Principles of Bone Biology

SECOND EDITION

Volume 1

EDITED BY

John P. Bilezikian

Lawrence G. Raisz

Gideon A. Rodan

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Preface to the Second Edition

The success of the first edition of *Principles of Bone Biology* clearly indicated that this text met an important need in our field. Well-worn copies (often with a cracked spine!) can be found on the shelves of bone biology research laboratories and offices throughout the world. We knew from the outset that undertaking the first edition would include a commitment to producing a second one. Advances in bone biology over the past five years have moved forward at a dizzying pace, clearly justifying the need for a second edition at this time. The elucidation of the molecular interactions between osteoblasts and osteoclasts is one of many examples documenting this point. Studies of animals in which critical genes have been deleted or over-expressed have produced some surprises and added still further complexity to what we have already recognized as an extremely complex regulatory system controlling the development and maintenance of skeletal structures. These and many other advances have provided the background for further development of effective therapeutic approaches to metabolic bone diseases.

In preparing the second edition, we have asked all authors to provide extensive revisions of their chapters. Additionally, the second edition features new authors who have written 10 new chapters. Some chapters from the first edition have been consolidated or otherwise reconfigured to keep the total number of chapters essentially the same as in the first edition. Although the number of chapters and their organizational structure has been retained, the extraordinary amount of new information has led to an increase in size of many of the chapters along with more extensive referencing. As a result, the substantially larger second edition is being published in two volumes. Each volume contains a full table of contents and full indexing to help the reader find specific information. The somewhat smaller individual volumes should be easier to handle and hold up better to the extensive use we expect from readers.

As was the case in the first edition, we asked our authors to meet a tight schedule so that the text would be as up-to-date as possible. We are indebted to our many authors who successfully met this challenge. The updated chapters as well as the new ones have, therefore, been written in such a way that the newest and most exciting breakthroughs in our field are still fresh. This task could not have been completed without the help of the staff at Academic Press. We acknowledge, in particular, Jasna Markovac and Mica Haley. They have been enormously helpful in all phases of this effort.

We have enjoyed very much the task of bringing this second edition to you. We trust that this second edition will be even more useful to you than the first. Enjoy the book!

John P. Bilezikian
Lawrence G. Raisz
Gideon A. Rodan

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Preface to the First Edition

The world of modern science is undergoing a number of spectacular events that are redefining our understanding of ourselves. As with any revolution, we should take stock of where we have been, where we are, and where we are going. Our special world of bone biology is participating in and taking advantage of the larger global revolution in modern science. Often with shocking but delightful suddenness, we are gaining new insights into difficult issues, discovering new concepts to explain old observations, developing new approaches to perennial mysteries, and applying novel technological advances from other fields to our own. The pace with which the bone world is advancing is impressive not only to the most ardent optimists, who did not expect so much so soon, but also to the more sober minded who, only several years ago, would have brushed off the notion that progress could come with such lightening speed.

The rationale for this book is rooted in the recognition of the revolution in bone biology. We need a new repository of knowledge, bringing us both to the core and to the edge of our universe. Our goal is to provide complete, truly up-to-date, and detailed coverage of this exciting and rapidly developing field. To achieve this, we assembled experts from all over the world and asked them to focus on the current state of knowledge and the prospects for new knowledge in their area of expertise. To this end, *Principles of Bone Biology* was conceived. It is designed to be useful to students who are becoming interested in the field and to young investigators at the graduate or postgraduate level who are beginning their research careers. It is also designed for more established scientists who want to keep up with the changing nature of our field, who want to mine this lode to enrich their own research programs, or who are changing their career direction. Finally, this book is written for anyone who simply strives for greater understanding of bone biology.

This book is intended to be comprehensive but readable. Each chapter is relatively brief. The charge to each author has been to limit size while giving the reader information so complete that it can be appreciated on its own, without necessary recourse to the entire volume. Nevertheless, the book is also designed with a logic that might compel someone to read on, and on, and on!

The framework of organization is fourfold. The first 53 chapters, in a section titled "Basic Principles," cover the cells themselves: the osteoblast, the osteoclast, and the osteocyte; how they are generated; how they act and interact; what turns them on; what turns them off; and how they die. In this section, also, the biochemistry of collagenous and noncollagenous bone proteins is covered. Newer understandings of calcium, phosphorus, and magnesium metabolism and the hormones that help to control them, namely, parathyroid hormone, vitamin D metabolites, calcitonin, and related molecules, are presented. A discussion of other systemic and local regulators of bone metabolism completes this section.

The second section of this book, "Molecular Mechanisms of Metabolic Bone Diseases," is specifically devoted to basic mechanisms of a variety of important bone diseases. The intention of these 17 chapters is not to describe the diseases in clinical, diagnostic, or therapeutic terms but rather to illustrate our current understanding of underlying mechanisms. The application of the new knowledge summarized in Part I to pathophysiological, pathogenetic, and molecular mechanisms of disease has relevance to the major metabolic bone disorders such as osteoporosis, primary hyperparathyroidism, and hypercalcemia of malignancy as well as to the more uncommon disorders such as familial benign hypocalciuric hypercalcemia, pseudohypoparathyroidism, and osteopetrosis.

The third section of this book, "Pharmacological Mechanisms of Therapeutics," addresses the great advances that have been made in elucidating how old and new drugs act to improve abnormalities in bone metabolism. Some of these drugs are indeed endogenous hormones that under

specified circumstances are useful therapies: estrogens, vitamin D, calcitonin, and parathyroid hormone are representative examples. Others agents such as the bisphosphonates, fluoride, and calcium are reviewed. Finally, agents with therapeutic potential but still in development such as calcimimetics, insulin-like growth factors, transforming growth factor, bone morphogenetic protein, and fibroblast growth factor are presented with a view to the future. The intent of this 12-chapter section is not to provide step-by-step "how-to" instructions for the clinical uses of these agents. Such prescribing information for established therapies is readily found in other texts. Rather, the underlying mechanisms by which these agents are currently believed to work is the central point of this section.

The fourth and final section of this book, "Methods in Bone Research," recognizes the revolution in investigative methodologies in our field. Those who want to know about the latest methods to clone genes, to knock genes out, to target genes, and to modify gene function by transfection and by transcriptional control will find relevant information in this section. In addition, the selection and characteristics of growth conditions for osteoblastic, osteoclastic, and stem cells; animal models of bone diseases; assay methodologies for bone formation and bone resorption and surrogate bone markers; and signal transduction pathways are all covered. Finally, the basic principles of bone densitometry and bone biopsies have both investigative and clinical relevance. This 15-chapter section is intended to be a useful reference for those who need access to basic information about these new research technologies.

The task of assembling a large number of international experts who would agree to work together to complete this ambitious project was formidable. Even more daunting was the notion that we would successfully coax, cajole, and otherwise persuade authors of 97 chapters to complete their tasks within a six-month period. For a book to be timely and still fresh, such a short time leash was necessary. We are indebted to all the authors for delivering their chapters on time.

Finally, such a monumental undertaking succeeds only with the aid of others who helped conceive the idea and to implement it. In particular, we are grateful to Jasna Markovac of Academic Press, who worked tirelessly with us to bring this exciting volume to you. We also want to thank Tari Paschall of Academic Press, who, with Jasna, helped to keep us on time and on the right course. We trust our work will be useful to you whoever you are and for whatever reason you have become attracted to this book and our field. Enjoy the book. We enjoyed editing it for you.

John B. Bilezikian
Lawrence G. Raisz
Gideon A. Rodan

PART I

Basic Principles

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CHAPTER 1

Structure and Development of the Skeleton

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Introduction

This brief overview of the structure and development of the skeleton focuses primarily on bone and its cells (Fig. 1). We review the structure and function of these cells, their divisions of labor within the skeleton, the emerging complexities of their changing regulation with age, and the emerging knowledge of the molecular regulation of the skeleton. We also look briefly at emerging knowledge of molecular regulation of the skeleton. Because neither the complexities of the cellular microenvironment nor the influences of nonosseous tissues on bone cells can be duplicated *in vitro,* these parameters of bone metabolism must be evaluated eventually in the complexities of the *in vivo* environment. To assist both the reader and the investigator in interpreting these and other studies of the structure, development, and regulation of bone, we offer a brief critical analysis review of various methods used to examine bone metabolism.

The interested reader is referred to reviews of this topic from different perspectives (Buckwalter *et al.,* 1996a,b; Hall, 1987; Marks and Popoff, 1988; Schenk, 1992).

Cells of The Skeleton: Development, Structure, and Function

Bone is a highly specialized form of connective tissue that is nature's provision for an internal support system in all higher vertebrates. It is a complex living tissue in which the extracellular matrix is mineralized, conferring marked rigidity and strength to the skeleton while still maintaining some degree of elasticity. In addition to its supportive and protective functions, bone is a major source of inorganic ions, actively participating in calcium homeostasis in the body. There is increasing evidence that the central control of development and renewal of the skeleton is more sophisticated than previously appreciated (Ducy *et al.,* 2000).

Bone is composed of an organic matrix that is strengthened by deposits of calcium salts. Type I collagen constitutes approximately 95% of the organic matrix; the remaining 5% is composed of proteoglycans and numerous noncollagenous proteins (see chapters to follow). Crystalline salts deposited in the organic matrix of bone under cellular control are primarily calcium and phosphate in the form of hydroxyapatite.

Morphologically, there are two forms of bone: cortical (compact) and cancellous (spongy). In cortical bone, densely packed collagen fibrils form concentric lamellae, and the fibrils in adjacent lamellae run in perpendicular planes as in plywood (Fig. 2). Cancellous bone has a loosely organized, porous matrix. Differences between cortical and cancellous bone are both structural and functional. Differences in the structural arrangements of the two bone types are related to their primary functions: cortical bone provides mechanical and protective functions and cancellous bone provides metabolic functions.

