Contributors

VOLUME 1

Pamela E. Ginn *BS, DVM, Diplomate ACVP*
Department of Infectious Diseases and Pathobiology
College of Veterinary Medicine
University of Florida
Gainesville, Florida
USA

Joanne E. K. L. Mansell *DVM, MS, Diplomate ACVP*
Department of Veterinary Pathobiology
College of Veterinary Medicine
Texas A&M University
College Station, Texas
USA

M. Grant Maxie *DVM, PhD, Diplomate ACVP*
Animal Health Laboratory
Laboratory Services Division
University of Guelph
Guelph, Ontario
Canada

Pauline M. Rakich *DVM, PhD, Diplomate ACVP*
Athens Veterinary Diagnostic Laboratory
College of Veterinary Medicine
University of Georgia
Athens, Georgia
USA

Keith Thompson *BVSc, PhD, Diplomate ACVP*
Pathobiology Section
Institute of Veterinary, Animal and Biomedical Sciences
Massey University
Palmerston North
New Zealand

Beth A. Valentine *DVM, PhD, Diplomate ACVP*
Oregon State University
College of Veterinary Medicine
Corvallis, Orlando
USA

John F. Van Vleet *DVM, PhD, Diplomate ACVP*
School of Veterinary Medicine
Purdue University
West Lafayette, Indiana
USA

Brian P. Wilcock *DVM, PhD*
Histovet Surgical Pathology
Guelph, Ontario
Canada
(Formerly: Department of Pathobiology, Ontario
Veterinary College, University of Guelph, Canada)

Sameh Youssef *BVSc, MVSc, PhD, DVSc, Diplomate ACVP*
Animal Health Laboratory
Laboratory Services Division
University of Guelph
Guelph, Ontario
Canada

VOLUME 2

Dale C. Baker *DVM, PhD, Diplomate ACVP, Diplomate ABT*
Department of Toxicology and Pathology
Portola Pharmaceuticals, Inc.
South San Francisco, California
USA
(Formerly: Department of Pathology, Colorado State
University, USA)

Ian K. Barker *DVM, PhD*
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

Corrie C. Brown *DVM, PhD, Diplomate ACVP*
Department of Pathology
College of Veterinary Medicine
University of Georgia
Athens, Georgia
USA

Jeff L. Caswell *DVM, DVSc, PhD, Diplomate ACVP*
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

Jennifer A. Charles *BVSc, MVS, Diplomate ACVP*
Department of Veterinary Science
Faculty of Veterinary Science
University of Melbourne
Werribee, Victoria
Australia

M. A. (Tony) Hayes *BVSc, PhD, Diplomate ACVP*
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

M. Grant Maxie *DVM, PhD, Diplomate ACVP*
Animal Health Laboratory
Laboratory Services Division
University of Guelph
Guelph, Ontario
Canada

Shelley J. Newman *DVM, DVSc, Diplomate ACVP*
Department of Pathology
College of Veterinary Medicine
University of Tennessee
Knoxville, Tennessee
USA

Margaret J. Stalker *DVM, PhD, Diplomate ACVP*
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

Kurt J. Williams *DVM, PhD, Diplomate ACVP*
Department of Pathobiology and Diagnostic Investigation
College of Veterinary Medicine
Michigan State University
East Lansing, Michigan
USA

VOLUME 3

Charles C. Capen *DVM, PhD, Diplomate ACVP*
Department of Veterinary Biosciences
Ohio State University
Columbus, Ohio
USA

Robert A. Foster *BVSc, PhD, MACVSc, Diplomate ACVP*
Department of Pathobiology
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

Patricia A. Gentry *PhD*
Department of Biomedical Sciences
Ontario Veterinary College
University of Guelph
Guelph, Ontario
Canada

Philip W. Ladds *BVSc, MVSc, PhD, FACVSc, FRCPath, Diplomate ACVP*
Goonellabah, New South Wales
Australia

M. Grant Maxie *DVM, PhD, Diplomate ACVP*
Animal Health Laboratory
Laboratory Services Division
University of Guelph
Guelph, Ontario
Canada

Richard B. Miller *DVM, PhD, Diplomate ACVP*
School of Veterinary Medicine
Ross University
St. Kitts, West Indies

Wayne F. Robinson *BVSc, MVSc, PhD, Diplomate ACVP, MACVSc*
Vice Chancellor's Office
University of Ballarat
Ballarat, Victoria
Australia

Donald H. Schlafer *DVM, PhD, Diplomate ACVP, Diplomate ACVM, Diplomate ACT*
Department of Biomedical Sciences
College of Veterinary Medicine
Cornell University
Ithaca, New York
USA

V. E. O. (Ted) Valli *DVM, PhD, Diplomate ACVP*
Department of Pathobiology
College of Veterinary Medicine
University of Illinois at Urbana-Champaign
Urbana, Illinois
USA

Preface

It was an honor to be asked by Drs. Jubb, Kennedy, and Palmer to assume the editorship of *Pathology of Domestic Animals*. I studied from the first edition in Saskatoon as a DVM student, used the second edition as a working pathologist, and encouraged production of, and contributed to, the third and fourth editions. I'm delighted to have been able to be part of this continuum. In this age of instantaneous global communication and virtually universal access to databases through powerful search engines, does the need continue for such print versions of textbooks? I firmly believe that printed books continue to serve a useful function, partly through their portability and ease of use, but primarily through provision of a measured overview of important topics, with the relevance of competing topics put into perspective by authorities in the field. In particular, this latest edition of these volumes is offered as a comprehensive, and we hope beneficial, overview of the diseases of the major domestic mammals.

The literature and knowledge of veterinary pathology have grown tremendously since publication of the fourth edition in 1993. However, the foundations of these volumes remain sound, and are firmly rooted in the detailed descriptions of gross and histologic pathology recorded by Jubb and Kennedy in the first edition. Etiologies and pathogeneses have been clarified over time, with the major revisions of the fifth edition involving the addition of knowledge gained from molecular biology. Diseases dating from antiquity, such as tuberculosis, are still with us, diseases thought to be under control, such as leptospirosis, have re-emerged as significant concerns, and new diseases and agents have evolved, such as porcine circovirus-associated disease caused by *Porcine circovirus 2*. I hope that we have captured significant changes, and have synthesized this new knowledge to provide a balanced overview of all topics covered. Keeping pace with changing agents and their changing impacts is of course a never-ending challenge. We have used current anatomical and microbial terminology, based on internationally accepted reference sources, such as the Universal Virus Database of the International Committee on Taxonomy of Viruses http://www.ncbi.nlm.nih.gov/ICTVdb/index.htm. Microbial taxonomy is, of course, continually evolving, and classifications and names of organisms can be expected to be updated as newer phylogenetic analyses are reported.

My thanks to the primary contributors to the 5th edition for their rigorous perusal of the literature in their areas of interest, for their addition of insightful information to their chapters, and for their inclusion of many new figures. Additional contributors are acknowledged in individual chapters, but I also offer my thanks to others who have labored in the background and provided helpful suggestions and advice, including Dr. Ron Slocombe (University of Melbourne) and Dr. Murray Hazlett (University of Guelph). The chapter format of the 5th edition is similar to previous editions, with the exception of including Peritoneum within the Alimentary system chapter. We have attempted to improve readability and usability by increased use of highlighting of text (boldfacing, italicization), use of bullet points, and updating of the graphic design, including reorganization of tables of contents. The complete index to all 3 volumes is printed in each volume, again as an aid to readers. Of necessity, bibliographies have been pruned to save space, with references being presented as entry points to the literature through computerized search and retrieval systems embodied in major databases, such as PubMed.

My thanks to Elsevier, and formerly Academic Press, for their help and support throughout this project, with particular thanks to Rita Demetriou-Swanwick (Associate Editor, Health Professions) and Joyce Rodenhuis (Commissioning Editor, Veterinary Medicine). Louisa Welch (Editorial Assistant, Health Sciences) has done a fine job of assembling permissions for use of previously published material. We have attempted to contact all contributors of figures from previous editions, and apologize to any that we were unable to contact or overlooked. My sincere thanks to Sue Nicholls, Senior Production Editor, Keyword Group Ltd, for her diligent checking and cross-checking of text and figures, and for her coordination of copyeditors, typesetters, and proofreaders.

Grant Maxie,
Guelph, Ontario, 2006

Dedication

Drs. Palmer, Jubb, and Kennedy, while working on the 3rd edition, Melbourne, 1983. (Courtesy of University of Melbourne.)

These volumes are dedicated to Drs. Kenneth V. F. Jubb, Peter C. Kennedy, and Nigel C. Palmer, and to my family – Laura, Kevin, and Andrea.

1 Bones and joints

Keith Thompson

Diseases of bones

ACKNOWLEDGMENTS

This update of Chapter 1 is based on the foundation developed in previous editions by Dr Ken Jubb and, more recently, Dr Nigel Palmer. It is an honor to follow in their footsteps. I am grateful to the many pathologists who have contributed illustrations to this chapter, in particular Dr Rob Fairley. Significant contributions have also come from the transparency collections of pathology departments at Ontario Veterinary College, Texas A&M University, Massey University, North Carolina State University, Ohio State University, University of California-Davis, and Western College of Veterinary Medicine.

Diseases of bones

GENERAL CONSIDERATIONS

Bone is a highly specialized connective tissue, its properties depending largely on the unique nature of its extracellular matrix. In addition to providing mechanical support and protecting key organ systems from traumatic injury, bone is intimately involved in the homeostasis of calcium, an essential cation in a wide range of bodily functions. In spite of their apparent inertia, *bones are dynamic organs, undergoing constant remodeling throughout life.* Even in mature individuals, bone tissue is continually undergoing localized resorption and replacement in response to the demands of mineral homeostasis and alterations in mechanical forces. The dynamic nature of bones is well illustrated by their impressive powers of repair following injury.

Because of the difficulties associated with processing mineralized tissue, the study of bones, both by researchers and diagnosticians, has lagged behind that of most other organ systems. The skeleton is seldom examined in detail during routine necropsy and it is highly likely that many disorders go undiagnosed. Even in cases where a bone disease is suspected, many veterinary pathologists do not feel confident in their approach to making a diagnosis. *Familiarity with the gross and microscopic anatomy of bones, factors regulating bone formation and resorption, and an understanding of the responses of bone to injury are key to an appreciation of the pathogenesis and pathology of bone diseases.* The initial sections of this chapter will therefore focus on these aspects and outline an approach to examining the skeleton at necropsy.

