

1

Bone Structure and the Response of Bone to Stress

Mark D. Markel

Comparative Orthopaedic Research Laboratory, Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

INTRODUCTION

The skeleton serves many essential purposes in the body, including the protection of internal organs, the provision of rigid kinematic links to allow for locomotion, and the storage of calcium and phosphorous, acting as a dynamic mineral reserve bank.^{20,26,30} Bone, the principal component of the skeleton, is living connective tissue made rigid by the orderly deposition of minerals on an organic matrix.¹ Bone has many unique structural characteristics which allow it to fulfill these functions. It is one of the hardest substances in the body, following only dentin and enamel of teeth. Its intricate structural organization, combined with its high metabolic activity, allows bone to respond rapidly to both physical and biochemical demands. Additionally, bone is highly vascular with an excellent capacity for self-repair. The surfaces of bone are covered with osteoblasts and osteoclasts, which are responsible for constant bone turnover through simultaneous bone formation and bone resorption.³ Osteocytes, the third major cellular component of bone, reside within bone tissue and communicate with adjacent osteocytes and osteoblasts through channels called canaliculi. All three cell types help bone respond quickly to mechanical and metabolic demands.

This chapter will describe the structure and function of bone and its response to stress, focusing on the cellular and mechanical characteristics of bone structure.

BONE STRUCTURE

On the microscopic level, two types of bone are found in the mature skeleton. Hard, compact cortical bone occurs in the shafts of the long bones. Cancellous, or trabecular, bone is composed of a network of fine, interlacing parti-

tions, called trabeculae, enclosing the cavities within the bone that contain either hematopoietic or fatty marrow. Cancellous bone is found in most of the axial skeleton and in the ends of the long bones. Bones of the appendicular skeleton are generally long and cylindrical, with relatively narrow mid-portions. The length of equine bones increases the moment arm of each muscle as it acts on the limb, enhancing a horse's speed and power. The expanded ends of long bones diminish the stresses that act on the articular surfaces by distributing loads over a larger cross-sectional area.

An immature long bone is divided into four distinct regions (Figure 1.1). The central region of bone is called the diaphysis, with the physis, epiphysis, and metaphysis at either end. The physis, present in one or both ends of the bone, separates the epiphysis and metaphysis, and is responsible for the majority of long bone growth in young animals through a process called endochondral ossification. As an animal matures, the physis ceases growth and closes, at which stage the entire expanded end of the bone is represented by the metaphysis, which is composed of trabecular (cancellous or spongy) bone surrounded by cortical and dense subchondral bone. The diaphysis is a hollow tube of cortical bone with a central cavity that contains the major arterial and venous blood supply to the bone and fatty marrow. Most of the hematopoiesis in the body occurs in the metaphyseal cancellous bone and in the bones of the axial skeleton, although the fatty marrow of the diaphysis does contain hematopoietic elements.

Bones of the appendicular skeleton are covered by periosteum, except in regions covered by articular cartilage or where ligaments, tendons, or joint capsules attach.^{1,3} The periosteum has two layers: an outer, fibrous layer permeated by blood vessels and nerves which act in a supportive capacity, and an inner, osteogenic layer which provides the osteoprogenitor cells necessary for fracture

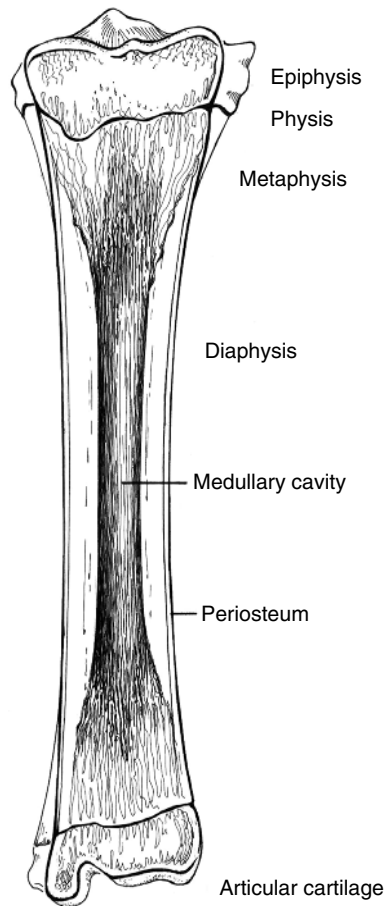


Figure 1.1 Immature equine tibia showing the different regions of a long bone.

healing and is responsible for appositional growth prior to skeletal maturity. During growth, the osteogenic layer of the periosteum is thick, highly vascular, and adhered to the bone. With maturity, the osteogenic layer thins and becomes only loosely adhered to the bone.

The microstructure of bone can be divided into three principal components which are intimately associated with one another to allow for rapid response to the mechanical and homeostatic requirements of the body. These components include the cells, the organic extracellular matrix, and the inorganic portion of bone.

Cellular Components

Osteoblasts

Osteoblasts, which develop from fibroblastic osteoprogenitor or mesenchymal cells, cover the majority of bone surfaces and are responsible for the formation of the organic matrix, called osteoid (Figure 1.2).^{3,17,27,39} Osteoblasts deposit osteoid on bone surfaces, enveloping themselves in osteoid seams.