Bone Cell Structure and Function

Bone is composed of four different cell types (Fig. 1). Osteoblasts, osteoclasts, and bone lining cells are present on bone surfaces, whereas osteocytes permeate the mineralized interior. Osteoblasts, osteocytes, and bone-lining cells originate from local osteoprogenitor cells (Fig. 3A), whereas osteoclasts arise from the fusion of mononuclear

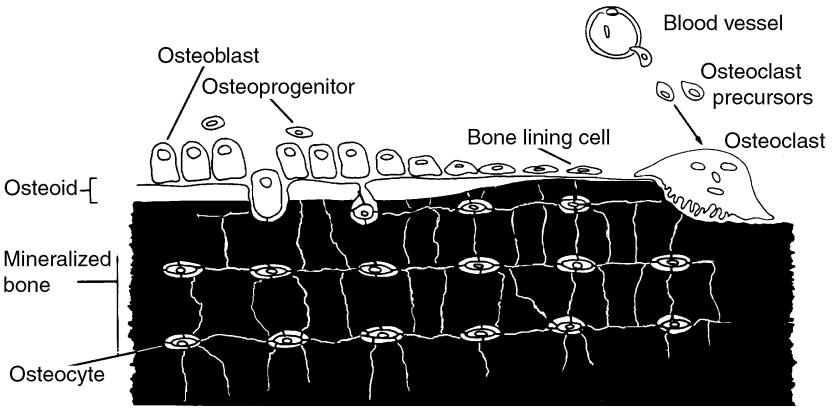

Figure 1 The origins and locations of bone cells. Taken from Marks and Popoff (1988). Reprinted by permission of John Wiley and Sons, Inc.

precursors, which originate in the various hemopoietic tissues. The apical and basal surfaces of bone cells are defined in an opposite sense from those of epithelia. Apical surfaces are those that are attached to the extracellular matrix and basal surfaces are those that are away from the matrix.

Osteoblasts are fully differentiated cells responsible for the production of the bone matrix. Portions of four osteoblasts are shown in Figs. 2 and 3B. An osteoblast is a typical protein-producing cell with a prominent Golgi apparatus and well-developed rough endoplasmic reticulum. It secretes the type I collagen and the noncollagenous proteins of the bone matrix (see Chapters 4 and 5). The staggered overlap of the individual collagen molecules provides the characteristic periodicity of type I collagen in bone matrix. Numerous noncollagenous proteins have been isolated from bone matrix (Sandberg, 1991), but to date there is no consensus for a definitive function of any of them.

Osteoblasts regulate mineralization of bone matrix, although the mechanism(s) is not completely understood. In woven bone, mineralization is initiated away from the cell surface in matrix vesicles that bud from the plasma membrane of osteoblasts. This is similar to the well-documented role of matrix vesicles in cartilage mineralization (Hohling *et al.,* 1978). In lamellar bone, the mechanism of mineralization appears to be different. Mineralization begins in the hole region between overlapped collagen molecules where there are few, if any, matrix vesicles Landis *et al.,* 1993) and appears to be initiated by components of the collagen molecule itself or noncollagenous proteins at this site. Whatever the mechanisms of mineralization, collagen is

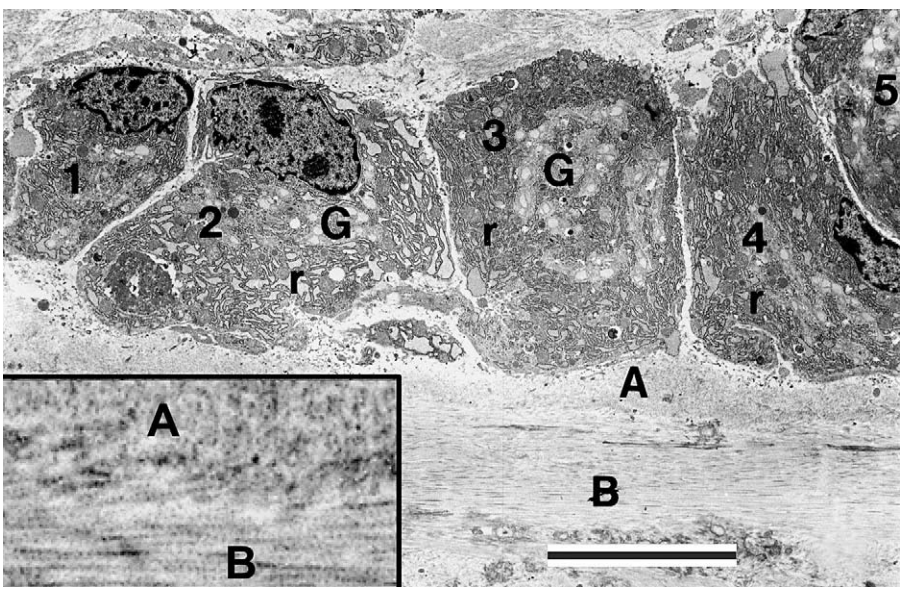

Figure 2 Transmission electron micrograph of osteoblasts (numbered) on a bone surface in which the collagenous matrix has been deposited in two layers (A and B) at right angles to each other. The Golgi apparatus (G) and rough endoplasmic reticulum (r) are prominent cytoplasmic organelles in osteoblasts. (Original magnification: ×2800. Bar: 0.1 μm.)

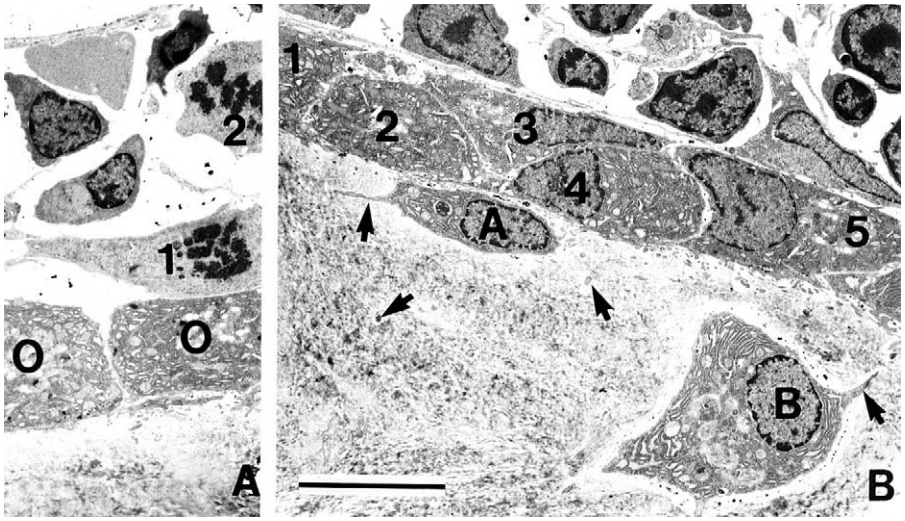

Figure 3 (A) Transmission electron micrograph of an osteoblast (O) and daughter cells (1 and 2) of a dividing osteoprogenitor cell. (Original magnification: ×2100.) (B) Transmission electron micrograph of five osteoblasts (numbered) and two osteocytes (A and B) in the process of being embedded in bone matrix. Arrows identify processes extending from the osteocytes and within the bone matrix that will serve as their metabolic and regulatory lifelines via gap junctions between adjacent cells. (Original magnification: ×2100. Bar: 0.1 μm.)

at least a template for its initiation and propagation and there is always a layer of unmineralized bone matrix (osteoid) on the surface under osteoblasts. Matrix deposition is usually polarized toward the bone surface, but periodically becomes generalized, surrounding the osteoblast and producing the next layer of osteocytes. Deposition of mineral makes the matrix impermeable, and to ensure a metabolic lifeline, osteocytes establish numerous cytoplasmic connections with adjacent cells before mineralization.

The osteocyte (Fig. 3B) is a mature osteoblast within the bone matrix and is responsible for its maintenance (Buckwalter *et al.,* 1996a). These cells have the capacity not only to synthesize, but also to resorb matrix to a limited extent. Each osteocyte occupies a space, or lacunae, within the matrix and extends filopodial processes through canaliculi in the matrix (Figs. 4A and B) to contact processes of adjacent cells (Figs. 5A and B) by means of gap junctions. Because the diffusion of nutrients and metabolites through the mineralized matrix is limited, filopodial connections permit communication between neighboring osteocytes, internal and external surfaces of bone, and with the blood vessels traversing the matrix. The functional capacities of osteocytes can be easily ascertained from their structure. Matrix-producing osteocytes have the cellular organelles characteristic of osteoblasts (Fig. 5A), whereas osteolytic osteocytes contain lysosomal vacuoles and other features typical of phagocytic cells (Fig. 5B). (For a review of osteocyte functions, see Chapter 6.)

Bone lining cells are flat, elongated, inactive cells that cover bone surfaces that are undergoing neither bone formation nor resorption (Fig. 6). Because these cells are inactive, they have few cytoplasmic organelles. Little is known regarding the function of these cells; however, it has been speculated that bone lining cells can be precursors for osteoblasts.

Osteoclasts are large, multinucleated cells that resorb bone (Fig. 7). When active, osteoclasts rest directly on the bone surface and have two plasma membrane specializations: a ruffled border and a clear zone. The ruffled border is the central, highly infolded area of the plasma membrane where bone resorption takes place. The clear zone is a microfilament-rich, organelle-free area of the plasma membrane that surrounds the ruffled border and serves as the point of attachment of the osteoclast to the underlying bone matrix. Active osteoclasts exhibit a characteristic polarity. Nuclei are typically located in the part of the cell most removed from the bone surface and are interconnected by cytoskeletal proteins (Watanabe *et al.,* 1995). Osteoclasts contain multiple circumnuclear Golgi stacks, a high density of mitochondria, and abundant lysosomal vesicles that arise from the Golgi and cluster near the ruffled border. A molecular phenotype for osteoclasts is emerging (Horne, 1995; Sakai *et al.,* 1995) (see Chapters 7, 8, and 9).