STRUCTURE AND FUNCTION OF BONE TISSUE

Cellular elements

Bone tissue consists of four cell types: *osteoblasts, osteocytes, lining cells, and osteoclasts*. The first three are derived from primitive

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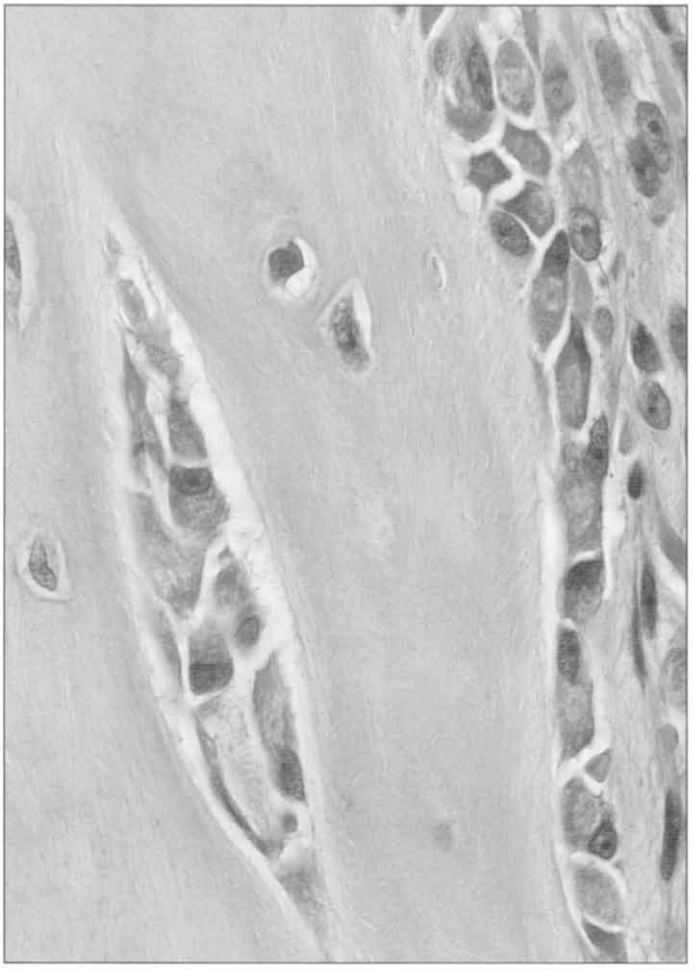

Figure 1.1 Active osteoblasts at a site of rapid bone formation in a newborn kitten. Note the eccentric nuclei, basophilic cytoplasm and prominent Golgi zone in many of the cells. Some osteoblasts have surrounded themselves with osteoid to become osteocytes.

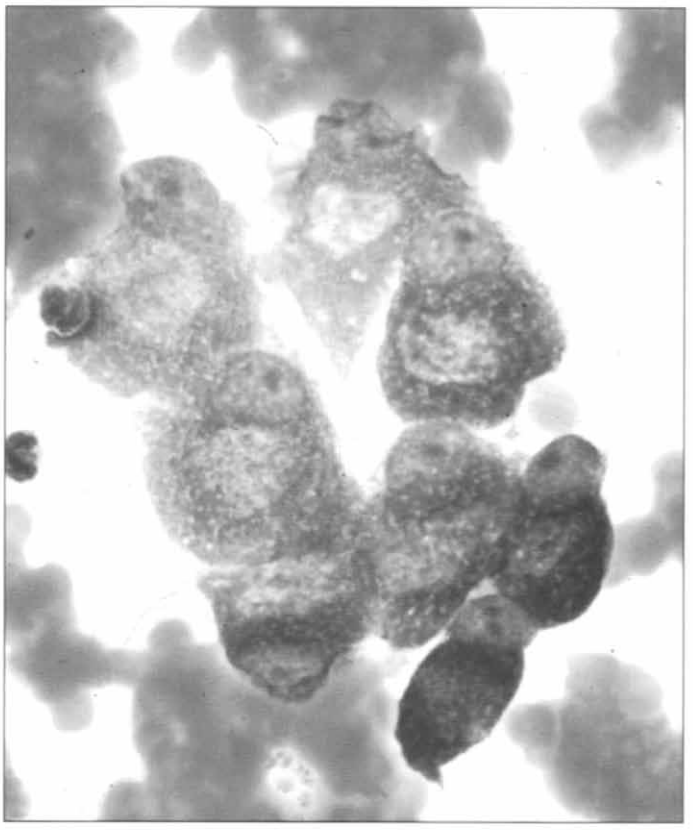

Figure 1.2 Active osteoblasts in a cytological preparation showing the characteristic eccentric nucleus, basophilic cytoplasm and prominent Golgi zone.

osteoprogenitor cells of mesenchymal origin, which are present in bone as well as other tissues, and which can be induced to differentiate into osteoblasts under the influence of appropriate paracrine and autocrine stimuli. Osteoclasts are derived from hematopoietic stem cells, probably of the monocyte series.

Osteoblasts *are responsible for manufacturing osteoid*, the organic component of bone matrix. Active osteoblasts, which line surfaces where bone formation is occurring, have abundant rough endoplasmic reticulum and a prominent Golgi apparatus, reflecting their role in protein synthesis. Histologically they appear as plump, cuboidal cells with basophilic cytoplasm, their nuclei sometimes being polarized away from the adjacent bone surface (Fig. 1.1). The typical morphologic features of osteoblasts are often more easily appreciated in cytologic preparations (Fig. 1.2). Not only do osteoblasts produce the osteoid, *they play a role in initiating its mineralization*, although the mechanism is not fully understood. The membranes of osteoblasts are rich in alkaline phosphatase, which appears to be involved in mineralization of both osteoid and cartilage. The high level of alkaline phosphatase activity measured normally in the serum of rapidly growing young animals presumably reflects the intense osteoblastic activity that is occurring in the developing skeleton. There is mounting evidence that the osteoblast is the central cell through which bone resorption and formation are mediated. In addition to osteoid, they produce an array of *regulatory factors* that are deposited in bone matrix and which play a critical role in bone remodeling.

Inactive osteoblasts, or **bone-lining cells**, are flattened cells with few organelles that cover endosteal bone surfaces that are undergoing neither formation nor resorption. Although barely visible in histological sections, these are the most abundant cells on the endosteal surface of the adult skeleton and link with each other to *form a functional barrier* between the extracellular fluid compartment of bone tissue and that of surrounding tissues. Bone-lining cells are believed to be involved in calcium homeostasis by regulating the exchange of calcium and other ions, such as sodium and magnesium, between bone fluid and extracellular fluid, under the influence of parathyroid hormone. It is likely that bone-lining cells can produce osteoid and contribute to bone deposition should the demand arise at a site of previous inactivity. Endosteal osteoprogenitor cells may be residents of this population of bone-lining cells.

During active bone formation, about 10–20% of osteoblasts at regular intervals along a bone-forming surface surround themselves with osteoid and become **osteocytes** (see Fig. 1.1). *These are the most abundant cells of bone tissue, residing in small spaces (lacunae) within the mineralized matrix*. Newly formed osteocytes retain some morphological and functional characteristics of osteoblasts, but as they mature and become embedded deeper in the mineralized matrix, the amount of rough endoplasmic reticulum in their cytoplasm is reduced considerably and they develop features more typical of phagocytic cells. Osteocytes maintain contact with adjacent osteocytes, and with bone-lining cells or osteoblasts on the surface, by a

network of branching cytoplasmic processes extending through *canaliculi*. In routinely stained histological sections, the canaliculi are not visible and only the nuclei of osteocytes are usually apparent.

Osteocytes are thought to be capable of producing and resorbing bone in their immediate vicinity, thereby decreasing or increasing the size of their lacunae. This process is known as *osteocytic osteolysis*. The perilacunar bone matrix is less heavily mineralized and more labile than that in other areas. The action of osteocytes on this perilacunar bone is believed to be important in regulating the concentration of calcium and other minerals in the bone fluid compartment. Because of the large surface area of mineralized bone exposed to either osteocytes or their canaliculi, significant quantities of calcium can be mobilized from this source very rapidly in response to the demands of calcium homeostasis. Although osteocytes can enlarge their lacunae by resorbing perilacunar bone matrix, this process of osteocytic osteolysis does not play a significant role in structural modifications of bones, or in the development of bone lesions associated with disease processes.

Osteocytes probably survive for several years in the mature skeleton but eventually die, leaving empty lacunae. The gradual loss of osteocytes is presumably a normal phenomenon, compensated by the construction of new bone as part of the remodeling process that occurs throughout life. In disease states in which bone necrosis is a feature, there is no immediate loss in structural integrity of the dead bone tissue, but efforts to remove the remaining mineralized matrix and replace it with new bone suggest that its function is impaired.

Osteoclasts *are primarily responsible for resorption of bone tissue.* They are probably related to monocytes and macrophages, which are capable of resorbing bone in vitro, but are sufficiently different histochemically to suggest that they may possess a distinct stem cell. Osteoclasts are rich in acid phosphatase and a range of other acid hydrolases, packaged in primary lysosomes. The acid phosphatase isoenzyme present in osteoclasts is tartrate-resistant, unlike the tartrate-sensitive acid phosphatase found in monocytes and macrophages. Osteoclasts are easily recognizable histologically as large, multinucleated cells with eosinophilic cytoplasm, typically situated on bone surfaces and often within shallow pits called **Howship's lacunae**. The presence of Howship's lacunae on a bone surface is convincing evidence of previous resorption at that site, even if no osteoclasts are present at the time of observation. Although not always apparent histologically, osteoclasts involved in active bone resorption have a highly specialized "ruffled" or brush border contiguous with the bone surface (Fig. 1.3). A clear zone adjacent to the brush border is free of organelles but contains actin-like filaments, which may assist in anchoring the cell to the bone matrix. This attachment of active osteoclasts to the bone surface is an essential requirement for resorption to occur, as is the activity of a specific intracellular, membrane-bound tyrosine kinase. Deletion of the gene coding for this enzyme in mice has been shown to induce osteopetrosis, a disease characterized by defective osteoclastic activity.

During **osteoclastic bone resorption**, an acid environment is created in the narrow space between the cell and the bone surface. Hydrogen and bicarbonate ions are generated from carbon dioxide and water by the action of carbonic anhydrase II on the brush border membrane of osteoclasts. An ATP-mediated proton pump, also located on the brush border membrane, actively transfers hydrogen ions into the extracellular space. The acidity of the local environment not only induces demineralization of the bone, it enhances the activity of the acid hydrolases released from osteoclasts when primary lysosomes fuse with the cell membrane of the brush border. Fragments of degraded matrix are endocytosed by osteoclasts and further digested within secondary lysosomes.