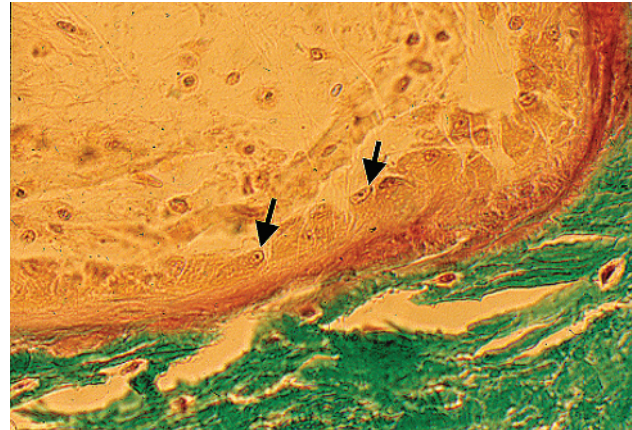


Figure 1.2 Light microscopic image of a 5 μm undecalcified section of bone showing osteoblasts (arrows) laying down osteoid on bone surface (Goldner Stain, magnification $\times 80$).

Ultrastructurally, osteoblasts contain abundant endoplasmic reticulum, ribosomes, Golgi apparatus, and mitochondria. These cellular components are responsible for the osteoblasts' high metabolic activity and productivity. Osteoblasts produce the majority of the organic components of bone, including collagen, proteoglycans, and other noncollagenous proteins.

Osteocytes

Approximately 10% of the osteoblastic population become enclosed in matrix and are then referred to as osteocytes (Figure 1.3).¹⁷ Compared with osteoblasts, osteocytes have less endoplasmic reticulum and fewer cytoplasmic organelles. Osteocytes have numerous cytoplasmic processes that extend into the surrounding matrix and fill the canaliculi of bone (Figure 1.3). These processes contact the processes of other osteocytes and osteoblasts to form an intricate transport and communication system within the bone. This interconnection of deeply embedded osteocytes and surface-lining osteoblasts regulates the flow of mineral ions from the extracellular space surrounding the osteoblasts to the osteocytes, from the osteocytes to the extracellular fluid surrounding them, and finally from this fluid to the mineral surrounding the osteocytes. This organizational structure allows the large surface area of the osteocyte population to regulate the exchange of mineral ion between the extracellular fluid and the bone by means of the canalicular system.

Osteoclasts

The cell type responsible for the majority of bone resorption is the osteoclast.¹⁷ Osteoclasts are large, multinucleated cells on or near bone surfaces that reside within concavities called Howship's lacunae, which are the active sites of bone resorption (Figure 1.4).

Figure 1.3 Brightfield (left) and grayscale composite of Z-stacked confocal photomicrographs (right) of osteocytes surrounding an osteon Haversian canal (large arrow) in the third metacarpal bone in a racing Thoroughbred. A dense syncytial network of vital osteocytes and their canaliculi are demonstrated (small arrows) connecting adjacent lacunae before ultimately reaching the Haversian canal. Scale bar = 50 μ m. Source: Courtesy Peter Muir, University of Wisconsin-Madison.

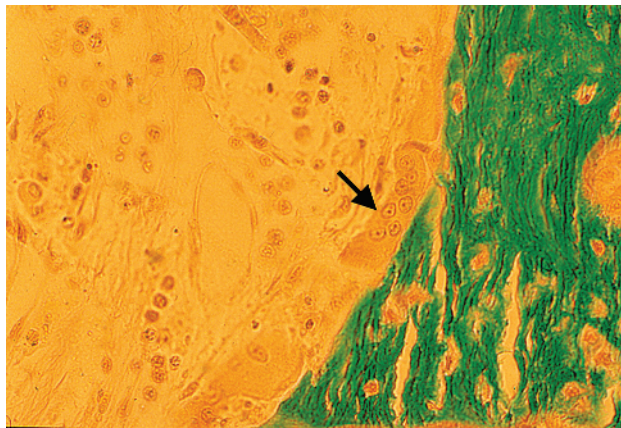
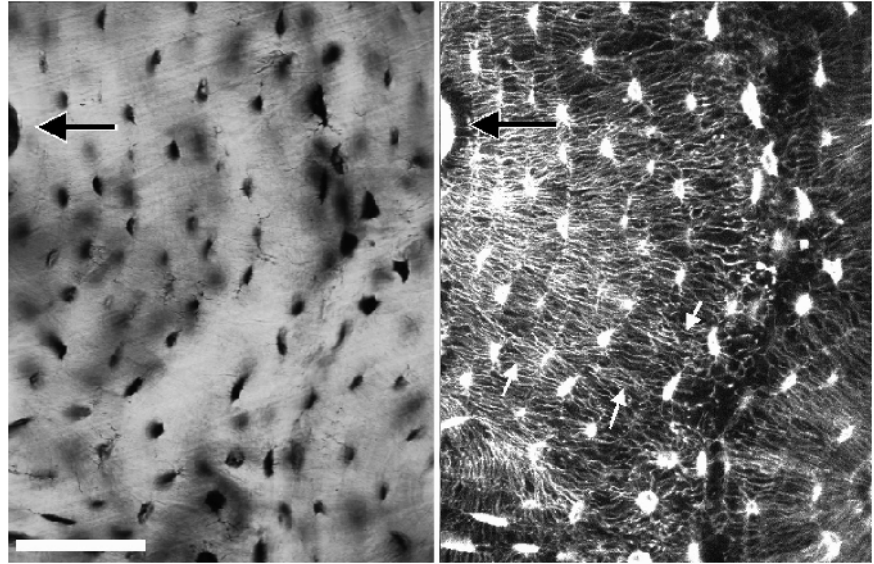