Cellular Divisions of Labor within the Skeleton

Cartilage and bone are two tissues that comprise the skeleton. Despite their shared supportive functions, these tissues are dramatically different (i.e., matrix composition and mineralization state). The cellular activities that occur in each of the two tissues, however, are limited to matrix formation, matrix mineralization, and matrix resorption. In each tissue, different cell types perform different, yet sometimes overlapping, functions (Fig. 8). In cartilage, matrix is produced and mineralized by chondrocytes. Mineralization and resorption of cartilage are activities associated with hypertrophied chondrocytes.

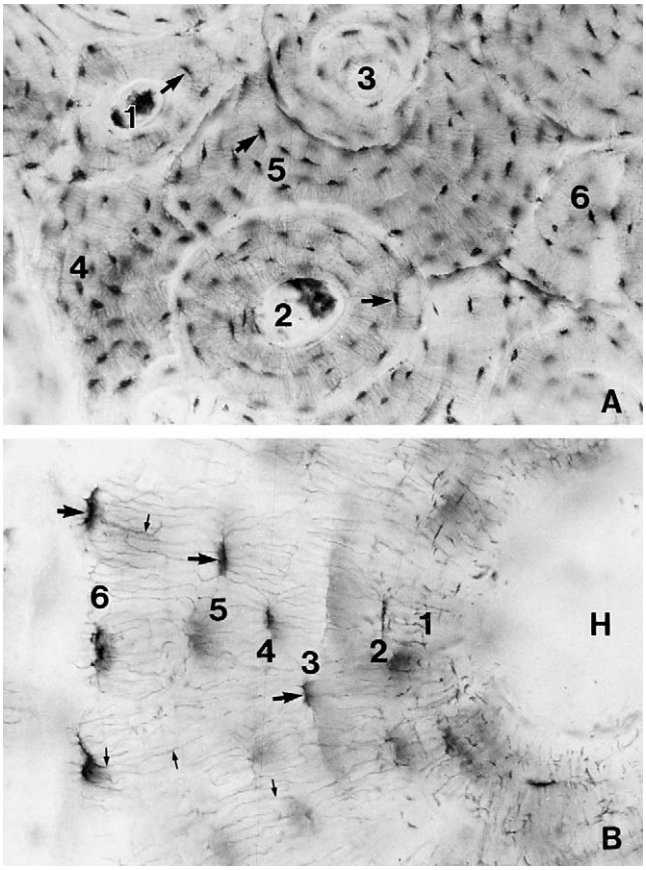

Figure 4 (A) A thin-ground crosssection of human cortical bone in which osteocyte lacunae (arrows) and canaliculi have been stained with India ink. Osteocytes are arranged around a central vascular channel to constitute Haversian systems. Active Haversian systems (1, 2, and 3) have concentric lamellae in this plane. Older Haversian systems (4, 5, and 6) have had parts of their original territories invaded and remodeled. This is seen most clearly where 2 and 3 have invaded the territory originally occupied by 5. (Original magnification: ×185. Bar: 50 μm.) (B) Higher magnification of part of a Haversian system showing the successive layering (numbers) of osteocytes (large arrows) from the central core (H) that contains the vasculature. Small arrows identify the canaliculi that connect osteocyte lacunae in different layers. (Original magnification: ×718. Bar: 50 μm.)

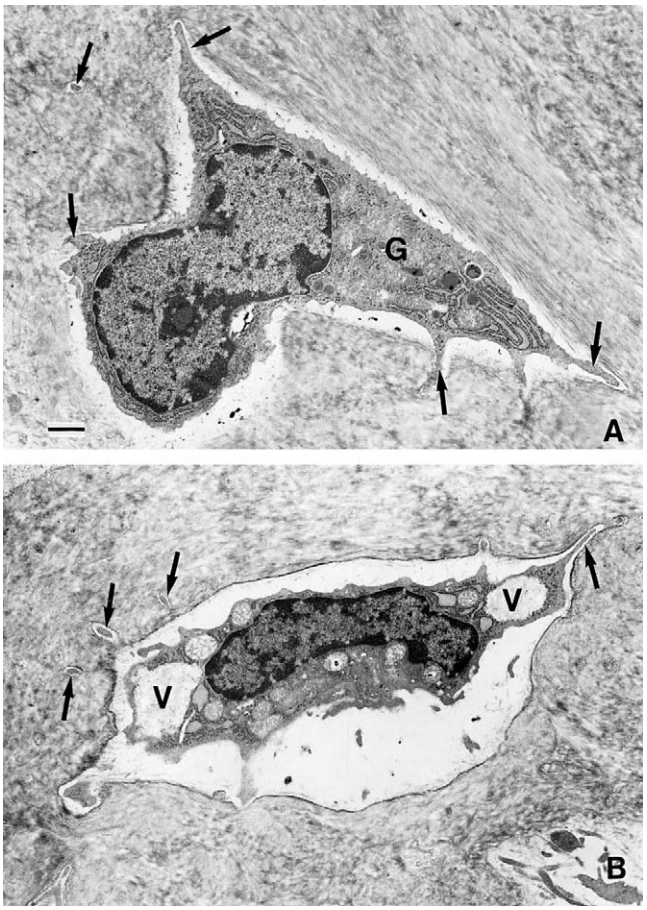

Figure 5 Transmission electron micrographs of two osteocytes of different phenotype and functional states. Young osteocytes (A) have nuclear and cytoplasmic features of osteoblasts: a euchromatic nucleus with a prominent nucleolus, a large Golgi apparatus (G), prominent rough endoplasmic reticulum, and numerous cytoplasmic processes (arrows) projecting into the surrounding matrix. Some older osteocytes (B) can have an osteolytic phenotype with increased lacunar volume, an electron-dense lacunar surface, condensed nuclei, and numerous cytoplasmic vacuoles. (Original magnification: ×7000. Bar: 0.01 μm.)

However, cartilage mineralized in the growth plate is resorbed by osteoclasts (see Figs. 12 and 13). In bone, matrix is produced and mineralized by osteoblasts and osteocytes. Resorption occurs primarily by osteoclasts, but localized perilacunar resorption may occur around osteocytes (Fig. 5B).

Coordination of Cellular Activities during Skeletal Development and Maturation

Variable Activities of Skeletal Cells

The activities of skeletal cells vary considerably over the life span of the organism. This is necessary to build a mineralized tissue where there was none before and to maintain it after reaching maturity. The variable activities of bone formation and resorption in relation to each other during the human life cycle are summarized in Fig. 9. The first two decades are devoted to development of the skeleton, called modeling. During this period, bone formation necessarily precedes and exceeds bone resorption. Thus, although these activities are related temporally and spatially, they are uncoupled in the sense that they are unequal. During the next three decades (and beyond) the adult skeleton is maintained by removing and replacing a fraction each year. This remodeling begins with a localized resorption that is succeeded by a precisely equal formation of bone at the same site (Parfitt, 1994). Thus, bone formation equals bone resorption, a process called coupling (see the section that follows, Fig. 10). In compact bone, resorption by osteoclasts produces a cutting cone through Haversian systems, and the subsequent reformation of these systems produces osteons of unequal age, size, and configurations (Fig. 4A). Sometime after the fifth decade, the formative phase of the remodeling sequence

Figure 6 Transmission electron micrograph of bone lining cells (asterisks). These flat cells have few organelles and form a thin cellular layer on inactive bone surface that is often hard to resolve by light microscopy. (Original magnification: ×3000. Bar: 0.1 μm.)

fails to keep pace with resorptive activity and skeletal mass, including the connectivity of trabecular bone, decreases. This reduces skeletal strength and increases the risk of fracture over time, depending on the magnitude by which resorption and formation are uncoupled. Given the apparent inevitability and universality of an osteoporotic trend with age, therapy has focused on increasing skeletal mass during development and/or slowing resorption after the fifth decade. What is needed is a selective, predictable, locally active anabolic agent. This discovery may be more likely if we focus more on skeletal development than its pathology.

It is clear that the coordination of the activities of skeletal cells is a local event. Local factors recruit specific cells and local factors regulate their activity. Furthermore, multiple factors in a precise sequence and concentration are needed for the full expression of a cell's potential, and these factors and their concentrations differ for bone formation and bone resorption. It is also clear that more than one cell type can produce many of these factors and that normal skeletal development is a collaborative effort of cells from diverse lineages (Marks and Popoff, 1988; Yamazaki and Eyden, 1995; Yoder and Williams, 1995). The complexities of skeletal development and maintenance are now being acknowledged along with the poverty of our understanding of these relationships. This book is an attempt to put these factors and cells in some order that has both theoretical (functional) and practical (therapeutic) significance.

General Regulation of Cellular Activities

Most simply put, the challenges of understanding the complexities of skeletal modeling and remodeling, coupling and uncoupling, are illustrated by the influences that osteoblasts have on osteoclasts and vice versa (Marks and Popoff, 1988; Mundy, 1994). These are illustrated schematically in Fig. 10. Osteoblasts, the progeny of local osteoprogenitor cells, produce factors that influence the differentiation and function of osteoclasts (Martin and Ng,

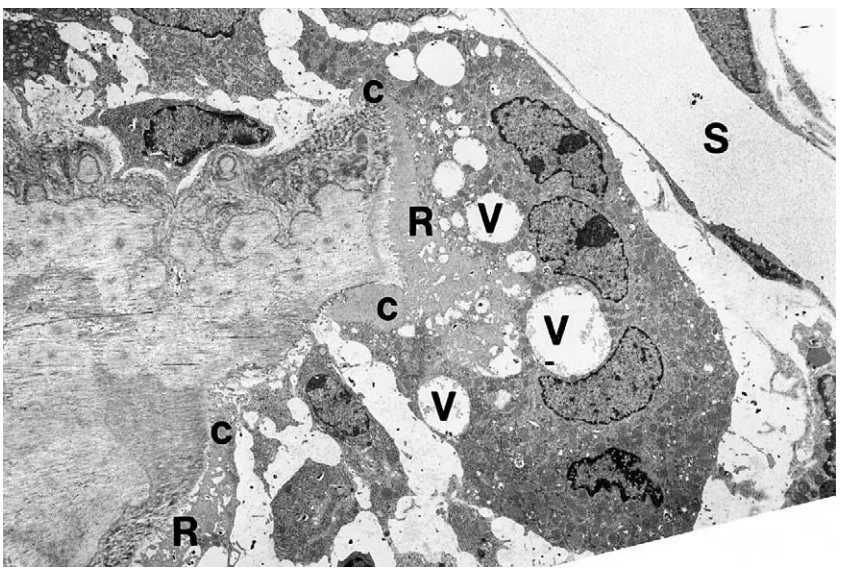

Figure 7 Transmission electron micrograph of parts of two osteoclasts. These multinucleated cells attach to bones at clear zones (C), which create a three-dimensional seal around the ruffled border (R) working area. Active cells have large vacuoles in the cytoplasm next to the ruffled border. S, vascular sinus. (Original magnification: ×2240. Bar: 0.1 μm.)