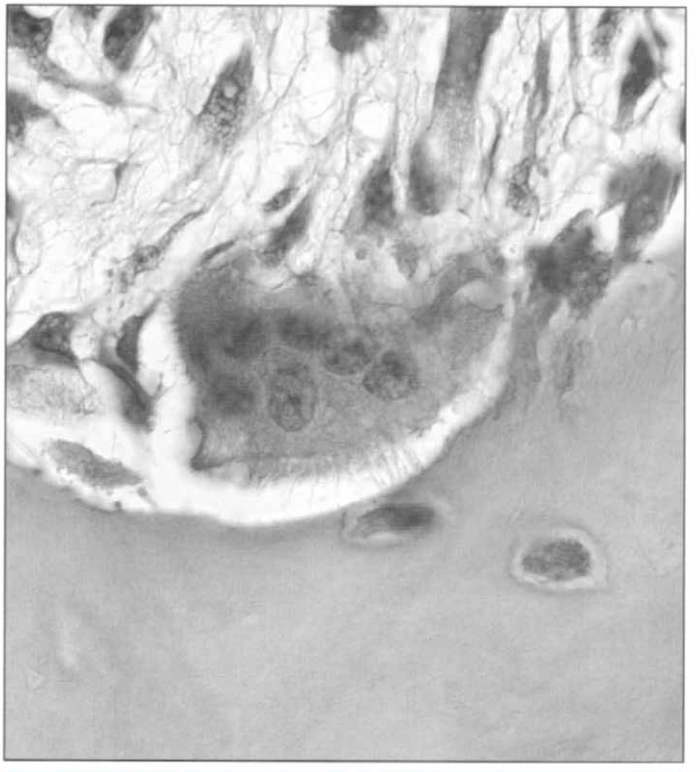

Figure 1.3 Multinucleated osteoclast in a shallow pit (Howship's lacuna) on a bone surface undergoing resorption. Note the ruffled border of the osteoclast adjacent to the bone.

The potential rate of removal of bone by osteoclasts is much greater than the rate of formation by osteoblasts. An individual osteoclast can erode approximately 400 μm^3 of bone, and travel 100 μm across a bone surface, per hour. As a result, localized or generalized removal of bone during the normal physiological processes of modeling and remodeling, or in disease states, can occur very rapidly.

Once osteoclasts have completed their required phase of resorption, they most likely undergo apoptosis and disappear from resorption sites. This is characterized by condensation of nuclear chromatin, loss of the ruffled border and detachment from the bone surface. Drugs that inhibit bone resorption have been shown to induce osteoclast apoptosis both in vitro and in vivo. Importantly, parathyroid hormone promotes the survival of osteoclasts and in cases of either nutritional or renal hyperparathyroidism, surviving osteoclasts are found in medullary spaces mixed with fibroblastic elements. The abnormal persistence of osteoclasts in these sites is an important aid to the diagnosis of these conditions.

Bone matrix

Bone matrix consists of an *organic component, called* **osteoid**, *and an inorganic component comprised predominantly of hydroxyapatite crystals.* The main constituent of osteoid (approximately 90%) is **type I collagen**, which is also the predominant form of collagen in tendons,

ligaments, dentine, and the ocular sclera. Each collagen molecule consists of three polypeptide chains assembled into a triple helix, which is a highly stable configuration, resistant to proteolytic degradation, and which forms the basic unit of all collagenous structures. The strength of bone and other collagenous structures is due in part to the manner in which individual collagen molecules are aggregated into fibrils, with each fibril overlapping its neighbor by about one quarter of its length. This creates a characteristic banding pattern, clearly evident on transmission electron microscopy. The tensile strength of collagenous structures is further enhanced by intermolecular cross-links, which form by the oxidative deamination of either lysyl or hydroxylysyl residues under the influence of the copper-dependent enzyme lysyl oxidase. The number of these cross-links in bone collagen is greater than that of the collagen types found in soft tissues. Interference with the formation of cross-links, as occurs in copper deficiency or certain toxicity diseases (see below), may significantly alter the mechanical properties of bone and other connective tissues.

Several *noncollagenous proteins* are also produced by osteoblasts and form part of the organic matrix of bone. The most abundant of these is **osteonectin**, a phosphoprotein that interacts with both type I collagen and hydroxyapatite, and has been shown to facilitate the mineralization of type I collagen in vitro. Osteonectin concentration is highest in mature bone, especially in areas with the highest degree of mineralization. **Osteocalcin**, also referred to as *bone Gla protein* because of its γ-carboxyglutamic acid (Gla) residues, is also abundant in bone, accounting for up to 10% of total noncollagenous proteins. Its synthesis by osteoblasts is vitamin K-dependent and is stimulated by 1,25 dihydroxyvitamin D. Osteocalcin is deposited in osteoid shortly before mineralization and binds strongly to calcium ions and to hydroxyapatite, suggesting that it is important in the mineralization process. There is also evidence to suggest that it may be involved in the recruitment of osteoclasts to sites of bone resorption or remodeling. Interestingly, depletion of osteocalcin concentration to less than 1% of normal in rats fed warfarin is not accompanied by a reduction in the mechanical strength of bone. Osteocalcin is also found in plasma, where it serves as a marker for osteoblastic activity. A second Gla-containing protein, *matrix Gla protein*, occurs in bone as well as several other tissues. Its function is not known but it appears early in skeletal development when osteocalcin levels are still low, suggesting a possible role in bone development.

The **proteoglycans** of bone matrix are considerably smaller and less abundant than those found in cartilage matrix, possessing a relatively small protein core and only one or two glycosaminoglycan (chondroitin sulfate) side-chains. The bone proteoglycans are concentrated near the mineralization front where they are believed to play a *key role in the organization and mineralization of the matrix*. During this process, the protein core is degraded, leaving the chondroitin sulfate side-chains, which persist in the mineralized matrix of bone.

Several other noncollagenous proteins have been detected in bone matrix, including *osteopontin*, a sialoprotein that binds strongly to hydroxyapatite, and many other glycoproteins and phosphoproteins whose functions are unknown.

Bone matrix also contains a *variety of growth factors* that are capable of inducing mitogenic responses in a range of cell types, including bone cells. These factors, which probably play an important role in bone development, modeling and remodeling, especially at the local level, include: bone morphogenetic proteins, fibroblast growth factors, platelet-derived growth factors, insulin-like growth factors, and transforming growth factors β.

The **inorganic (mineral) component** of bone matrix is known to consist largely of **hydroxyapatite** [$Ca_{10}(PO_4)_6(OH)_2$], but its structure and properties are poorly understood. In addition to calcium and phosphate, bone mineral contains considerable quantities of *carbonate, magnesium, sodium and zinc*, not all of which are available for exchange. *Fluoride* is also present in small amounts in bone matrix. Ultrastructurally, hydroxyapatite is present in bone matrix either as thin, needle-like crystals oriented in the same direction as collagen fibrils, or as an amorphous, granular phase, depending on the type of bone.

Matrix mineralization

The mineralization of skeletal tissues is a highly complex process, and is only partly understood. In organ systems throughout the body, extracellular tissue fluids in equilibrium with plasma are supersaturated with respect to hydroxyapatite. Many also contain type I collagen similar to that in bone, but mineralization does not normally occur. This is most likely due to the presence of potent inhibitors, which must be enzymatically degraded before mineralization can be initiated. In bone, the selective and localized degradation of such inhibitors, and the synthesis by osteoblasts of unique molecules that promote mineralization, could account for the orderly manner in which mineral deposition occurs in this tissue. However, the presence of substrates that promote nucleation at humoral solute concentrations is also required.

There is no doubt that **matrix vesicles**, *tiny extracellular organelles originating as cytoplasmic blebs from osteoblasts, chondrocytes and odontoblasts*, play an important role in initiating the mineralization process, particularly in cartilage undergoing endochondral ossification. These vesicles are rich in calcium-binding phospholipids and proteins, alkaline phosphatase, pyrophosphatases, phospholipase A_2 and in metalloproteinases that degrade proteoglycans, potential inhibitors of mineralization. Although the mechanism is still uncertain, the initial nucleation of hydroxyapatite crystals occurs on the inner surface of matrix vesicle membranes, at least in mineralizing cartilage matrix. Phospholipids in vesicles are believed to sequester calcium, while pyrophosphatases and metalloproteinases inactivate local inhibitors and alkaline phosphatase generates phosphates, allowing mineralization to proceed.

Although mineral deposition in some tissues such as growth plate cartilage appears to be mediated almost exclusively by matrix vesicles, this is not the case for bone matrix. Unlike cartilage, where mineralization of collagen fibrils does not occur, hydroxyapatite crystals are deposited in the type I collagen fibrils of bone. Evidence suggests however that collagen fibrils alone are not capable of initiating primary nucleation. The adsorption to collagen of bone-specific noncollagenous proteins such as osteonectin and osteocalcin, both of which are strong binders of Ca^{2+} ions, may create appropriate sites for nucleation. Once initiated, the mineral spreads in an orderly manner throughout collagen fibers until the entire aqueous space of the fiber is filled with hydroxyapatite crystals. The mineralization of individual fibers occurs rapidly, as evidenced by the sharp division between highly and sparsely mineralized matrix at the junction between mineralized bone and osteoid seams.

Figure 1.4 Transverse (slightly oblique) section of cortical bone viewed under polarized light to show the **osteons or Haversian systems**, which consist of concentric lamellae of bone surrounding a central vascular canal.

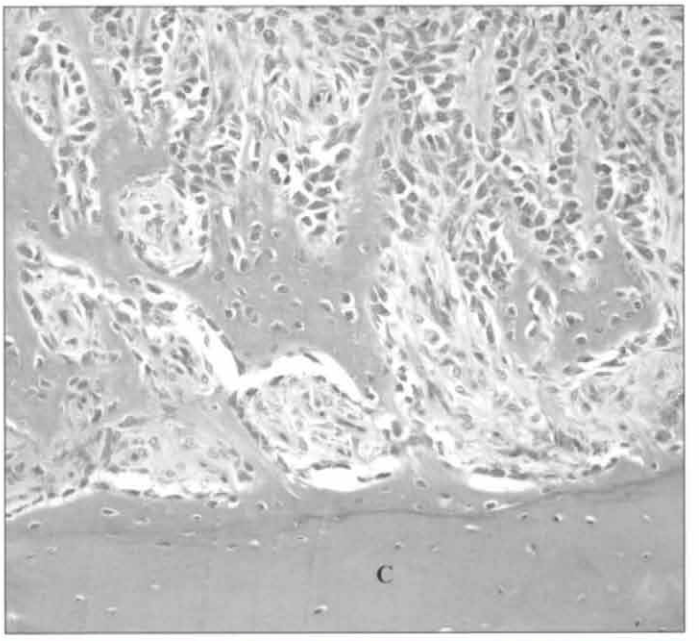

Figure 1.5 Trabeculae of **woven bone** emerging from the cortex (C) beneath an elevated periosteum. The osteocytes in the woven bone are more numerous than in the mature lamellar bone of the cortex and are irregularly distributed. The matrix of the woven bone is slightly more basophilic than that of the mature bone.