Figure 1.4 Light microscopic image of a 5 μ m undecalcified section of bone showing an osteoclast (arrow) residing within a Howship's lacuna where bone is resorbed (Goldner Stain, magnification $\times 80$).

Osteoclasts originate from blood monocytes, which circulate in the vascular system before arriving in bone. The size and number of nuclei in osteoclasts vary, but each nucleus usually is associated with a perinuclear Golgi apparatus, in which Golgi vesicles exist in various stages of development. Osteoclasts contain little endoplasmic reticulum and few ribosomes, but they do have abundant mitochondria, Golgi apparatus, and Golgi vesicles. The contact area between osteoclasts and bone consists of two regions, the ruffled border and the sealing zone. The ruffled border is composed of finger-like membranous folds that extend varying distances into the cytoplasm and are responsible for bone resorption. The sealing zone is characterized by a dense, homogenous cytoplasmic membrane that lies in close apposition to the bone and isolates the ruffled border, preventing leakage

and concentrating the lysosomal enzymes and hydrogen ions produced by the osteoclast at the site of resorption. Osteoclasts produce acid phosphatase and collagenase to first dissolve mineral and then remove the organic matrix to a depth of 1–2 μ m. Hydroxyapatite crystals and collagen fibers can be observed in the extracellular space between the cytoplasmic folds of the ruffled border. After being degraded, these components are taken up via endocytosis, transported across the cell, and then extruded into the extracellular space.

Organic Matrix

Collagen

The organic matrix of bone acts as a supporting structure for the deposition and crystallization of inorganic salts. Organic matrix is 21% of the bone by weight, with the remainder of the bone made up of inorganic material (71%) and water (8%). Approximately 95% of the organic matrix is collagen, with type I collagen the predominant collagen in bone. Collagen is the most abundant protein in mammals, accounting for 20–50% of the dry weight of adult long bones, approximately 70% of the dry weight of skin, and approximately 90% of the dry weight of tendon.^{3,17,34}

Collagen's unique ultrastructure makes it exceedingly strong in tension. Collagen is composed of three tightly folded polypeptide chains, called alpha chains, each consisting of approximately 1000 amino acids. The basic unit of collagen is tropocollagen, composed of three procollagen polypeptide alpha chains, each coiled in a left-handed helix, and the alpha chains are then further coiled around each other into a right-handed triple helix (Figure 1.5). Tropocollagen molecules, which are approximately 1.4 nm in diameter and 300 nm long, polymerize

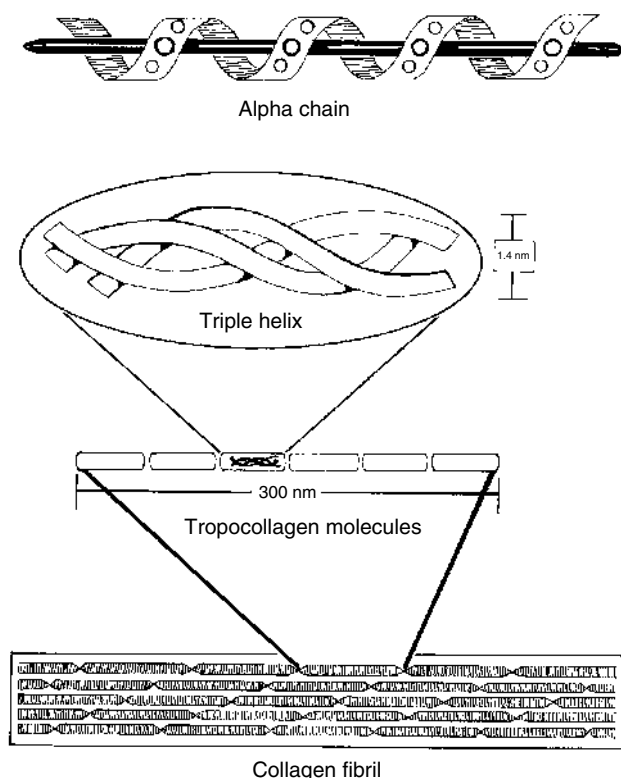


Figure 1.5 Molecular features of collagen structure from the alpha chain to the fibril. The flexible amino acid sequence in the alpha chain allows these chains to wind tightly into a right-handed triple helix, forming the tropocollagen molecule. This tight triple helical arrangement contributes to the high tensile strength of the collagen fibril. The parallel alignment of the individual tropocollagen molecules, in which each molecule overlaps the other by about one-quarter of its length, results in a repeating banded pattern of the collagen fibril. *Source:* Adapted from Nordin and Frankel 1989.³⁰ Reproduced with permission of John Wiley and Sons.

into larger collagen fibrils. Covalent cross-links form between the tropocollagen molecules, adding to the fibrils' high tensile strength. Individual fibrils are aligned in a quarter-staggered array with fibril lengths of 640 Å. The fibrils are separated by 400 Å gaps called hole zones. The hole zones are thought to serve as the initial mineralization site within collagen.