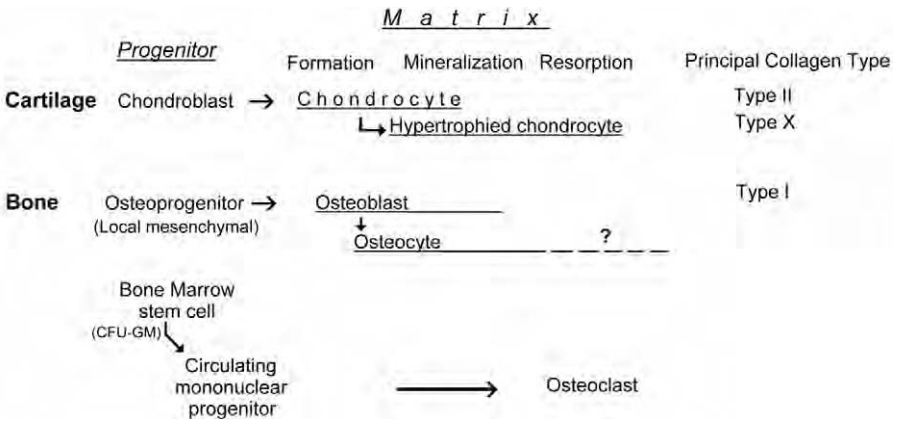

Figure 8 Cellular division of labor in the skeleton. Schematic of the major cells and their functions in cartilage and bone.

1994). Some of these are deposited in bone matrix itself, whereas others appear to be secreted locally in response to hormones or local factors. These conclusions are based on the facts that receptors for most osteolytic factors are found on osteoblasts, not osteoclasts (Rodan and Martin, 1981), that osteoclasts resorb bone in response to factors released into culture media by activated osteoblasts (McSheehy and Chambers, 1986), and that some components of the extracellular matrix of bone can attract and/or activate osteoclasts (Thesingh and Burger, 1983). Osteoclasts, however, are derived from hemopoietic stem cell progeny (monocytes) that use vascular routes to migrate to skeletal sites (Marks, 1983). After exiting the vasculature at specific locations in the skeleton, these mononuclear precursors either fuse with each other or other multinucleated cells to become osteoclasts. Their activation depends in large part on local signals derived from other cells, including but not limited to osteoblasts. However, bone resorption itself produces factors that recruit and activate osteoblasts. Indeed, the ability of supernatants of resorbing bone organ cultures to promote the proliferation and differentiation of osteoblast progenitors began the current interest in identifying the coupling factor(s) (Drivdahl *et al.,* 1981; Farley *et al.,* 1982).

It is clear from the foregoing that the activities of skeletal cells in a particular site change with age, that these changes are controlled by local factors, including weight bearing, and that we have much to learn about the identity and sequence of action of these agents in the changing dynamics of skeletal metabolism (Frost and Jee, 1994; Weryha and Leclere, 1995).

	Development	Maintenance	The Osteoporoses
Age	0-20	20-50	50+
Sequence	BF → BR Modeling	BR→ BF Remodeling	BR → BF
Activity	BF > BR	BF = BR	BF < BR

Figure 9 Development, maintenance, and pathology of the skeleton. Summary of the relative levels of skeletal cell activity during the human life cycle.

Formation of the Skeleton

Formation of the skeleton (ossification) occurs by either a direct (intramembranous) or an indirect (endochondral) process. Both require a solid base and a well-developed vascular supply for the elaboration and mineralization of the extracellular matrix. Mobility or low oxygen tension at the site favors the differentiation of chondrocytes or fibroblasts.

Intramembranous ossification occurs during embryonic development by the direct transformation of mesenchymal cells into osteoblasts. This type of ossification for entire bones is restricted to those of the cranial vault, some facial bones, and parts of the mandible and clavicle. The flat bones of the skull grow toward each other from primary ossification centers in each and meet at sutures. Sutures are fibroelastic cellular domains (Fig. 11) composed of the periostea of adjacent bones. The center of a suture contains a proliferating cell population whose progeny differentiate and move toward adjacent bone surfaces, becoming osteoblasts. During this migration these cells produce type III collagen at low levels, types V and XI transiently, and finally type I, the major bone collagen (Wurtz *et al.,* 1998). This mechanism provides a steady source of osteoblasts and allows bones to expand at their edges. When growth is complete, sutures remain as fibrous connections or disappear, depending on the suture site.

Bones that participate in joints and bear weight form by endochondral ossification, a method by which the unique properties of cartilage and bone are exploited to provide a mechanism the for formation and growth of the skeleton during growth of the individual. In such bones the condensed embryonic mesenchyme transforms into cartilage, which reflects in both position and form the eventual bone to be formed at that site. In the central part of such a bone, endochondral ossification provides for a linear, interstitial proliferation of columns of chondrocytes. Their progressive hypertrophy, mineralization of the intercolumnar cartilage matrix in the long axis of the bone, and the persistence of mineralized cartilage after disappearance of its cells acts as an elongating scaffold for the deposition of subchondral

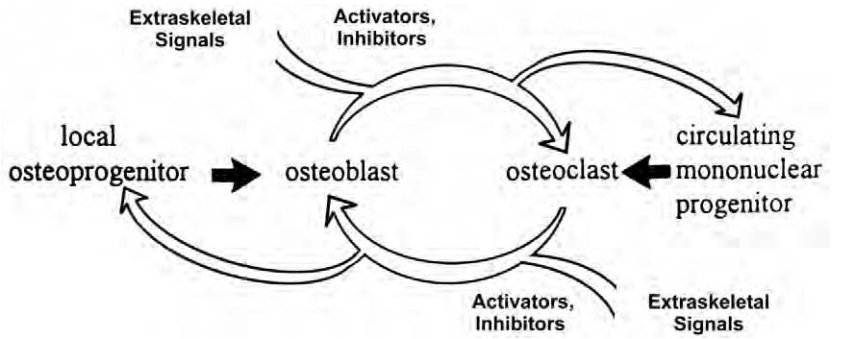

Figure 10 Cellular coordination of skeletal development. Schematic of the divergent origin and interrelated function of the principal bone cells.

(metaphyseal) bone (Hunziker, 1994). In the circumference of such a bone, starting initially at the center and progressing toward the ends, the investing cartilage cells and stroma (perichondrium) transform into osteoblasts that form a periosteal collar after the underlying chondrocytes have hypertrophied and mineralized the matrix. The peripheral osteoblasts (periosteum) arrive with a blood supply whose vessels penetrate the central hypertrophied, mineralized cartilage core and carry to the interior the skeletal cell progenitors for the formation and turnover of bone. Thus, peripherally extension of the periosteum and centrally mineralization of cartilage, hypertrophy, and disappearance of chondrocytes and bone formation on the mineralized cartilagenous scaffold proceed toward the end of each growing long bone.

The cellular events of long bone growth in length by endochondral ossification are illustrated in Figs. 12 and 13. At the top of the figures, chondrocyte proliferation and matrix elaboration in the direction of bone growth and the hypertrophy of these cells are the primary mechanisms for the linear growth of bones (Hunziker, 1994). Chondrocytes mineralize the intercolumnar matrix, producing a rigid scaffold that persists in the metaphysis and becomes the solid base upon which osteoblasts deposit and mineralize bone matrix. The closely packed mineralized cartilage septae at the chondroosseous junction are thinned to about one-third their density (Schenk *et al.,* 1967, 1968) by osteoclasts at this site (Fig. 12), providing space for new bone and a longitudinally oriented vasculature in the metaphysis (Aharinejad *et al.,* 1995). The final component of longitudinal bone growth is resorption of the central (marrow cavity) ends of metaphyseal trabeculae.

The fate of hypertrophied chondrocytes is controversial. Earlier reports of universal cell death conflicted with biochemical data and were perpetuated by poor fixation methods that produced pyknotic cells. Better fixation preserves the morphology of these cells, and it is clear that at least some hypertrophied chondrocytes survive (Farnum *et al.,* 1990; Hunziker and Schenk, 1984; Takechi and Itakura, 1995) after vascular penetration of their lacunae (Figs. 12 and 13) and can differentiate into osteoblasts (Galotto *et al.,* 1994; Roach *et al.,* 1995; Thesingh *et al.,* 1991) at least *in vitro* but that the percentage of such cells may vary among species (Gibson *et al.,* 1995).