Osteoid does not become mineralized for 5–10 days after deposition. As a result, *a thin layer of unmineralized osteoid*, the **osteoid seam**, covers the surfaces where bone is being formed. Although not always apparent histologically in decalcified tissue sections, the osteoid seam is usually more eosinophilic than previously mineralized bone tissue and, in lamellar bone, separated from it by *a basophilic line*, the **mineralization front**. The osteoid seam may be 5–15 μm in depth, depending on its rate of formation. Once mineralization of osteoid begins, it occurs very rapidly, with over 60% of the matrix becoming mineralized almost immediately. However, the remaining deposition of mineral is a slow cumulative process that can take weeks to complete.

Structural organization of bone tissue

Although the cellular elements of bone tissue, and the basic composition of the matrix, are relatively constant, there is variation in the organization of these components both at the macroscopic and microscopic level. The adult skeleton consists predominantly of mature **lamellar bone**, where the collagen fibers of the bone matrix are oriented in parallel layers. This pattern is clearly apparent in histological sections viewed under polarized light (Fig. 1.4). Osteocytes are present in small slit-like lacunae between layers in a regular pattern, their distribution reflecting the orderly manner in which osteoblasts manufacture lamellar bone. In dense cortical bone, the lamellae are organized into **osteons** or **Haversian systems**, consisting of concentric lamellae surrounding a central vascular canal (Fig. 1.4). *Osteons run longitudinally through the cortex and are cemented together by interstitial lamellae.* The trabecular or spongy bone of medullary cavities consists of variable numbers of lamellae arranged parallel to the surface rather than organized into osteons.

The alternating pattern of birefringent and nonbirefringent layers in lamellar bone has traditionally been interpreted as reflecting a 90° switch in orientation of collagen fibers between successive layers, creating a structure with physical strength similar to plywood. This model has remained unquestioned since the early 20th century, but has been challenged by recent studies using scanning electron microscopy. An alternative model proposes that lamellar bone consists of alternating layers of collagen-rich (dense) and collagen-poor (loose) lamellae, only the former showing birefringence.

A variant of lamellar bone is often seen on the weight-bearing aspects of long bones of rapidly growing animals, especially young ruminants. In these areas, the outer cortex is often arranged in laminar arrays rather than conventional Haversian systems, and is known as **laminar bone**.

In the developing fetus, and at sites of rapid bone formation during postnatal life, the *collagen fibers in bone matrix are arranged in a haphazard, interwoven fashion.* This immature form of bone tissue is referred to as **woven bone**, or coarse-bundle bone. Its matrix is more basophilic than that of lamellar bone and the osteocytes are larger, more numerous, and are irregularly arranged (Fig. 1.5). During skeletal maturation and remodeling, woven bone is resorbed and replaced with lamellar bone, which has greater strength, but it is seen in adults at sites where bone is produced rapidly in response to injury, inflammation or neoplasia. Fracture calluses invariably contain this form of bone tissue, as do bone-forming tumors.

A third type of bone, **chondroid bone**, arises directly from fibrocartilaginous origins and is found in ossifying tendon sheaths, of bone derived from neural crest origins and probably from some mixed tumors.

Formation of cranial bones

The pathology of the cranial bones often differs from bones elsewhere in the body, and some of the cranial bones may be spared in disorders that affect other bones. This is probably due to the complex origins of the cranial bones. The base of the cranium develops from a hyaline cartilage model that mineralizes and subsequently undergoes endochondral ossification. The remainder of the cranial bones and also the facial bones arise from mesenchyme directly by the process of intramembranous bone formation. Experimental studies using chimeric chick embryos and transgenic mice indicate that neural crest cells populate the sutures of membranous cranial bones, contribute to tooth morphogenesis and the formation of odontoblasts, dentine and cementum of teeth, and in the formation of the mandible and its cartilaginous articulation with the temporal bones. Chondroid bone that develops from neural crest cells populates the cranial bone sutures, and also forms as an intermediate step in the formation of intramembranous skull bones. Sutures remain as active sites of intramembranous bone production, and are probably the site of origin for the distinctive tumor that arises in the skull, the multilobular tumor of bone.

The embryology and development of the vertebral column is also complex, and in most vertebrae there are eight separate ossification centers. However, the atlas and axis differ in their structure and development.

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Regulation of bone formation and resorption

The regulation of bone cell function is accomplished by the action of a variety of systemic hormones and the local action of cytokines, which not only influence the recruitment and action of differentiated osteoblasts and osteoclasts, but also may stimulate the proliferation of their precursors. A list of systemic and local factors involved in bone remodeling is presented in Table 1.1.

Table 1.1 Factors controlling bone remodeling

Systemic factors	Local factors
Polypeptide hormones • Parathyroid hormone • Calcitonin • Insulin • Growth hormone	**Cytokines** • Interleukins (IL-1, IL-6, IL-11) • Insulin-like growth factors (IGF-I, IGF-II) • Transforming growth factors (TGF-β) • Bone morphogenetic proteins • Fibroblast growth factors • Platelet-derived growth factor • Tumor necrosis factors • Interferons • Colony stimulating factors (M-CSF, GM-CSF)
Steroid hormones • Vitamin D (1,25-dihydroxyvitamin D_3) • Sex hormones • Corticosteroids	**Prostaglandins** • Prostaglandin E2
Thyroid hormones	**Nitric oxide**

Systemic hormones known to have either direct or indirect effects on the skeleton include: parathyroid hormone, 1,25-dihydroxyvitamin D_3, calcitonin, glucocorticoids, growth hormone, insulin, sex steroids, and thyroid hormones. **Parathyroid hormone** (PTH) is a potent activator of osteoclastic bone resorption, but its action appears to be mediated through osteoblasts or bone-lining cells as PTH receptors have been identified on osteoblasts but not osteoclasts. Furthermore, in vitro studies have demonstrated that the activation of osteoclasts by PTH depends on the presence of osteoblasts. The TNF-family molecule RANK-L (receptor activator of NF-kappaB ligand) has recently been identified as an essential signal in osteoclast development, activation, and survival. RANK-L is highly expressed in areas of bone remodeling and at sites of pathological bone loss in a variety of disease states in man and animals.

Osteoclasts are poorly able to resorb unmineralized bone matrix and since a thin layer of osteoid covers all bone surfaces, except those undergoing resorption, this layer must be removed before resorption can be initiated. Osteoblasts also may be involved in this process through activation of a latent form of collagenase, the synthesis and secretion of which are enhanced by PTH. In addition to its effect on bone resorption, PTH has been shown experimentally both to inhibit and stimulate bone formation, depending on the duration of administration and the dose rate. High doses cause increased bone resorption leading to fibrous osteodystrophy. In contrast, low and intermittent doses of PTH that are too small to affect serum calcium concentration have been shown to promote bone formation and increase bone mineral density. This anabolic effect of PTH is mediated through inhibition of apoptosis of osteoblasts and osteocytes, thereby increasing their lifespan.

Like PTH, **1,25-dihydroxyvitamin D_3** ($1,25(OH)_2D_3$), *the active form of vitamin D_3, is a potent stimulator of osteoclastic bone resorption.* It promotes the differentiation and fusion of osteoclast progenitors and activates mature osteoclasts, probably by a mechanism similar to, but independent of, PTH. Receptors for $1,25(OH)_2D_3$ do not occur on osteoclasts but are present on osteoblasts, supporting the involvement of osteoblasts in mediating osteoclastic resorption triggered by $1,25(OH)_2D_3$. The effect of $1,25(OH)_2D_3$ on bone formation is complex, but it appears to be required for normal bone growth and mineralization. This effect on mineralization is partly related to maintaining adequate serum concentrations of calcium and phosphorus, but evidence for a direct effect of $1,25(OH)_2D_3$ on mineralization has been provided by experiments in mice and rats. The synthesis of osteocalcin by osteoblasts is stimulated by $1,25(OH)_2D_3$, but there is confusion over the exact role of this peptide hormone and other metabolites of vitamin D_3 on bone formation.

Calcitonin *has a direct, but transient, inhibitory effect on osteoclastic bone resorption* but does not appear to influence bone formation. Following exposure to calcitonin, active osteoclasts rapidly lose their ruffled border and become physically separated from the underlying bone surface. There is also an inhibitory effect on osteoclast formation through reduced proliferation of progenitor cells and reduced differentiation of committed precursors. Calcitonin is capable of inhibiting PTH-induced bone resorption in vitro, but the mechanism and significance of this action is unclear.

Insulin does not regulate bone resorption but *plays an important role in the synthesis of bone matrix, and in cartilage formation.* As such, it has a major influence on normal skeletal growth. Rather than influence bone cell replication, insulin stimulates bone matrix synthesis by differentiated osteoblasts. **Growth hormone** promotes longitudinal

growth of bones in immature individuals and appositional bone growth in adults, but its action is indirect, being mediated through insulin-like growth factors produced by the liver.

Glucocorticoids *have a significant catabolic effect on the skeleton* through actions on both osteoblasts and osteoclasts. There is direct inhibition of osteoblastic activity, thereby reducing bone formation, and an indirect enhancement of osteoclastic resorption. The latter is probably due to inhibition of enteric calcium absorption and impaired renal tubular calcium reabsorption, leading to reduced serum ionized calcium concentration and secondary hyperparathyroidism. Reduction in bone mass is a recognized sequel to long-term corticosteroid therapy both in human and animal patients.

Estrogens and **androgens** appear to be *important regulators of skeletal growth and maturation*. Androgen receptors are present on osteoblasts, but at low densities. During puberty, androgens stimulate bone growth, while in male adults they are involved in the maintenance of the skeleton. Androgens do not affect osteoclasts directly, but may inhibit bone resorption indirectly by inhibiting the recruitment of osteoclast precursors from bone marrow. A significant effect of estrogens appears to be to inhibit bone resorption. Immediately after menopause, a decline in circulating estrogen levels in some women is accompanied by a rapid acceleration in bone loss, leading to osteoporosis. Treatment of postmenopausal women with estrogen has been shown to significantly reduce the risk of osteoporosis-related fractures. Estrogen may inhibit bone resorption by reducing the synthesis of cytokines such as interleukin-6, although the exact mechanism is unknown.