Individual alpha chains of type I collagen consist of repeating tripeptides, composed of the amino acid sequence glycine-x-y, where x and y can be proline, hydroxyproline, or hydroxylysine.⁴ Glycine accounts for one-third of all the constituent amino acids in type I collagen, because it is the only amino acid small enough to fit in the center of the collagen triple helix. Type I collagen consists of two identical alpha chains and one alpha chain of a different amino acid composition, $[\alpha 1(I)]_2\alpha 2(I)$.

Proteoglycans and Glycosaminoglycans

The remaining 5% of organic matrix is ground substance. The predominant constituents of ground substance are

proteoglycans and their constituent glycosaminoglycans. Proteoglycans are high molecular weight molecules, with acidic glycosaminoglycan side chains that provide flexibility and resilience to the connective tissue matrix.⁴

Proteoglycans are made up of a central protein core to which acidic glycosaminoglycan side chains are covalently attached. The individual glycosaminoglycans are large anionic molecules of repeating basic and acidic disaccharides. In bone, proteoglycans do not form the large aggregates that predominate in other tissues such as cartilage. Glycosaminoglycans also serve as the cementing substance between layers of mineralized collagen fibers in lamellar bone.

Inorganic Component

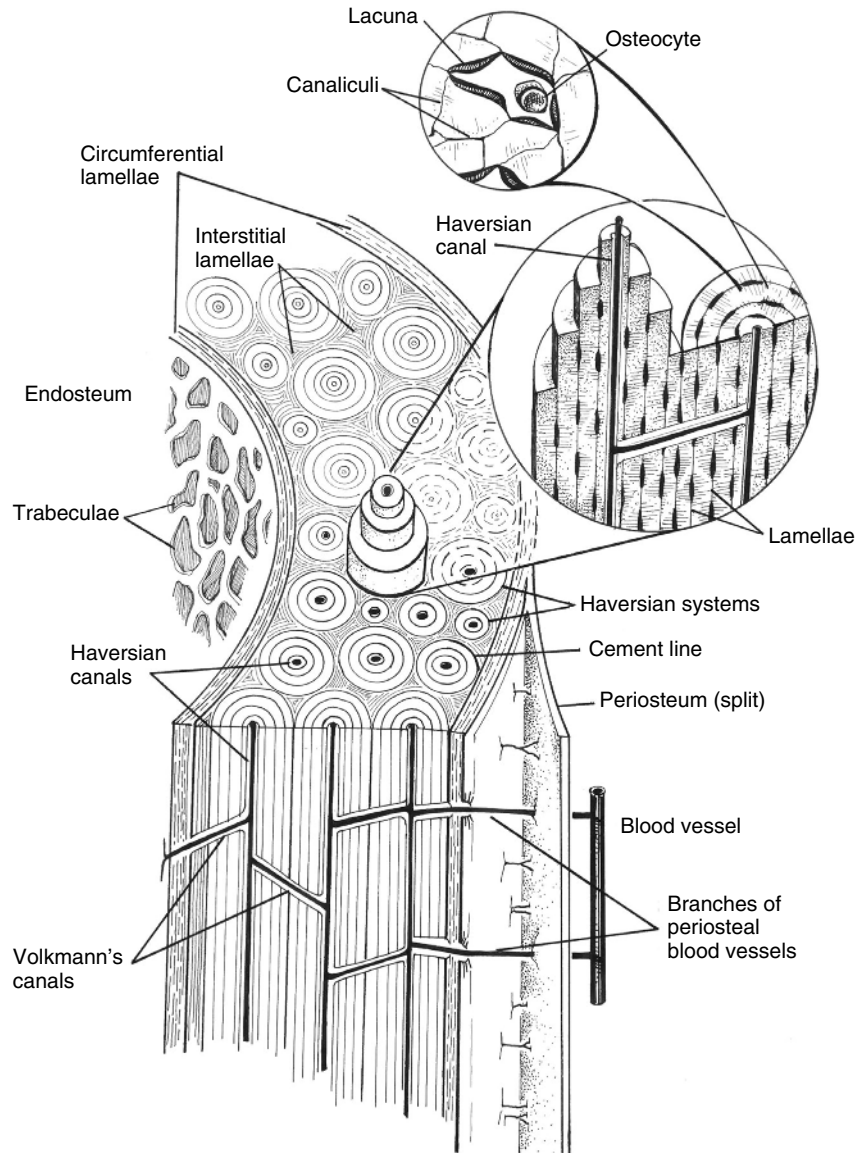
The mineral portion of bone consists primarily of calcium and phosphate, mainly in the form of small crystals that resemble synthetic hydroxyapatite crystals and have the composition $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$.^{3,13} Bone mineral crystals are extremely small, ~25–75 Å in diameter and 200 Å in length, in contrast to geological apatite crystals which are much larger.²⁹ Because of their small size, the microscopic crystals found in bone mineral are more soluble than geological apatites. They also contain more impurities than pure hydroxyapatite crystals. In addition to calcium and phosphorous, bone mineral contains carbonate, magnesium, fluoride, and citrate in variable amounts. This structure of bone (hydroxyapatite in intimate apposition to the organic matrix) is responsible for its mechanical strength.