Longitudinal bone growth is a precise balance between chondrocyte proliferation, cartilage matrix production and mineralization, and hypertrophy and vascular invasion of the lacuna of the terminal hypertrophied chondrocyte after re-

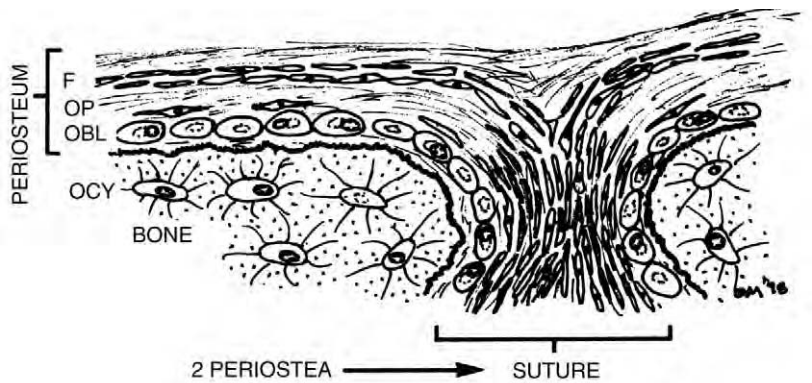

Figure 11 Cellular relationships in a periosteum and a suture. F, fibroblast; OP, osteoprogenitor cell; OBL, osteoblast; OCY, osteocyte. Reprinted from Marks *et al.* (1999), with permission of John Wiley & Sons.

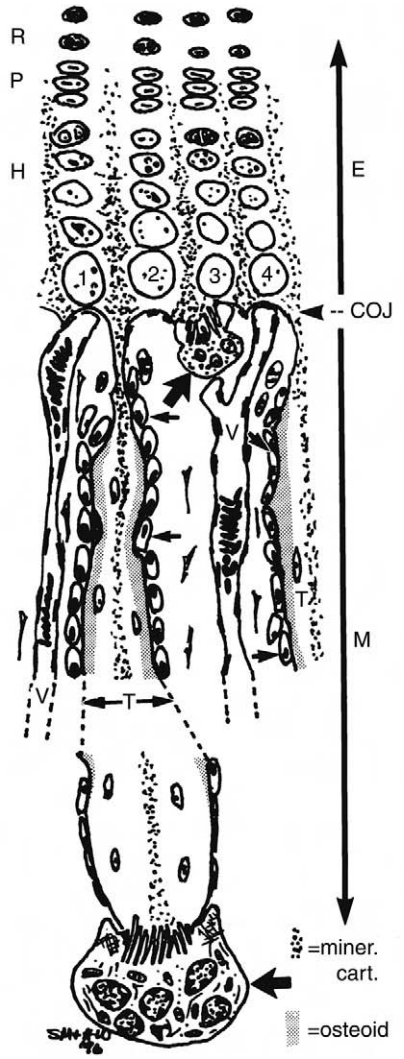

Figure 12 Schematic drawing of cellular locations and activities at the chondroosseous junction (COJ) of growing bone. The physis, or epiphyseal plate (E), consists of resting (R), proliferating (P), and hypertrophied (H) chondrocytes. In the metaphysis (M), trabeculae (T) alternate longitudinally with vascular channels (V). Osteoblasts (small arrows) line trabecular surfaces beginning just below the COJ, and osteoclasts (large arrows) are found in two locations: at the COJ and at the marrow cavity ends of the trabeculae. Chondrocytes are aligned in columns (four are numbered), and their alignments are maintained by mineralization of the longitudinal interterritorial matrix between columns that begins in the zone of proliferating chondrocytes and gets denser in the zone of hypertrophy. These mineralized cartilaginous struts are the surfaces in the metaphysis on which osteoblasts differentiate, produce, and mineralize the extracellular matrix of bone. All trabeculae in the metaphysis have a mineralized cartilage core, which is then resorbed, together with bone, by osteoclasts at the margin of the marrow cavity (bottom). Other osteoclasts at the COJ resorb about two of every three cores of mineralized cartilage that extend from the epiphysis. This provides space in the metaphysis for bone deposition and vascular invasion. The latter is an important regulator of the thickness of the zone of hypertrophied chondrocytes by penetrating the horizontal septum between the oldest such chondrocytes and the metaphysis (illustrated for cells 1 and 4). Reprinted from Marks (1998), with permission of C.V. Mosby.

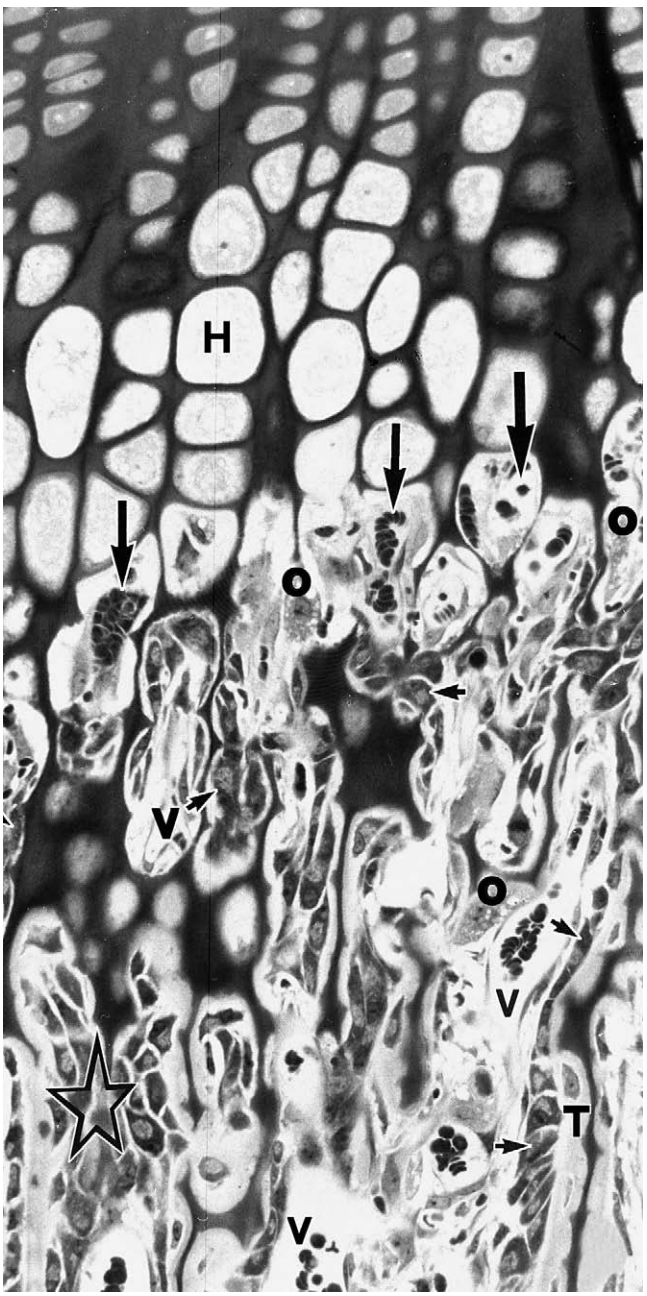

Figure 13 Photomicrograph of the chondroosseous junction in a young rat. The physis is composed primarily of hypertrophied (H) chondrocytes in this field. Vascular invasion of chondrocyte lacunae is occurring at many sites (vertical arrows) along the COJ, and vascular channels (V) are common. Mineralized cartilage in the metaphysis stains darkly. The typical trabecular cross section of a central cartilage core, bone, osteoid, and osteoblasts is clear at the lower right (T) but is obscured in much of the rest of the field due to the obliquity of their planes of section. Osteoblasts (small arrows) can be identified on most of the metaphyseal surfaces, and a large group (star) appears where trabeculae converge just out of the plane of this section. Several osteoclasts (O) can be seen near the COJ. (Toluidine blue stain; ×500.) Reprinted from Marks (1998), with permission of C. V. Mosby.

sorption of the horizontal septum within a column by mononuclear cells (Hunziker, 1994; Price *et al.,* 1994) rich in cathepsin B and with a distinct morphology (Lee *et al.,* 1995). Cartilage proliferation is under the direct influence of a variety of hormones (growth, thyroid, corticosteroids, and parathyroid) and local growth factors (insulin-like growth factors and basic fibroblast growth factor) (Nilsson *et al.,* 1994). Because most studies have been done *in vitro* where three-dimensional relationships of cells and matrices and the complex physiological landscape cannot be duplicated, it is

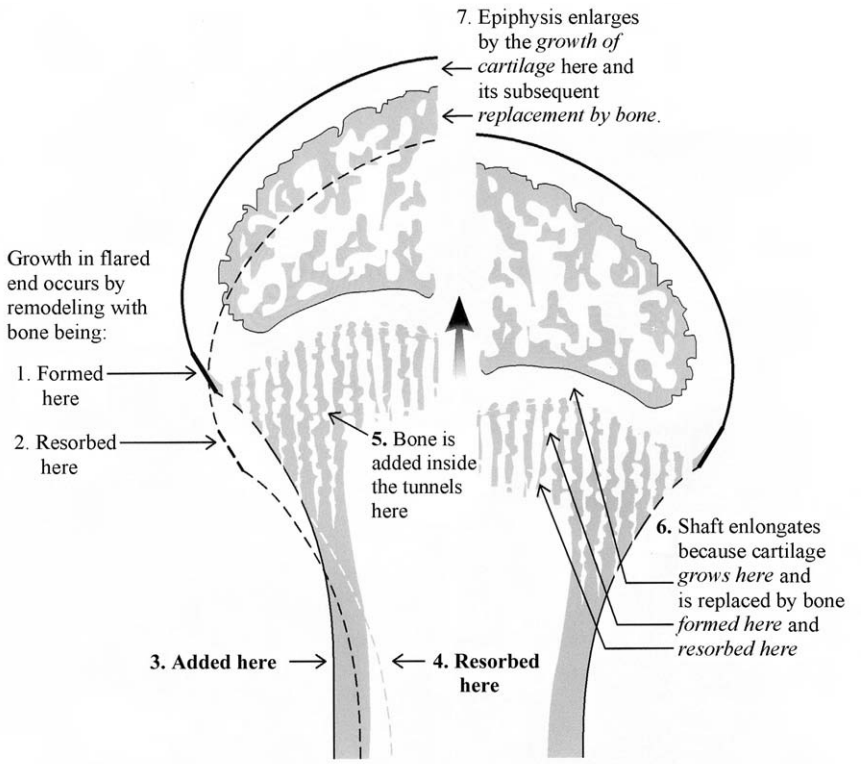

Figure 14 Diagram of regional changes in cartilage and bone that produce growth in the length (large arrow) and width of long bones. Reprinted from Marks (1998), with permission of C. V. Mosby.

not surprising that reports of the effects of individual factors on bone growth conflict and give us incomplete information at best.