Thyroid hormones are important for normal skeletal development, primarily through *stimulation of cartilage growth*, but they also stimulate bone resorption. In human patients, accelerated bone resorption in hyperthyroidism may result in hypercalcemia and increased risk of osteoporosis. Hypothyroidism during fetal development has been linked to congenital abnormalities of the skeleton in animals. Reduced longitudinal bone growth, delayed ossification and impaired bone resorption were reported in lambs following fetal thyroidectomy, while delayed ossification of carpal bones in newborn foals has been described in association with hyperplastic goiter.

In addition to the range of systemic hormones capable of influencing bone formation and resorption, several locally produced **growth regulatory factors** or **cytokines** with paracrine, autocrine, or juxtacrine functions have been identified. In fact, these factors are likely to be more important than the systemic hormones in initiating physiologic bone resorption and remodeling, which occurs in discrete localities throughout the skeleton. Many of these growth factors become stored in the matrix and their release in active form during future episodes of bone resorption may stimulate the differentiation and proliferation of osteoblast precursors. They can be grouped into distinct families based on their action and target cell, but there is considerable overlap and redundancy in their activities.

The **insulin-like growth factors** (IGF-I and IGF-II) are growth hormone-dependent polypeptides produced by several tissues, including bone. *They are known to have powerful systemic and local effects on bone formation and maintenance of bone mass*. IGF-I, which is more potent than IGF-II, stimulates the proliferation of osteoblast precursors and enhances matrix synthesis by differentiated osteoblasts. It also inhibits the degradation of bone collagen, most likely by inhibiting the expression of interstitial collagenase by osteoblasts. The synthesis of IGF-I is stimulated by PTH and other agents that stimulate cAMP in bone cells, but is inhibited by glucocorticoids. The reduced bone mass that occurs in association with glucocorticoid excess may be due in part to this inhibition of IGF-I.

Transforming growth factor-β (TGF-β), another family of polypeptide hormones produced both in bone and other tissues, *has powerful effects on both osteoclasts and osteoblasts, and probably plays a key role in bone remodeling*. During bone resorption, TGF-βs are released from the bone matrix in active form. They inhibit the proliferation and differentiation of osteoclast precursors, as well as the activity of mature osteoclasts. TGF-βs also stimulate the replication of osteoblast progenitors, increase collagen synthesis by differentiated osteoblasts and induce osteoblast chemotaxis in vitro.

Bone morphogenetic proteins (BMPs) are a *large subgroup of signaling molecules* within the TGF-β superfamily and have been the subject of intense research in recent years. Approximately 30 different BMPs with overlapping expression patterns have been identified. BMP-specific antagonists, such as noggin and chordin, have also been identified. BMPs appear early in embryogenesis and are involved in the induction of bone and cartilage development during organogenesis. They stimulate osteoblast differentiation and have a unique property of inducing heterotopic bone formation in vivo. This has led to considerable interest in the possible therapeutic use of BMPs in disease conditions where enhanced bone formation is desirable.

Acidic and basic **fibroblast growth factors** (FGFs) also have been shown to *stimulate bone formation* but, unlike TGF-βs, they do not influence bone resorption. FGFs probably generate additional osteoblast precursors, leading to increased numbers of differentiated osteoblasts, and are effective in stimulating new bone formation to restore bone mass. Abnormalities in FGF receptors have been identified in human patients with certain inherited skeletal disorders, including achondroplasia. **Platelet-derived growth factor** (PDGF) is a *potent stimulator of new bone formation, but also promotes bone resorption*.

Estrogen depletion induced by ovariectomy in a rat model markedly increases the synthesis of **interleukin-6** (IL-6) and **interleukin-11** (IL-11) by osteoblasts or their precursors in the bone marrow stroma. *These cytokines appear to play a crucial role in the recruitment, proliferation, and differentiation of osteoclast progenitors that eventually lead to reduced bone mass in estrogen deficiency*. Further support is derived from studies in mice following deletion of the IL-6 gene. Unlike normal controls, mice lacking the IL-6 gene do not show any reduction in bone mass after ovariectomy. **Interleukin-1** (IL-1) and **tumor necrosis factor-α** (TNF-α) are related cytokines that act synergistically on bone and also have been implicated in the pathogenesis of postmenopausal osteoporosis. Both are potent stimulators of osteoclastic activity in vitro and in vivo and may be involved in mediating focal bone resorption in certain inflammatory disorders. Unlike other cytokines, **γ-interferon** does not stimulate bone resorption. In fact, it selectively inhibits the resorption stimulated by IL-1 and TNF, probably through inhibition of prostaglandin synthesis. **Osteoprotegerin** (OPG), a newly described receptor-like protein, is a member of the TNF receptor family, and acts as a decoy receptor for RANK-L. Its major role appears to be in regulating bone remodeling through a negative effect on the maturation and activation of osteoclasts.

Several **colony-stimulating factors** (CSFs) influence bone resorption by regulating the proliferation and differentiation of osteoclast precursors. Their importance is highlighted by studies in

mice with the *op/op* variant of osteopetrosis, where there is impaired production of macrophage colony-stimulating factor (M-CSF or CSF-1). A decrease in osteoclast formation, leading to reduced bone resorption and osteopetrosis, can be reversed transiently by the administration of M-CSF. Granulocyte macrophage colony-stimulating factor (GM-CSF) can also increase osteoclast differentiation from their precursors, thereby promoting bone resorption. Osteoblasts are capable of secreting GM-CSF following exposure to bacterial endotoxin or PTH.

Prostaglandins (PGs), in particular those of the E series, are another group of *important mediators of local bone resorption*. They are characterized by restricted, local activity before being rapidly degraded, making studies of their in vivo action extremely difficult. Consequently, their precise role has not been determined. PGE_2 appears to directly stimulate osteoclastic activity and may be a mediator of bone resorption regulated by osteoblasts. Local production of PGE_2 in response to inflammation, mechanical trauma, or neoplasia is likely to contribute to the bone resorption that is often associated with such conditions. PGE_2 has also been linked to the bone resorption and hypercalcemia associated with certain malignancies.

Nitric oxide, generated by the nitric oxide synthase group of enzymes, may be an important mediator of bone cell function. In vitro studies have revealed a biphasic effect on bone resorption. At low concentrations, nitric oxide potentiates IL-1-induced osteoclastic bone resorption, suggesting that small amounts of nitric oxide may be required for normal osteoclast activity. In contrast, high concentrations of nitric oxide strongly inhibit bone resorption both in organ cultures and in cultures of isolated osteoclasts. This inhibitory effect appears to be due to apoptosis of osteoclast progenitors, induced by the action of nitric oxide derived from osteoblasts. The effect of nitric oxide on osteoblast function is less clear, although estrogen has been shown to stimulate nitric oxide synthase activity in both endothelial cells and osteoblasts, suggesting a possible role of nitric oxide in mediating the protective effects of estrogen on bone. At high concentrations, nitric oxide inhibits osteoblast growth and differentiation. This provides a possible explanation for the reduced bone formation and osteoporosis that occurs in association with certain inflammatory conditions. The role of nitric oxide in vivo has yet to be established, but there is increasing evidence to support its role as an important regulator of bone turnover.

Remodeling of bone tissue

Throughout postnatal life, old or defective bone is constantly being replaced with new bone tissue by a process of **remodeling**. This occurs at a local level and involves the coordinated activities of osteoclasts and osteoblasts in so-called **bone remodeling units**, presumably mediated by a complex interaction between systemic hormones and locally acting cytokines. The remodeling sequence starts with activation of osteoblasts (probably bone-lining cells) by external signals such as hormones, cytokines, or growth factors to induce accelerated formation of osteoclasts from their precursors. The osteoclasts attach to the bone surface, seal it off from the extracellular space, and resorb bone matrix and mineral. Then follows a reversal phase during which resorption ceases, the osteoclasts detach from the bone and disappear, probably by apoptosis. Osteoblasts are attracted by an unknown mechanism to the resorption site where they deposit new bone.

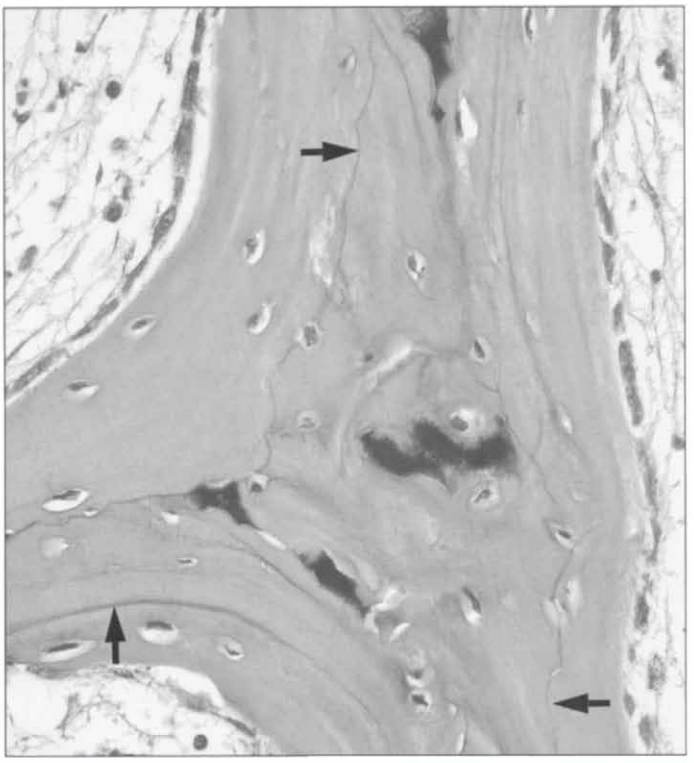

Figure 1.6 Cementing lines in a segment of trabecular bone. The smoothly contoured lines (vertical arrow) are referred to as **resting lines** and indicate sites at which bone formation had ceased for a period then recommenced. The scalloped lines (horizontal arrows) are **reversal lines** and reflect previous resorption, followed by deposition of new bone.

In the cortex, activated osteoclasts form a "cutting cone" which bores longitudinally through the dense, primary bone, creating a *resorption canal*. As the canal advances, it becomes lined by osteoblasts, which fill the space with concentric layers of new lamellar bone, creating a *secondary osteon or Haversian system*. This process provides a mechanism for on-going internal replacement of cortical bone without altering its gross form or function. Remodeling of trabecular bone follows a similar sequence, but from the trabecular surface, without the formation of resorption canals.