Osteon

At the microstructural level, the fundamental unit of bone is the osteon or Haversian system (Figure 1.6). At the center of each osteon is a small channel, called a Haversian canal, that contains blood vessels, nerve fibers, and lymphatic-type channels. Surrounding the central canal is a concentric series of layers, or lamellae, of mineralized bone (Figure 1.6). Along the boundaries of each lamella are small spaces known as lacunae, each of which contains individual osteocytes. Canaliculi radiate from these lacunae and connect with adjacent lamellae before ultimately reaching a Haversian canal (Figure 1.3). Cell processes extend from the osteocytes into the canaliculi, allowing nutrients from the blood vessels in the Haversian canal to reach the osteocyte.

At the periphery of each osteon is a cement line, a narrow area of cement-like ground substance composed primarily of glycosaminoglycans. The canaliculi of the osteon do not cross this cement line. Like the canaliculi, the collagen fibers in the bone matrix interconnect from one lamella to another within an osteon, but do not cross the cement line. This intertwining of collagen fibers within the osteon increases the bone's resistance to mechanical stress and probably explains why the cement line is the weakest portion of the bone's microstructure.

Figure 1.6 Microstructural arrangement of a long bone depicted without the marrow cavity. Secondary osteons are apparent as the structural units of cortical bone. In the center of the osteons are the Haversian canals, which form the main branches of the circulatory network. Each osteon is bounded by a cement line and consists of lamellae, formed by concentric rings of mineral matrix surrounding the Haversian canal. Along the boundaries of the lamellae are small cavities known as lacunae, each of which contains a single bone cell, or osteocyte. Radiating from the lacunae are tiny canals, or canaliculi, into which the cytoplasmic processes of the osteocytes extend. *Source:* Adapted from Nordin and Frankel 1989.³⁰ Reproduced with permission of John Wiley and Sons.



A typical osteon is 200 μm in diameter, so no portion of an osteon is more than 100 μm from the centrally located blood supply. In the appendicular skeleton, the osteons run longitudinally, but they branch frequently and anastomose extensively with each other.

Interstitial lamellae span the regions between complete osteons and are continuous with the osteons. As in osteons, no point in the interstitial lamellae is farther than 100 μm from its blood supply. The interfaces between these lamellae contain numerous osteocytes lying within lacunae, which interconnect with each other through the canalicular system.

There are two distinct types of osteons present in lamellar bone, primary and secondary. Primary osteons form during appositional bone growth, when the bone is increasing in diameter.^{9,40} Osteoblasts on the surface of the bone deposit successive lamellae of new bone,

progressively diminishing the caliber of each vascular space. The resulting anastomosing, convoluted areas of bone, occupying what were previously vascular spaces, are called primary Haversian systems, or primary osteons.^{8,9,40} Primary osteons usually turn parallel to the long axis of the bone, may contain one to several vascular canals, and are always surrounded by woven bone.^{9,15}

Secondary osteons form during the continuous process of remodeling that occurs throughout life.^{6,9,14,33,34} This process is initiated by the osteoclastic resorption of bone via a structure called a cutting cone, and results in anastomosing tubular cavities that are oriented longitudinally (Figure 1.7). Osteoblasts on the inner surface of the cutting cone then deposit successive layers of lamellae with an orderly fiber orientation. The caliber of each cavity is thereby gradually reduced until only a single small vascular canal remains. The newly formed cylinders

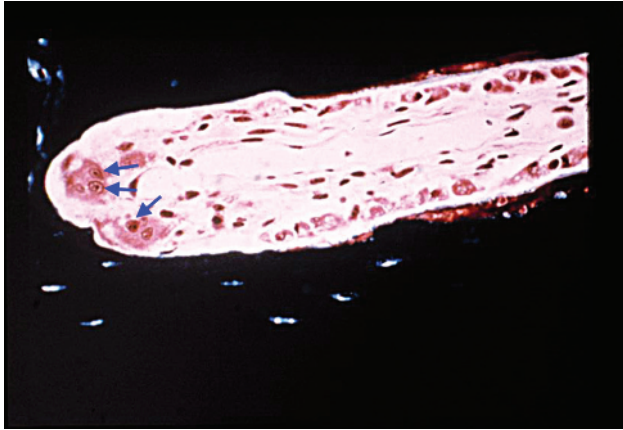


Figure 1.7 Light microscopic image of a 100 μm undecalcified section of a bone-cutting cone with osteoclasts resorbing bone at the apex of the cone (arrows) (Goldner Stain, magnification $\times 80$).