Bone growth in diameter (Fig. 14) is accomplished most basically by formation externally (periosteum) and resorption internally (endosteum). This is strictly true only for the central portion of long bones and only if the bone is cylindrical. Because most bones are asymmetrical cylinders centrally and are expanded (flared) unevenly at each end, growth in diameter is more complex than depicted in the process just described and varies by region according to the dynamic changes in bone shape at that site.

At the flared ends of a growing long bone the periosteal collar externally surrounds part of the growth plate cartilage and extends much farther peripherally than the central bone (Fig. 14) of the shaft. Thus, during bone growth, with extension of the new periosteal collar, the old periosteal collar has to be removed and reformed toward the center. This is accomplished by resorption on the periosteal surface and formation on the endosteal surface at this site, a polarization of these activities that is opposite that seen at the center of the shaft. In summary, the succession of metabolic activities on the periosteal surface is (1) formation at the periosteal collar, (2) resorption, and (3) formation toward the center of the shaft. In general, activities in the peripheral endosteum are the opposite. In the metaphysis, bone formation on the mineralized cartilage scaffold takes place after osteoclasts thin the longitudinal mineralized cartilage remnants of the growth plate. This increases the thickness and strength of these trabeculae, which remain until their central ends are resorbed to accommodate longitudinal expansion of the marrow cavity during bone growth.

Bone growth involves the coordination of a variety of cellular activities in specific sites whose onset and rates vary among bones and even within a single bone during its development. These activities are under the influence of a variety of humoral and local factors whose relative concentrations, sites, and sequences of appearance vary during development.

The complexities of skeletal maintenance are unlikely to be substantially less complicated than those of development. Thus, the multiplicity and redundancy of the biological controls of skeletal metabolism need to be appreciated as we seek to interpret all experimental data.

Molecular Regulation of Skeletal Development

The principal physiologic processes of skeletal formation and maintenance might be summarized as pattern formation, transition from cartilage to bone, bone matrix synthesis and secretion, and bone resorption and remodeling. Genes with crucial roles in all these processes have been discovered recently, giving both new depth to our understanding of normal bone biology and hopes for novel clinical strategies and interventions in disease or injury. Some of these discoveries came as surprises in gene knockout or transgenic studies conceived with quite different expected outcomes, demonstrating the critical importance of evaluating gene function in the living organism. Genes essential for bone synthesis,

normal patterning, and bone resorption are discussed in the following brief overview. Subsequent chapters treat these in much greater detail.

Bone Formation

A molecular event crucial for the synthesis and secretion of bone matrix, i.e., for the fully differentiated activity of osteoblasts, is the production by osteoprogenitor cells of the DNA-binding transcription factor cbfa-1. Independent investigations led to its simultaneous discovery by three groups (Ducy *et al.,* 1997; Komori *et al.,* 1997; Mundlos *et al.,* 1997; Otto *et al.,* 1997). People and mice with a haploid insufficiency of the *cbfa-1* gene suffer from skeletal defects that include a ridged skull and lack of clavicles, known clinically as cleidocranial dysplasia. The dramatic, and lethal, phenotypic consequences of diploid defects of *cbfa-1* were seen in knockout mice. Those mice were able to construct a nearly complete cartilage model of the skeleton, but having lost all osteoblastic bone matrix production, failed to mineralize the cartilage model. Clearly, cbfa-1 acts as a master switch in osteoblast differentiation and bone synthesis. In turn, its induction or inhibition by local and systemic factors is central to bone formation. This area has been reviewed by Ducy *et al.,* (2000) and is treated in greater depth in Chapters 3, 4, and 5.

Patterning and Endochondral Ossification: The Changeover from Cartilage to Bone

Growth of the long bones, the spine, and ribs proceeds via the construction of a cartilage model that is then remodeled into bone (see Figs. 12, 13, and 14). This process begins before birth and continues throughout the growth phase. Interestingly, some advances in understanding the complexities of its regulation owe much to basic research done with organisms that have no endoskeleton. The *hedgehog* gene, discovered in *Drosophila melanogaster* as a regulator of body segment polarity, has been conserved through evolution and is present in three versions in mammals, called sonic, desert, and Indian hedgehog. The hedgehog proteins regulate axis polarity and pattern formation in early cartilage modeling. Indian hedgehog, partially through communication with the parathyroid hormone-related protein and its receptor, helps maintain the exquisitely balanced regulation of chondrocyte proliferation and hypertrophy that determines bone growth in the epiphysis (Kronenberg *et al.,* 1997; Philbrick *et al.,* 1996; St-Jacques *et al.,* 1999; van der Eerden *et al.,* 2000; Vortkamp *et al.,* 1998). See Chapter 3 for more information on this process.

Bone Resorption: An Exception to the Redundancy of Critical Functions Rule

The advent of gene knockout technology has necessitated a reevaluation of our thinking about bone resorption. Many genes were knocked out in mice by researchers in various fields who anticipated phenotypic consequences consistent with important gene functions inferred from results of cell culture experiments, only to find that the missing gene's function could be compensated for by other redundant pathway components. While this was not always the case, it did occur with some frequency and produced a general appreciation that evolution has selected for redundancy in many critical functions.

The phenotype of osteopetrosis, however, which results from defective osteoclast development or function, was found unexpectedly in several gene knockout experiments. These include the protooncogenes *c-src* (Soriano *et al.,* 1991) and *c-fos* (Wang *et al.,* 1992); a transcription factor identified in immune system cells, NF-κB (Franzoso *et al.,* 1997; Iotsova *et al.,* 1997); and the hematopoietic transcription factor PU.1 (Tondravi *et al.,* 1997). In addition, genes critical for osteoclast function have been identified in studies of naturally occurring osteopetrotic mutations: the cytokine M-CSF, or CSF-1, in the *op* mouse (Yoshida *et al.,* 1990); and microphthalmia, a transcription factor also active in pigment and mast cells, in the *mi* mouse (Steingrimsson *et al.,* 1994) and the *mib* rat (Weilbaecher *et al.,* 1998). In addition to these, knockouts of osteoclast-specific genes for cathepsin K (Saftig *et al.,* 1998), a cysteine protease, and the vacuolar proton pump Atp6i (Li *et al.,* 1999) also result in osteopetrosis.

The field of immunology contributed another key discovery recently in our understanding of osteoclast formation and activity, the identification of a tumor necrosis factor family member produced by T cells called TRANCE (also known in the literature as RANKL, ODF, and OPGL) (Anderson *et al.,* 1997; Kong *et al.,* 1999; Wong *et al.,* 1997; Yasuda *et al.,* 1998). TRANCE is also produced by osteoblasts, and knockout mice lack both osteoclasts and lymph nodes. The TRANCE receptor (also called RANK) and its intracellular-associated signaling molecule TRAF-6 are both required for osteoclast formation, shown by the severe osteopetrosis in mice in which either of those genes are knocked out (Dougall *et al.,* 1999; Lomaga *et al.,* 1999; Naito *et al.,* 1999). More information about osteoclasts, their formation, and activation is presented in Chapters 7, 8, and 9.

Together, these findings demonstrate that, in contrast to some bodily processes that have redundant means to ensure they take place, bone resorption does not. Bone resorption may in fact be thought of as a highly regulated and specialized form of autoimmunity. It appears that evolution has favored a scenario in which the commitment to resorb bone, which is a unique and potentially debilitating process, requires that many signaling pathways all agree.

Methods for Studying Skeletal Development and Regulation

Mineralization in the skeleton has made cellular access difficult and has impeded progress in understanding bone cell biology. A century ago, studies of the skeleton had to

focus on either the mineral or the cellular components because one had to be destroyed to study the other. Improvements in methods were not sufficient to study both bone cells and their mineralized environment until the advent of electron microscopy, which provided durable embedding media and thin-sectioning procedures. Bone cell cultures were developed later than those for other tissues for similar reasons. As a result, considerable attention was paid to cells outside the skeleton for clues about bone cell function (Kahn *et al.,* 1978). Unfortunately, these data often supported erroneous conclusions because of two facts: cells of a particular family operate differently in different tissues (even the same cells are known to function differently in different site; Cecchini *et al.,* 1994) and no culture conditions *in vitro* can duplicate the complex cell/matrix/humoral interactions that occur in the organism *in vivo*. Detailed discussions of these points have been published (Fox *et al.,* 2000; Marks, 1997; Marks and Hermey, 1996). These principles need to be remembered when trying to reconcile discrepancies between studies of bone cells. The method(s) used will determine or limit the results that are possible.

Fortunately, it is now possible to study the effects of both genes and proteins *in vivo*. Analyses are complex and the results often surprising, but, unlike many *in vitro* studies, the validity of data is unquestioned. The relative ease with which animals can be produced in which a gene has been eliminated, modified, or overexpressed or in which there is cells- or tissue-specific expression has produced a variety of *in vivo* models to study the authentic biological effects of genes and their products. A number of these transgenic and knockout mutations have had surprising skeletal phenotypes. Many of the new developments in bone biology described in this book have been derived from these new discoveries in organismal molecular biology. In short, these targeted gene manipulations, combined with the numerous spontaneous skeletal mutations and an understanding of the predictable, orderly, local events in normal skeletal development, provide a new series of reality checks for bone biologists, replacing the earlier overreliance on *in vitro* methods. Many sites in the body exhibit localized, precisely timed displays of skeletal metabolism, including cell recruitment, activation, function, and senescence and as such are places where the informed investigator can intelligently dissect the crucial elements of these events. Two such sites are the postnatal development of the caudal vertebrae in rodents (unpublished work by Cecchini in Marks and Hermey, 1996) and the skeletal events around erupting teeth (Marks and Schroeder, 1996). We can expect to learn much more about the basic and applied biology of the skeleton using these systems, some of which are reviewed in Chapters 87–96.