In histological sections, the separate units of secondary bone that form during remodeling can be distinguished from each other, and from adjacent primary bone, by the presence of deeply basophilic **cementing lines** (Fig. 1.6). These lines are created by the deposition of a thin layer of highly mineralized, collagen-free matrix at sites where bone resorption or formation ceases. Two types of cementing lines are recognized. Those with a scalloped appearance are termed **reversal lines**, and indicate a site where previous bone resorption had occurred, then new bone deposited in its place. Smoothly contoured cementing lines, or **resting lines**, mark sites where bone formation ceased for a period, then re-commenced. The number and pattern of cementing lines may provide useful information on the recent history of an area of bone, in particular regarding the rate of turnover.

Markers of bone remodeling

Although not used routinely in veterinary medicine, various **markers of bone remodeling** or turnover can be measured in

serum or urine and may add support to a clinical diagnosis, or be of value in research. *Serum alkaline phosphatase activity is a well-recognized indicator of osteoblastic activity*, increased levels occurring in diseases characterized by increased bone formation such as hyperparathyroidism. Its diagnostic value is limited however by the fact that high levels are also detected normally in rapidly growing young animals. Furthermore, other isoforms of alkaline phosphatase are commonly used as indicators of cholestatic liver disease in several species and hyperadrenocorticism in dogs. Another potentially useful indicator of osteoblastic activity is *serum osteocalcin*. Approximately 10–25% of the osteocalcin synthesized by osteoblasts escapes into the circulation and serum concentrations are proportional to the rate of osteoid synthesis.

Bone resorption, associated with increased osteoclastic activity, is reflected by increased serum activity of *tartrate-resistant acid phosphatase*, an enzyme released by osteoclasts during the degradation of bone matrix. Increased urinary concentration of *hydroxyproline* has long been considered a marker of bone resorption, but this amino acid is present in all types of collagen, not just the type I collagen of bone, and cannot therefore be considered specific. Recent interest has focused on measurement of urinary concentrations of the *pyridinium cross-links* that bind the nontriple helical portions of one collagen molecule to another. Of these molecules, hydroxylysyl pyridinoline is the most abundant, but is less specific for type I collagen than deoxypyridinoline. Both can be detected in urine as either free or peptide-bound forms using commercially available kits.

In dogs, horses, rats and humans, considerable diurnal variations in the serum and urinary concentrations of bone markers have been demonstrated. This may reflect circadian rhythms in the rates of bone formation and resorption. There has been increasing interest in such markers of bone resorption in human medicine as indicators of post-menopausal bone loss or monitoring the effectiveness of antiresorptive therapy, but their application in animals is still largely confined to research.

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Ectopic mineralization and ossification

Extraskeletal deposition of either amorphous calcium phosphate or hydroxyapatite crystals, referred to as **ectopic** or **heterotopic mineralization** (calcification), occurs in a range of disease conditions, and must be differentiated from **ectopic ossification**, where the mineral is deposited in the form of bone tissue.

Three main forms of ectopic mineralization are recognized in animals: metastatic, dystrophic, and idiopathic. **Metastatic mineralization** is a frequent complication of disease conditions associated with hypercalcemia and/or hyperphosphatemia, such as vitamin D toxicity, hypercalcemia of malignancy, primary hyperparathyroidism and uremia. In dogs, direct precipitation of mineral occurs when the *calcium-phosphate solubility product*, expressed in mg/dL, persistently exceeds 60. The mineral deposited in metastatic mineralization typically occurs in the outer medullary region of the kidney, the fundus of the stomach, and the lungs, all of which are involved in acid secretion and possess a local alkaline environment favoring mineral precipitation. Other favored sites include the media of large arteries and the elastic tissue of the endocardium, but the reason for such predilection is not clear.

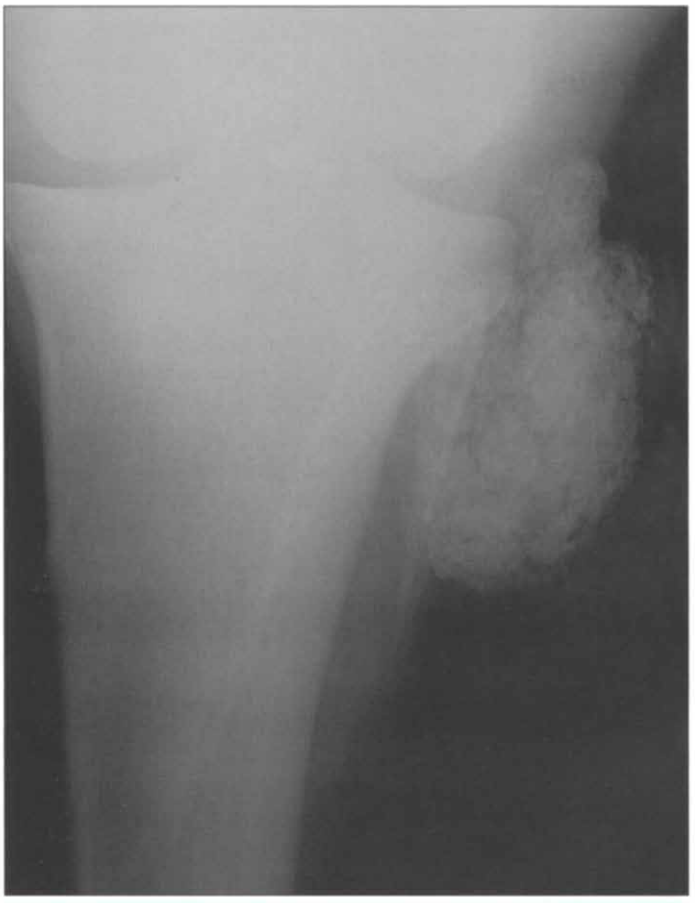

Figure 1.7 Tumoral calcinosis in a horse. A large, discrete mass lateral to the femoro-tibial joint contains multiple radiodense deposits.

Dystrophic mineralization occurs in association with *damaged tissue* rather than an elevated calcium-phosphate solubility product, probably due to the release or establishment of cellular components with nucleation properties following injury. Examples include mineral deposition in the caseous lesions of bovine tuberculosis and caprine paratuberculosis, and the mineralization of muscle fibers in animals with nutritional myodegeneration.

Idiopathic forms of ectopic mineralization occur in a variety of unrelated disease syndromes, including **calcinosis cutis**, which is characterized by widespread mineralization of dermal elastic fibers in association with hyperadrenocorticism, and **tumoral calcinosis** (*calcinosis circumscripta*), where tumor-like nodules of ectopic mineralization develop in soft tissues, often close to joints. Tumoral calcinosis in horses usually occurs in animals about 2–4 years of age, and 90% of lesions develop on the lateral aspect of the stifle (Fig. 1.7). The lesions may be single or multiple and are sometimes attached to the joint capsule, but not to the overlying skin. They usually appear as hard, well-circumscribed, nonpainful, subcutaneous swellings ranging in size from about 3–12 cm in diameter. Individual lesions consist of a tough, outer, fibrous capsule with collagenous trabeculae dividing the interior into numerous variable-sized locules consisting of finely granular, chalky white accumulations of calcium salts, surrounded by a rim of granulomatous inflammation cells. The lesions usually do not cause lameness and do not recur after surgical removal. Tumoral calcinosis in humans is also characterized by deposition of mineral in locular masses adjacent to major joints, particularly the shoulder and hip. Autosomal recessive inheritance is suggested to account for approximately one third of human cases, and hyperphosphatemia is often present, but the pathogenesis is unknown.

In dogs, the manifestations of calcinosis circumscripta are variable, and show breed preferences. In German Shepherds, the lesions are generally solitary, occurring most often in the skin near pressure points, but multiple lesions occur in some dogs, often attached to tendons, joint capsules, or the periosteum. Involvement of the vertebral processes of C-4 and C-5 was reported in three 5–6-month-old Great Dane puppies from the same litter, suggesting the possibility of a familial etiology. The lesions are usually nonpainful and nonprogressive, but may cause clinical signs if they interfere with the function of adjacent structures. For example, spinal cord compression caused by paravertebral calcinosis circumscripta occurred in two young dogs of different breeds. Although the pathogenesis is not known, repetitive trauma has been suggested as a predisposing factor, at least in some cases with skin involvement. Trauma is less likely to be responsible for lesions associated with vertebrae or involving the tongue, as has been reported.

A syndrome characterized by calcium hydroxyapatite deposition in the spinal canal of young Great Dane puppies has been reported to cause paraplegia and incoordination. Mineral deposition may also be present in joints and soft tissues of affected pups. In one report, the condition occurred in two litters of related Great Danes and was considered familial.

Ectopic ossification refers to the *formation of nonneoplastic trabecular bone in extraosseous sites*, presumably following the induction of pluripotential stem cells by appropriate growth factors. In most cases, ectopic bone is detected as an incidental finding at necropsy without an obvious predisposing cause. Typical sites include the pulmonary connective tissue of dogs and cattle, and the cervical and lumbosacral dura mater of aged dogs (ossifying pachymeningitis). Ectopic ossification also occurs in the supporting connective tissue of certain tumors in dogs, particularly mammary carcinomas, where it is very common, and to a lesser extent in thyroid and salivary carcinomas.