Figure 1.8 Backscatter electron microscopic image of a 100 μm undecalcified section of bone showing secondary osteons within cortical bone. Newly remodeled bone is darker, indicating less mineralization of the site (backscatter electron microscopy, magnification $\times 70$).

of bone are called secondary Haversian systems, or secondary osteons. Secondary osteons consist of concentric sheets of lamellar bone. Unlike primary osteons, secondary osteons are always bounded by cement lines, which are formed where osteoclastic activity ceases and osteoblastic bone formation resumes (Figure 1.8).

Lamellar bone, which is composed of osteons, is not the only type of bone within the body.^{2,9,40} The other type, called woven bone, is the first bone to appear in embryonic development and in the repair of fractures. Woven bone is gradually replaced by lamellar bone and serves only as a temporary structure, except in special locations such as the dental alveolus and osseous labyrinths. Woven bone is characterized by coarse fiber bundles, approximately 30 μm in diameter, running in a random or interlacing fashion. In contrast, lamellar bone consists of fine fiber bundles, 2–4 μm in diameter, that are arranged irregularly in parallel or concentric curving sheets.

On a macroscopic level, two types of bone are found in the mature skeleton: hard, compact cortical bone in the shafts of the long bones, and cancellous or trabecular bone which is composed of a network of fine, interlacing partitions, called trabeculae, enclosing the cavities within bone that contain either hematopoietic or fatty marrow. Cancellous bone is found in the majority of the axial skeleton and in the ends of the long bones.

RESPONSE OF BONE TO STRESS

Normal daily activity imposes a complex pattern of forces on the skeletal system that cause small deformations of the bone.⁹ The direction and magnitude of these deformations are dependent on the geometry of the bone, the direction and magnitude of the loads imposed on the bone, and the material properties of the bone tissue. The mechanical response of a bone to stress can be described by quantitatively assessing the relationships between various directional loads and their resultant deformations. These relationships reflect the structural behavior of the entire bone.

The imposition of forces on a bone also creates a complex pattern of internal forces and deformations throughout the bone structure. Local deformations within the bone are referred to as strains, and the local force intensities at these sites are the stresses, defined as a given force per unit area. The relationship between stress and strain at a particular point in the bone is governed by the material properties of the local bone tissue. If the whole bone is loaded with very high forces, the stresses and strains in one region may exceed the ultimate stresses or strains that the tissue can tolerate, and a fracture develops.

Osteonal Remodeling

In adult equine long bones, large areas of primary osteonal bone are often present in the cortex. This primary bone is lamellar in nature (although not truly lamellar),

consisting of layers of woven bone which are separated by layers of primary osteons. Studies of the relationship between the structure and the mechanical properties of bone have emphasized the comparison between primary and secondary osteonal (Haversian) bone. Many investigators have suggested that primary osteonal bone is stronger than secondary osteonal bone.^{8,9,12,16} Hert et al.²² showed that fully mineralized primary bone was as strong or stronger than secondary osteonal bone. Reilly and Burstein³⁷ demonstrated that bovine primary osteonal bone was significantly stiffer than secondary bone. Their finding was supported by Carter and Spengler,⁹ who showed that primary bovine bone was more fatigue resistant and was stronger in tensile tests than secondary bone. Carter et al.⁸ demonstrated that osteonal or Haversian remodeling of bovine primary osteonal bone reduces the tensile strength and fatigue resistance of bone by decreasing bone density and creating an inherently weaker structure.

Many investigators have demonstrated that equine bone remodels in response to the stresses that are placed on it. The nature of these stresses is important, especially given that not all mechanical stimuli result in the same effect. Bone adaptation is induced by dynamic strains rather than by static loading.^{25,41} In addition, the increase in cross-sectional area of bone induced by an osteogenic response was found to be highly correlated to the rate of bone deformation. These results suggest that training requires a high loading rate to elicit a maximal osteogenic response.

The rate at which musculoskeletal tissues adapt varies with tissue, age, and exercise regime.⁴¹ Age is a potent factor in determining the extent of adaptation. The immature skeleton is much more responsive than the adult skeleton, although this varies among species. Animals that show limited morphologic change with aging, such as rodents, likely exhibit less reduction in the responsiveness of the skeleton with age, compared to the more typical aging phenotypes such as the human and the horse. In bone, adaptation can be initiated through brief cyclic-loading periods given on a number of days per week. This type of exercise, if prolonged or introduced too rapidly, may also lead to fatigue damage and

microcrack development within the bone, with the potential to cause catastrophic fracture of the bone.^{5,18}

Several research studies have reported the impact of evaluated training regimens on bone modeling and fracture. Short bursts of high-speed gallop during training reduced the risk of fracture during subsequent racing.³² Nunamaker and colleagues⁵ proposed exercise regimens for young Thoroughbreds in training, based on experimental data where short-distance, high-speed gallops rather than longer, slower gallops led to improved bone strength and minimized fatigue fracture and the risk of catastrophic failure.