Conclusions

The skeleton is a complex association of metabolically active cells attached to, embedded in, or surrounded by a mineralized matrix. The potential activities of each cell type are understood in broad outline, but the complex cellular interactions during development and maturation of the skeleton are under intense scrutiny. These are the new frontiers of skeletal biology and will have required a shift in focus from the isolated *in vitro* cell systems of today to the complex *in vivo* environment. This is accomplished most efficiently by studying the development and progression of reproducible sites of skeletal maturation and the skeletal effects of targeted changes in expression of specific genes. This, in turn, can be facilitated by the application of new molecular and morphologic techniques, such as *in situ* hybridization, polymerase chain reaction, immunocytochemistry, and high-resolution three-dimensional reconstruction.

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CHAPTER 2

Biomechanics of Bone

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Introduction

Bone is a physiologically dynamic tissue whose primary functions are to provide a mechanical support system for muscular activity, provide for the physical protection of organs and soft tissues, and act as a storage facility for systemic mineral homeostasis. The resulting structure of the skeleton then is influenced heavily by mechanical principles, acting both as constraints and as driving forces in its architecture (Christiansen, 1999; Cullinane, 2000; Galileo, 1638; Thompson, 1946). A form–function relationship exists in the architecture of bone, and this relationship guides the evolution, embryogenesis, and continued ontological adaptation of the skeleton.

Since the observations of Galileo it has been recognized that the inherent architecture of bone is not only organized to accommodate normal loading, but also is influenced during ontogeny by the mechanical stresses associated with daily function. Thus, the skeleton is both evolutionarily adapted and has the capacity to adapt as a result of changes in daily activity (Carter, 2000). A formal description of the dynamic structure–function relationship between bone and mechanical load was established in the late 19th century in what has since become known as Wolff's law (Wolff, 1892). Wolff determined that the trabecular elements of the skeleton were not only designed to perform their specific functions, but also responded to load by altering their structural configuration during the lifetime of an individual. Wolff's law has become widely accepted as the general guiding principle of bone regulation, with some more recent modifications (Bertram and Swartz, 1991; Biewener *et al.,* 1996; Fyhrie and Carter, 1986) and recently proposed mechanisms (Carter, 2000; Martin, 2000; Mullender and Huiskes, 1995; Turner and Pavalko, 1998). The skeleton then is not only genetically programmed for a specific configuration, but a degree of morphological plasticity exists that is influenced heavily by an individual's mechanical loading history. These ontological adaptations modify the skeleton in order to optimize its functional capacity during locomotion or other mechanical duties.

To understand how the skeleton moves or how bone responds to impact, it is necessary to appreciate how the mechanical properties of bone determine skeletal responses to both physiological and mechanical load. If details on the structural configuration and the tissue level properties of bone are provided, this information can be used to predict the risk of fractures associated with normal daily activities, athletic activities, advancing age, or metabolic bone diseases. It is imperative then to apprecial that the mechanical behavior of the skeleton is contingent upon how bone functions as a tissue and a whole organ.

Basic Biomechanics

The mechanical behavior of bone may be studied at two levels: material and structural. The material, or tissue, level properties of bone are evaluated by performing standardized mechanical tests on uniform bone tissue samples. Depending on the level of resolution, tissue level testing is relatively independent of bone structure or geometry. Second, by examining the mechanical behavior of bones as whole anatomical units, the contributions of structural properties can be determined. Mechanical properties may also be estimated *in vivo* using densitometric projections, but these are less accurate than actual mechanical testing. Taken together, these two levels of mechanical properties represent the way bones respond to forces *in vivo* and can be observed by means of experiments on sections of bones

(for material properties) or on fully intact bones with normal geometry (for structural properties).

The assessment of bone mechanical properties can be made using techniques ranging from noninvasive imaging to *in vitro* mechanical tests of excised specimens or whole bones. The accuracy of any assessment, however, is contingent upon its degree of dependence on extrapolation from non-mechanical data and its reflection of actual, *in vivo* physiological stresses. Micro-computed tomography (CT), magnetic resonance imaging (MRI), and peripheral qualitative CT (pQCT) methods of imaging, especially in use with finite element models, continue to improve in their accuracy of mechanical property assessment, (Cody *et al.,* 1999; Moisio *et al.,* 2000), as does simple bone mineral density estimation (Toyras *et al.,* 1999), vibration analysis (Weinhold *et al.,* 1999), ultrasonic wave propagation (van der Perre and Lowet, 1996), and duel-energy absorptiometry (Sievanen *et al.,* 1996). Some of these techniques primarily reflect bone tissue level properties (DEXA, wave propagation, vibration, etc.), whereas others incorporate three-dimensional information from architecture with tissue mechanical property estimates to determine structural level properties (MRI, pQCT, and micro-CT with finite element models).

However, when performing mechanical tests on bone, it is important to bear in mind that the differences between tissue level and structural level properties are not always clear. If one considers a small cube of vertebral trabecular bone as a tissue section, then trabecular element preferred orientation may influence what is assumed to be a material property, when in fact it is largely influenced by structural configuration (Keaveny *et al.,* 2000). Likewise, both material and structural properties of bones can change with the level of resolution. As mentioned earlier, vertebral trabecular bone fails by creep as multiple individual elements fail, yet the failure mode of each individual trabecular element is more elastic. These two levels of mechanical properties in bone are also evident during fractures, when what appears to be a structural failure must be accompanied by a tissue level failure. It is important to realize then that a fracture represents a failure of bone tissue at both the material and the whole bone levels (Hayes, 1983).

Stress–Strain Relationships

Like other objects in nature, bone undergoes acceleration, deformation, or both when a force is applied to it. If the bone is constrained over one portion of its structure so that it cannot move when a force is applied or if equal and opposite forces are applied to it, deformation will occur, resulting in the generation of an internal resistance to the applied force. This internal resistance is known as *stress*. Stress is equal in magnitude but opposite in direction to the applied force and is distributed over the cross-sectional area of the bone (in a long bone example). It is expressed in units of force (Newtons = N) per unit area (meters squared = m^2):

$$\text{Stress} = \delta = \text{force/area}.$$

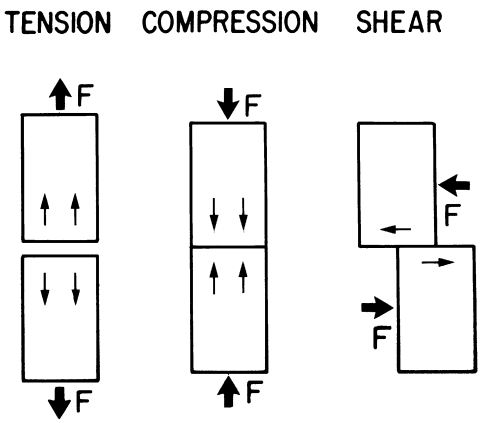

Figure 1 The three basic types of stress into which all complex stress patterns can be resolved: tension, compression, and shear. Reprinted with permission from Craig, R. G. (1989). "Restorative Dental Materials," p. 68. C. V. Mosby, St. Louis.

The standard international unit for stress is the Pascal, which is 1 N of force distributed over 1 m^2, which converts to 1.45×10^{-4} pounds per square inch (psi):

$$1 \text{ Pa} = 1N/m^2 = 1.45 \times 10^{-4} \text{ psi}.$$

Although an externally applied force can be directed at a specimen from any angle, producing complex stress patterns in the material, all stresses can be resolved into three types: tension, compression, and shear (Fig. 1). *Tension* is produced in a material when two forces are directed away from each other along the same line, with resistance to tensile forces coming from the intermolecular attractive forces that resist the material's being torn apart; ultimate tensile strength is a measure of this cohesive force. An example of

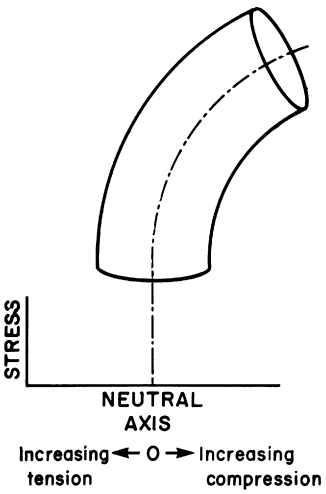

Figure 2 The bending of a simple cylinder results in tensile stresses on the convex side and compressive stresses on the concave side. The magnitude of these stresses increases proportionally to the distance from the neutral axis of bending. Reprinted with permission from Radin, E. L., Simon, S. R., Rose, R. N., and Paul, J. P. (1980). "Practical Biomechanics for the Orthopaedic Surgeon," p. 14. Wiley & Sons, New York.

tension occurs at the tendon–bone interface when a muscle acts via contraction. *Compression* results from two forces, again acting along the same line but directed toward each other; it is resisted by interatomic repulsive forces, which rise sharply at short interatomic distances. An example of compression occurring in the body is when a weight is carried on the head and the compressive load is transduced down the axis of the spine via the vertebral bodies. *Shear* forces occur when two loads act in parallel but in opposite directions from one another and can be linear or rotational. Shear occurs in a vertebral body when the superior end plate surface is loaded anteroposteriorly while the inferior end plate surface experiences a posteroanterior directed load. It must be noted that, *in vivo,* these individual stresses should be looked upon as a predominant rather than a singular stress for they almost always act in concert.