Specific disease entities associated with widespread ossification of soft tissues have been described both in human patients and animals and may involve overexpression or dysregulation of bone morphogenetic proteins. The temptation with such diseases in animals is to classify them according to the human syndrome that they most closely resemble. Such comparisons are usually subjective and may not be appropriate in all cases. A syndrome resembling human **fibrodysplasia ossificans progressiva** has been described in several cats and occasionally in the dog, although some of these cases more closely resemble myositis ossificans. In cats, the disease is characterized by *progressive, symmetrical, hyperplasia and ossification of connective tissue in the subcutis and epimysium of the neck, dorsum and limbs*. Affected cats have ranged in age from 5 months to 6 years. A feature of the disease in humans, and in some reported cases in animals, is the formation of bone in soft tissue sites by the process of endochondral ossification. In the 5-month-old cat referred to above, there was massive thickening of joint capsules and synovial membranes of several limb joints with disorganized hyaline cartilage (Fig. 1.8A, B). There was bilateral involvement of carpal and stifle joints in addition to several metatarsophalangeal and interphalangeal joints. Many foci of endochondral ossification were scattered throughout the hyperplastic

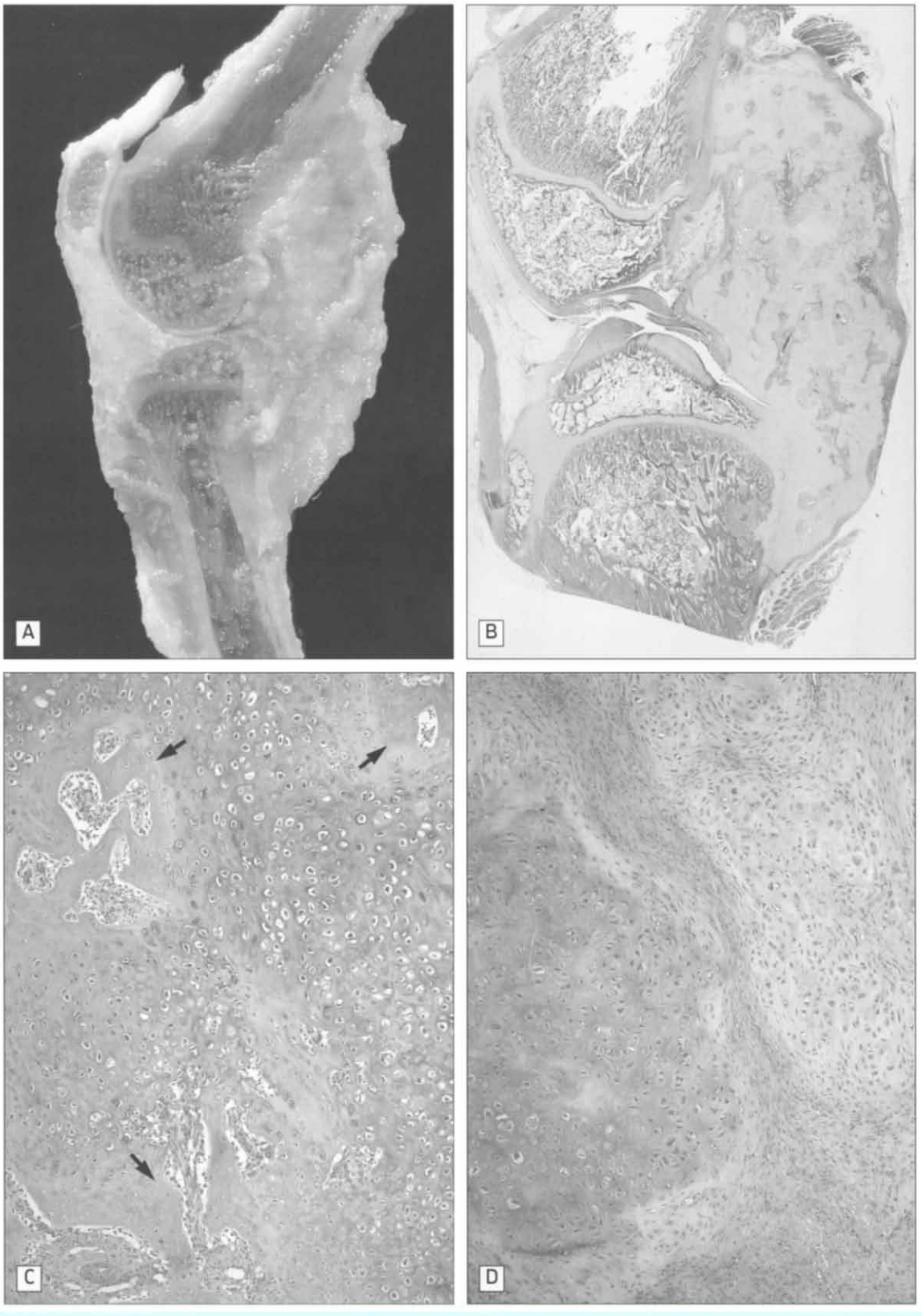

Figure 1.8 Fibrodysplasia ossificans progressiva in a 5-month-old kitten. **A.** Left stifle joint showing massive thickening of the joint capsule on the caudal aspect of the joint with tissue resembling cartilage. Similar lesions involved the right stifle, and several other limb joints. **B.** Subgross preparation of the same lesion, showing a cartilaginous mass bridging the stifle joint and attaching to the cortex of the adjacent femur and tibia in the region of the capsular insertions. **C.** Microscopically, the lesion consists of disorganized hyaline cartilage containing many plump chondrocytes and undergoing multifocal endochondral ossification (arrows). **D.** Biopsy of an early lesion from the same kitten, showing proliferating nodules of disorganized hyaline cartilage with features suggestive of chondrosarcoma.

cartilage, which, in some areas, had microscopic features suggestive of chondrosarcoma (Fig. 1.8C, D). The disease in man is associated with overexpression of at least one of the BMPs and is inherited as an autosomal dominant trait. Although there is no evidence of a genetic etiology in cats, there have been too few reported cases for this to be established.

Myositis ossificans has also been reported in cats and in a dog, but differs from fibrodysplasia ossificans in being *localized and asymmetric*. The lesions characteristically possess a peripheral zone of orderly maturation from fibrous tissue to mineralized osteoid, which is gradually replaced by lamellar bone. The prognosis following surgical removal is very good. Trauma is considered the initiating factor in most human cases of myositis ossificans, and the same probably applies to animals. Ossification of a hematoma may be the mechanism in some cases.

Generalized forms of myositis ossificans have been reported in cats and pigs but may have been more appropriately classified as fibrodysplasia ossificans. The disease in pigs was familial, occurring at 2–6 months of age in 34 of 115 offspring of an affected boar, and was characterized by widespread ossification of soft tissues, especially around vertebrae. The boar had developed similar lesions at 9 months of age.

Ectopic bone may form in lesions that have been mineralized for an extended period, possibly due to metaplasia of cells involved in the initial process of mineralization. This may account for the disseminated foci of metaplastic bone that form occasionally in the skin of dogs with hyperadrenocorticism and calcinosis cutis. Alternatively, the cutaneous osseous metaplasia in such cases may be due to local production of specific growth factors, including TGF-β, FGFs, and BMPs, by inflammatory cells in the dermis. TGF-β has been found in large quantities in inflammatory disorders of the skin of human patients with osteoma cutis, a condition similar to that described in dogs, and has been associated with the differentiation of fibroblastic cells into osteoblasts.

Where ectopic bone forms in close association with or becomes attached to a bone, differentiation of the lesion from a fracture callus or a parosteal osteosarcoma may prove difficult. Depending on its stage of maturation, a fracture callus is likely to contain remnants of cartilage or chondro-osseous bone, while *ectopic bone is composed exclusively of trabecular bone*. In parosteal osteosarcoma, the spaces between trabeculae will be populated with mesenchymal cells showing features of malignancy, rather than a single layer of osteoblasts lined up along the surface of bone trabeculae.

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BONES AS ORGANS

Development and anatomy

There are two distinct processes by which bone formation occurs in the developing fetus. *Flat bones of the skull develop by* **intramembranous ossification**. Mesenchymal progenitor cells migrate from the neural crest to form condensations at specific, highly vascular, sites in the head region and some other flat bones, where they differentiate directly into osteoblasts and produce anastomosing trabeculae of woven bone. These centers of ossification expand by on-going osteoblastic differentiation of mesenchymal cells at the periphery and apposition of new bone on the surface of trabeculae, to form a plate. A fibrous layer, *the periosteum*, separates the developing membrane bone from adjacent tissues and controls its shape. Individual bones of the developing skull are separated by connective tissue **sutures** until growth ceases, at which time a bony union forms. With maturity, the woven bone is remodeled and replaced by lamellar bone. Intramembranous bone formation also occurs at the periosteal surfaces of all bones during growth.

Most bones of the skeleton, including those of the limbs, vertebral column, pelvis, and base of the skull, develop by **endochondral ossification**. In this process, condensations of primitive mesenchymal cells differentiate into chondrocytes and produce crude cartilage models of the adult bone destined to form at that site. An avascular fibrous layer, the perichondrium, surrounds each cartilage model. As expansion of the model continues by interstitial growth, chondrocytes near the center become hypertrophic, and the matrix undergoes mineralization. Meanwhile, the perichondrium becomes invaded with capillaries, converting it into a periosteum, and a narrow cuff of bone forms by intramembranous ossification around the mid-shaft region of the developing bone. Capillaries and osteoclasts then invade the hypertrophic cartilage from the periosteum and establish a vascular network. Preosteoblasts also enter with the invading capillaries and differentiate into osteoblasts, which deposit osteoid on remnants of the mineralized cartilage, creating a **primary ossification center**. This process of endochondral ossification continues as the chondrocytes at either end of the developing bone continue to proliferate and the model expands in length and width. Once the bone reaches a certain stage of development, **secondary ossification centers** appear at one or both ends (depending on the bone), and expand by endochondral ossification to form the **epiphyses** of long bones. As the epiphyses expand, they remain separated from the primary ossification center, now occupying the **diaphysis** and **metaphysis** of the developing bone, by the **physis** or **growth plate**. Limited growth in size of the epiphysis continues by endochondral ossification beneath

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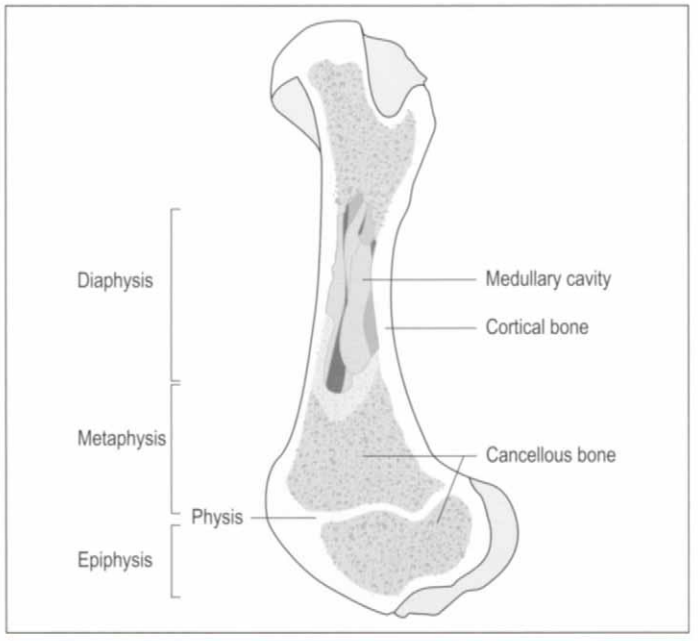

Figure 1.9 Drawing of the femur from a newborn calf, illustrating the **gross anatomy and terminology** of the different regions.