Track surface can also significantly affect the adaptive response of bone. Young et al.,⁴⁴ in a study evaluating the effects of training regimens and track surfaces on bone remodeling, found that the cancellous bone component of the equine proximal sesamoid bone of horses trained on dirt tracks had significantly lower porosity than that of untrained horses, enabling the bone to withstand the higher loads of racing.^{7,11,28,44} Track surface had a greater effect on the cancellous morphology of the proximal sesamoid bone than a variety of training regimens.^{33,44}

Young and coworkers⁴⁵ also demonstrated that equine carpal bone remodels in response to the stresses placed on it. This phenomenon, in which bone gains or loses cancellous or cortical bone or both in response to the level of stress sustained, is summarized as Wolff's law, which states that bone is laid down where it is needed and resorbed where it is not needed.^{10,12,24,31,36,38,43} Both theoretical and experimental evidence supports the prevailing hypothesis that trabeculae within bone align with the maximum and minimum principal stresses placed on the bone.^{7,19,23,36,42,44} Structural anisotropy is expected to be greatest in regions of primary tension or compression, in contrast to porosity and trabecular width, which correlate best with shear stress.²¹ The osteogenic response of bone to remodeling stimuli has been shown to be most dependent on strain magnitude and strain rate.^{24,38,44} Significant decreases in porosity have been demonstrated after controlled, impulsive loading of joints in rabbits and after exercising of sheep on concrete.^{35,36} The nature of the remodeling stimulus in these studies is believed to be associated with the high loading rate and the peak magnitude of the applied loads.³⁶

REFERENCES

- 1 Arnoczky, S.P. and Wilson, J.W. (1990). The connective tissues. In: *Canine Orthopedics* (ed. W.G. Whittick), 21–41. Philadelphia: Lea & Febiger.
- 2 Ascenzi, A., Bonucci, E., and Bocciarelli, D.S. (1965). An electron microscope study of osteon calcification. *J. Ultrastruct. Res.* 12: 287–303.
- 3 Boskey, A.L. (1981). Current concepts of the physiology and biochemistry of calcification. *Clin. Orthop.* 167: 225–257.
- 4 Boskey, A.L. (1985). Connective tissues of the musculoskeletal system. In: *Textbook of Small Animal Surgery* (ed. D.H. Slatter), 1926–1939. Philadelphia: WB Saunders.

- 5 Boston, R.C. and Nunamaker, D.M. (2000). Gait and speed as exercise components of risk factors associated with onset of fatigue injury of the third metacarpal bone in 2-year-old Thoroughbred racehorses. *Am. J. Vet. Res.* 61: 602–608.
- 6 Boyde, A. and Hobdell, M.H. (1969). Scanning electron microscopy of primary membrane bone. *Z. Zellforsch.* 99: 98–108.
- 7 Carter, D.R. and Hayes, W.C. (1977). The compressive behavior of bone as a two-phase porous structure. *J. Bone Joint Surg. Am.* 59: 954–962.
- 8 Carter, D.R., Hayes, W.C., and Schurman, D.J. (1976). Fatigue life of compact bone II. Effects of microstructure and density. *J. Biomech.* 9: 211–218.
- 9 Carter, D.R. and Spengler, D.M. (1978). Mechanical properties and composition of cortical bone. *Clin. Orthop.* 135: 192–217.
- 10 Chamay, A. and Tschantz, P. (1972). Mechanical influences in bone remodeling. Experimental research on Wolff's law. *J. Biomech.* 5: 173–180.
- 11 Cowin, S.C. (1983). The mechanical and stress adaptive properties of bone. *Ann. Biomed. Eng.* 11: 263–295.
- 12 Dempster, W.T. and Coleman, R.F. (1960). Tensile strength of bone along and across the grain. *J. Appl. Physiol.* 16: 355.
- 13 Eanes, E.D. and Posner, A.S. (1970). Structure and chemistry of bone mineral. In: *Biological Calcification* (ed. H. Schraer), 1–26. Amsterdam: North Holland.
- 14 Enlow, D.H. (1962). The functional significance of the secondary osteon. *Anat. Rec.* 142: 230.
- 15 Enlow, D.H. (1966). An evaluation of the use of bone histology in forensic medicine and anthropology. In: *Studies on the Anatomy and Function of Bone and Joints* (ed. F.G. Evans), 93–113. New York: Springer-Verlag.
- 16 Evans, F.J. and Bang, S. (1967). Differences and relations between the physical properties and the microscopic structure of human femoral, tibial, and fibular cortical bone. *Am. J. Anat.* 120: 79.
- 17 Fetter, A.W. (1985). Structure and function of bone. In: *Textbook of Small Animal Orthopaedics* (ed. C.D. Newton and D.M. Nunamaker), 9–12. Philadelphia: JB Lippincott.
- 18 Firth, E.C., Goodship, A.E., Delahunt, J. et al. (1999). Osteoinductive response in the dorsal aspect of the carpus of young Thoroughbreds in training occurs within months. *Equine Vet. J. Suppl.* 30: 552–554.
- 19 Fyhrie, D.P. and Carter, D.R. (1986). A unifying principle relating stress to trabecular bone morphology. *J. Orthop. Res.* 4: 304–317.
- 20 Hayes, W.C. and Carter, D.R. (1979). Biomechanics of bone. In: *Skeletal Research: An Experimental Approach* (ed. D.J. Simmons and A.S. Kunin), 263–300. New York: Academic Press.
- 21 Hayes, W.C. and Snyder, B. (1981). Toward a quantitative formulation of Wolff's law in trabecular bone. In: *Mechanical Properties of Bone*. AMD, vol. 45 (ed. S.C. Cowin), 43–68. New York: American Society of Mechanical Engineers.
- 22 Hert, J., Kucera, P., Vavra, M., and Volenik, V. (1965). Comparison of the mechanical properties of both primary and Haversian bone tissue. *Acta Anat.* 61: 412–423.
- 23 Lanyon, L.E. (1974). Experimental support for the trajectory theory of bone structure. *J. Bone Joint Surg. Br.* 56: 160–166.
- 24 Lanyon, L.E. (1982). Mechanical function and bone remodeling. In: *Bone in Clinical Orthopedics* (ed. G. Sumner-Smith), 273–304. Philadelphia: WB Saunders Co.
- 25 Lanyon, L.E. and Rubin, C.T. (1984). Static vs dynamic loads as an influence on bone remodeling. *J. Biomech.* 17 (12): 897–905.
- 26 Markel, M.D. (2006). Bone biology and fracture healing. In: *Equine Surgery*, 3e (ed. J.A. Auer and J.A. Stick), 991–1000. St. Louis: Saunders Elsevier.
- 27 Marks, S.C. (1983). The origin of osteoclasts: evidence, clinical implications and investigative challenges of an extra-skeletal source. *J. Oral Pathol.* 12: 226–256.
- 28 Martin, R.B. (1982). Porosity and specific surface of bone. *CRC Crit. Rev. Biomed. Eng.* 10: 179–222.
- 29 Menczel, L.J., Posner, A.S., and Harper, R.A. (1965). Age changes in the crystallinity of rat bone apatite. *Isr. J. Med. Sci.* 1: 251–252.
- 30 Nordin, M. and Frankel, V.H. (1989). Biomechanics of bone. In: *Basic Biomechanics of the Musculoskeletal System*, 2e (ed. M. Nordin and V.H. Frankel), 3–29. Philadelphia: Lea & Febiger.
- 31 Nunamaker, D.M., Butterweck, D.M., and Provost, M.T. (1989). Some geometric properties of the third metacarpal bone: a comparison between the Thoroughbred and Standardbred racehorse. *J. Biomech.* 22: 129–134.
- 32 Parkin, T.D., Clegg, P.D., French, N.P. et al. (2005). Risk factors for fatal lateral condylar fracture of the third metacarpus/metatarsus in UK racing. *Equine Vet. J.* 37: 192–199.
- 33 Pratt, G.W. (1982). The response of highly stressed bone in the race horse. In: *Proceedings of the American Association of Equine Practitioners*, vol. 27, 31–37. Lexington, KY: AAEP.
- 34 Pritchard, J.J. (1972). General morphology of bone. In: *The Biochemistry and Physiology of Bone*, vol. 1 (ed. G.H. Bourne), 1–20. New York: Academic Press.
- 35 Radin, E.L., Martin, R.B., Barr, D.B. et al. (1984). Effects of mechanical loading on the tissues of the rabbit knee. *J. Orthop. Res.* 2: 221–234.