Thus, most stress patterns are complex combinations of these three stress types. Bending, for example, produces a combination of tensile forces on the convex side of a structure or material and compression on the concave side (Fig. 2). *Torsion,* or twisting produces shear stress along the entire length of a structure or material, whereas tensile stresses elongate it and compressive stresses shorten it (Fig. 3). Bending in two directions (X and Y coordinates) simultaneously, even acting on a regularly shaped cantilevered beam, can combine to create more complex

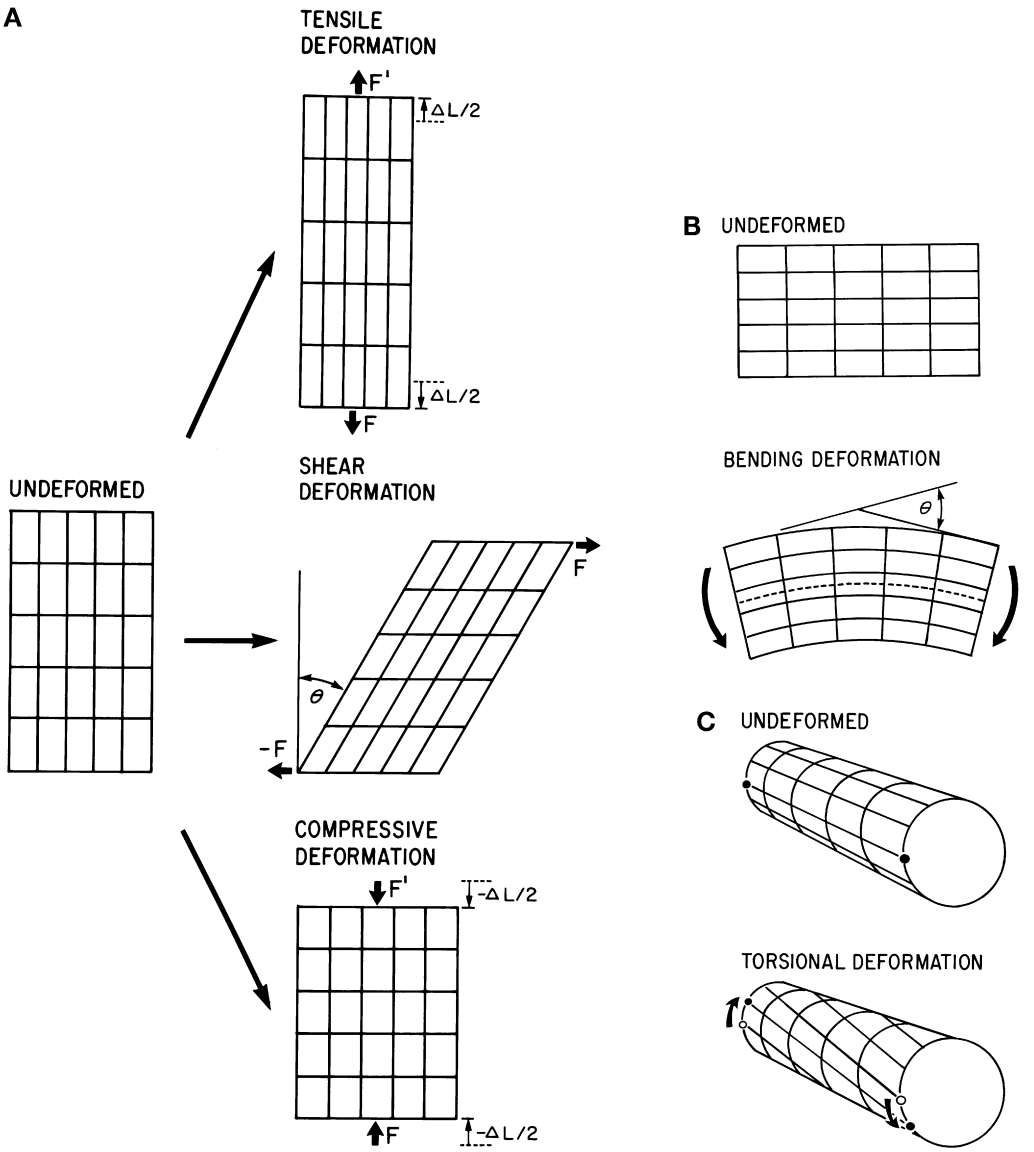

Figure 3 (A) Deformations produced by tensile, shear, and compressive stresses. F' and F are equal and opposite tensile or compressive forces, $-F$ and F are equal and opposite shear forces, θ is the angle of deformation, and Δ is change in length resulting from deformation. (B) Deformation produced by bending stress. θ is the angle of deformation. (C) Deformation produced by torsional stress. Reprinted with permission from Black, J. (1988). "Orthopaedic Biomaterials in Research and Practice." Churchill Livingston, New York.

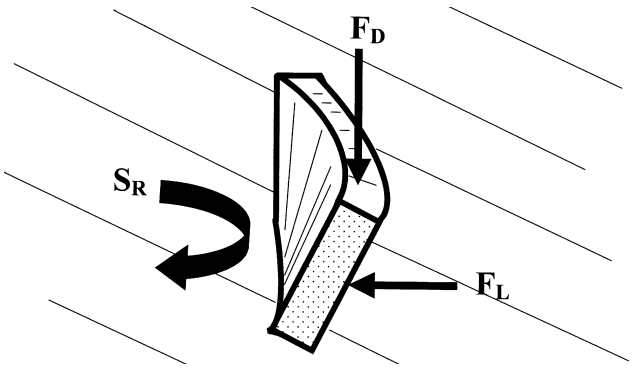

Figure 4 A simple cantilevered beam coming out of the page and with two bending forces applied. A combination of a force downward (F_D) from top and a lateral force from page right to page left (F_L) cause predictable bending in those two planes. However, the resultant strain (S_R) is a combination of bending in those two planes and an additional resultant axial torsion. Thus, even with very regular structures, combinations of even simple loads can induce complex strain behaviors. More complex biological structures like the femur would compound this strain reaction further.

stresses, including torsion along with the initial two simple bending stresses (Fig. 4). This complicating effect is even more apparent in irregularly shaped objects such as a long bone. The measurement of deformation resulting from any of these stresses, when normalized by the original configuration of the specimen, is called *strain:*

$$\begin{aligned}\text{Strain} = \varepsilon &= \text{change in length/original length} \\ &= (\text{deformed length} \\ &\quad - \text{original length})/\text{original length}.\end{aligned}$$

Strain is dimensionless and is therefore expressed as a percentage of change from the original dimensions or angular configuration of the structure. The application of these terms to bone can be made by considering the stresses and strains generated in the diaphysis of the femur (Fig. 5). For this purpose, the assumption must be that a very thin transverse section of bone behaves like a small cube, the top face of which may be designated *A*. Two types of internal forces can act on *A*, a perpendicular force *F* and shear force *S*. The former produces a normal stress gamma (δ), equal to *F/A*, whereas shear force results in a shear stress (*T*), equal to *S/A*. A normal stress might be applied toward the face of the cube, in which case it is called compression, or away from the face of the cube, in which case it is called tension.

The stresses described cause local deformation of the cube (Fig. 6). A normal compressive stress will cause shortening by a distance *L*, and the normal strain in the cube is then defined as the ratio of the change in length of the side of the cube ΔL to the original length *L* (strain = $\Delta L/L$). A shear stress applied to the top face of the cube will cause the front of the face to be deformed, and the resultant shear strain can be defined as the deviation of one side of the cube from its original angle, i.e., strain = $\Delta L/L$.

Considering that the cube represents a section of bone, the normal and shear strains experienced will be influenced

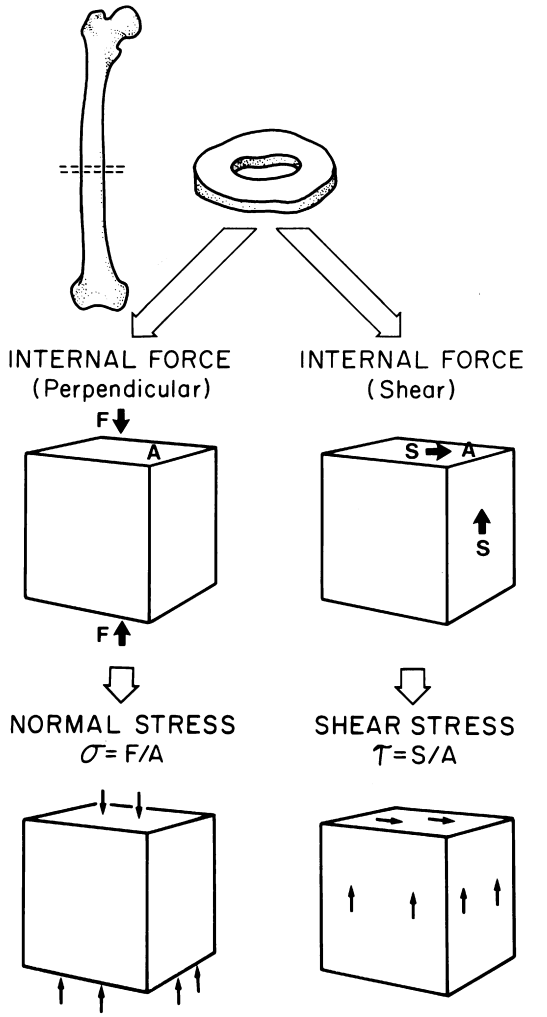

Figure 5 Schematic representation of the stresses acting on the diaphysis of the femur. In this example, a thin transverse section of the femur is considered to behave as if it were a small cube. *F,* perpendicular force; *S,* shear force; *A,* area on which force acts. Reprinted with permission from Einhorn, T. A. (1988). "Biomechanical Properties of Bone. Triangle," p. 28.

not only by the magnitude of the stresses applied, but also by the inherent material and structural properties of the bone. Stresses applied to normal, well-mineralized bone tissue will cause small strains, whereas the same stresses applied to poorly mineralized tissue, such as osteomalacic bone, will produce large strains. Likewise, if a bone experiences a bending stress in a direction in which it has a relatively greater areal moment (*I*), it will experience less strain than when loaded in a direction having a lower areal moment. It must be remembered, however, that in nature stresses are applied to bone not only from perpendicular and horizontal directions, but also from oblique angles and combinations of loads, resulting in a variety of complex mechanical relationships. Although strain is given most commonly in length dimensions, it may also be represented by angular deformation as well as other structural alterations such as volumetric changes.