. Simultaneously, osteoblasts in the perichondrium form a collar of compact bone around the diaphysis of the cartilage, locating the primary ossification center within a tube of bone. At one or both ends (epiphyses) of the cartilage, secondary ossification centers are formed, leaving a plate of cartilage (growth plate) between the epiphysis and diaphysis. Elongation of the long bone from the growth plate results from a coordinated sequence of chondrocyte proliferation, hypertrophy, and apoptosis. It is this choreographed process that creates the initial framework of trabecular bone and, thus in part, the foundation upon which the ontogenetic patterning and subsequent adult morphology rests. Concurrently, these processes are coordinated with growth in the epiphysis and radial periosteal appositional growth of the diaphysis (Olsen et al. 2000). Bone growth at the growth-plate cartilage or at an ossification center per se is insufficient to form the complex shapes of developing bones. This requires constant modeling and remodeling, which occurs within the bone tissue as well as in the endosteal, and periosteal bone envelopes in the form of bone deposition and resorption (Aiello and Dean 2002).

2.2.2 Morphological Features

Cartilage involved in endochondral ossification is found in both the growth plate (bounded by the epiphysis and metaphysis) and the articular-epiphyseal growth center. These are responsible for the extension of the primary and secondary centers of ossification (Mackie et al. 2008). This discussion will focus on the growth plate, which is organized into three morphologically distinctive zones, each with its own particular functional biology: the zones of resting (reserve), proliferative, and hypertrophic chondrocytes (Figure 2.1).

The resting zone is most distant from the ossification front and is the only zone that has a vascular supply, passing through the epiphysis and terminating at the upper end of