- 36 Radin, E.L., Orr, R.B., Kelman, J.L. et al. (1982). Effect of prolonged walking on concrete on the knees of sheep. *J. Biomech.* 15: 487–492.
- 37 Reilly, D.T. and Burstein, A.H. (1974). The mechanical properties of cortical bone. *J. Bone Joint Surg. Am.* 56: 1001–1022.
- 38 Rubin, C.T. and Lanyon, L.E. (1987). Osteoregulatory nature of mechanical stimuli: function as a determinant for adaptive remodeling in bone. *J. Orthop. Res.* 5: 300–310.
- 39 Simmons, D.J., Kent, G.N., Jilka, R.L. et al. (1982). Formation of bone by isolated, cultured osteoblasts in millipore diffusion chambers. *Calcif. Tissue Int.* 34: 291–294.
- 40 Smith, J.W. (1960). Collagen fibre patterns in mammalian bone. *J. Anat.* 94: 329–344.
- 41 Smith, R.K.W. and Goodship, A.E. (2008). The effect of early training and the adaption and conditioning of skeletal tissues. *Vet. Clin. North Am. Equine Pract.* 24: 37–51.
- 42 Townsend, P.R., Miegel, R.E., Rose, R.M. et al. (1976). Structure and function of the human patella: the role of cancellous bone. *J. Biomed. Mater. Res. Symp.* 7: 605–611.
- 43 Woo, S.L.-Y., Kuei, S.C., Amiel, D. et al. (1981). The effect of prolonged physical training on the properties of long bone: a study of Wolff's law. *J. Bone Joint Surg. Am.* 63: 780–787.
- 44 Young, D.R., Nunamaker, D.M., and Markel, M.D. (1991). Quantitative evaluation of the remodeling response of the proximal sesamoid bones to training-related stimuli in Thoroughbreds. *Am. J. Vet. Res.* 52: 1350–1356.
- 45 Young, D.R., Richardson, D.W., Markel, M.D. et al. (1991). Mechanical and morphometric analysis of the third carpal bone of Thoroughbreds. *Am. J. Vet. Res.* 52: 402–409.