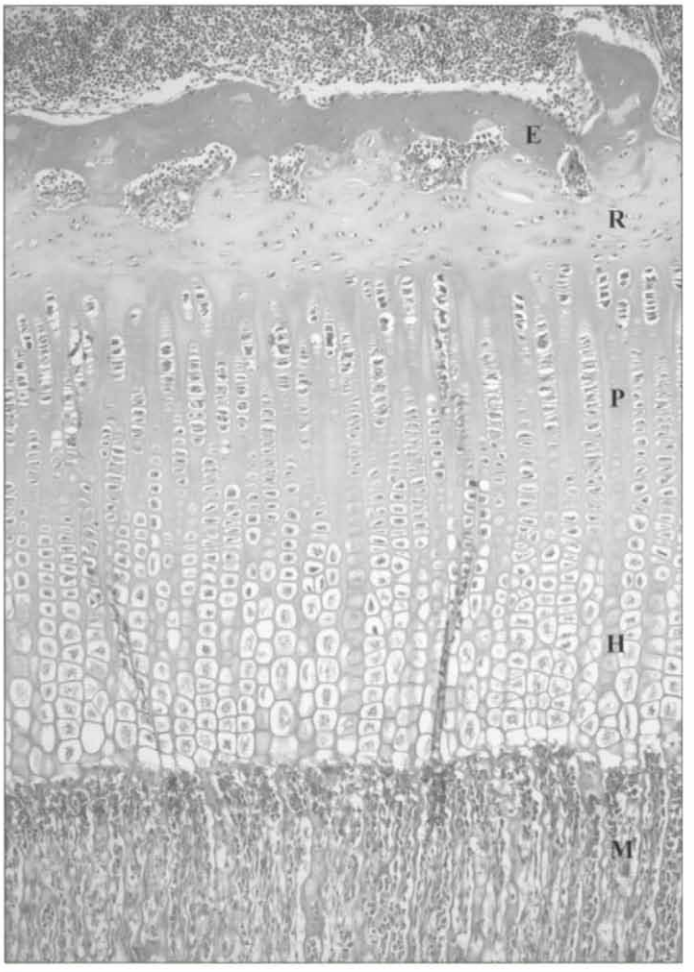

Figure 1.10 Physis or growth plate of a young animal showing the reserve (R), proliferative (P), and hypertrophic (H) zones. The reserve zone is anchored to trabecular bone of the epiphysis (E). Also note the abrupt transition from the hypertrophic zone of the physis to the metaphysis (M).

the articular cartilage at the articular–epiphyseal cartilage complex. The epiphyseal side of the growth plate soon becomes capped by a layer of trabecular bone, preventing further growth from that side, but proliferation of chondrocytes in the growth plate and endochondral ossification on the metaphyseal side continues until maturity. The gross anatomy and terminology of a developing long bone, in this case the femur of a newborn calf, is illustrated in Figure 1.9.

During active bone growth, *the hyaline cartilage of the growth plate is organized into three easily recognizable zones* (Fig. 1.10). A **reserve**, or **resting zone**, with irregularly dispersed chondrocytes and pale-staining matrix, is anchored to the trabecular bone of the epiphysis. The chondrocytes in this zone have the lowest concentration of intracellular ionized calcium, but the matrix has the highest concentration of type-II collagen. In the **proliferative zone**, the chondrocytes are tightly packed into longitudinal columns, the cell at the top being the progenitor cell for longitudinal growth of each column. The chondrocytes in this zone are actively dividing, accumulating glycogen, and synthesizing matrix proteoglycans. The columns of chondrocytes are separated by deeply basophilic cartilage matrix rich in aggregated proteoglycans, which inhibit mineralization in spite of the presence of matrix vesicles. Within columns, only thin matrix septa separate individual chondrocytes. The chondrocytes of the **hypertrophic zone** become enlarged, but remain metabolically active and are responsible for preparing the matrix for mineralization. They rely on anaerobic glycolysis for energy production because of the distance from epiphyseal blood vessels, which terminate at the top of the proliferative zone, and the inability of oxygen to diffuse from the metaphysis through the mineralized matrix of the lower hypertrophic zone. The energy is used primarily in the accumulation, storage, then release of calcium as part of the mineralization process. The lower region of the hypertrophic zone is commonly referred to as the *zone of degeneration*, as the chondrocytes appear to have separated from the pericellular matrix and become degenerate in sections prepared for histology and electron microscopy by routine methods. Following the development of more sophisticated techniques for preparing sections of cartilage for transmission electron microscopy, this concept has been challenged. Rather than being degenerate, these chondrocytes appear to be highly differentiated cells capable of synthesizing type-X collagen, chondrocalcin and other macromolecules that, together with matrix vesicles, are likely to be involved in initiating matrix mineralization. Mineralization of the cartilage matrix occurs in the deepest layer of the hypertrophic zone and is an essential event in the process of endochondral ossification. This mineralized layer is not evident in histological sections prepared after demineralization. The transition from growth plate to metaphysis is abrupt, and is designated by the last intact layer of chondrocytes.

Around the perimeter of the growth plate there is a wedge-shaped groove of cells, termed the **ossification groove of Ranvier**. The cells in this groove proliferate and are responsible for increasing the diameter of the physis during growth. A dense layer of fibrous tissue, the **perichondrial ring of LaCroix**, surrounds the groove of Ranvier and is continuous with the fibrous layer of the periosteum. As such, it provides strong mechanical support at the bone–cartilage junction of the growth plate, an area that is prone to injury in fast-growing young animals.

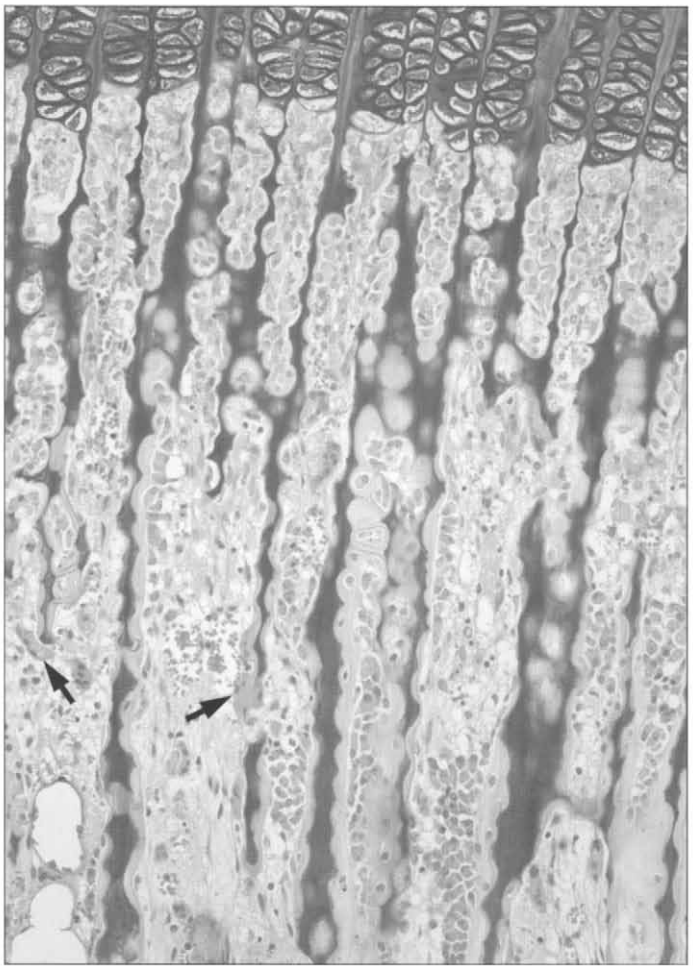

Figure 1.11 Primary spongiosa in a rapidly growing young animal. Basophilic spicules of calcified cartilage matrix extending into the metaphysis at right angles to the growth plate, form a lattice on which osteoblasts are lining up and depositing osteoid. Osteoclasts (arrows) are resorbing some trabeculae from the medullary end.

From the metaphyseal side of the growth plate, osteoclasts attack the mineralized cartilage matrix and rapidly remove the delicate transverse septa between individual chondrocytes within columns, allowing vascular invasion. The thicker longitudinal septa of mineralized cartilage matrix between columns of chondrocytes are not resorbed at this stage. Instead, they provide a framework on which newly differentiated osteoblasts line up and deposit a layer of woven bone (Fig. 1.11). *This lattice of trabeculae, with a basophilic core of mineralized cartilage covered by a thin, eosinophilic layer of bone is termed the* **primary spongiosa**. Trabeculae of the primary spongiosa extend at right angles to the direction of the growth plate, but deeper in the metaphysis, the trabeculae are remodeled by the coordinated action of osteoclasts and osteoblasts and are realigned in directions most suited to withstanding the mechanical forces acting on the bone. During this process, the cartilage cores and woven bone of the primary spongiosa are largely removed and replaced by thicker trabeculae of lamellar bone, which form the **secondary spongiosa**. While growth in length of a bone is continuing from the growth plate, osteoclastic resorption of trabeculae occurs at the metaphyseal–diaphyseal junction in order to *create the medullary cavity*.

The thickness of a growth plate is relatively constant across the width of the bone and is proportional to its rate of growth. So too is the distance to which trabeculae of the primary spongiosa extend into the metaphysis before they are remodeled. As growth slows, the different layers within the growth plate become narrow, and a transverse layer of trabecular bone forms on the metaphyseal side. The cartilage of the growth plate is then replaced with a bony scar, which is gradually remodeled into trabecular bone, blurring the margin between the epiphysis and metaphysis. The timing of growth plate closure varies both between and within bones and is controlled to a large degree by androgens and estrogens, but it is likely that nutritional factors can also play a role. In the radius, the distal growth plate remains open longer, and contributes significantly more to the length of the bone, than the proximal growth plate. In the humerus, femur, and tibia, the opposite is true. *The fastest-growing growth plates are the ones that are most likely to suffer damage due to trauma or nutritional imbalances*, and are therefore worthwhile sites to examine at necropsy, and to sample for histopathology.

The growth in width of the diaphysis in young animals occurs by intramembranous ossification beneath the **periosteum**, *which covers the surface of bones except at their articular ends and at insertion points of muscles and tendons*. The periosteum has a tough outer fibrous layer and a more cellular inner layer, *the cambium*, which contributes preosteoblasts for new bone formation (Fig. 1.12). Where muscle fibers and tendons insert onto bones, dense collagen fibers termed **Sharpey's fibers** become embedded in the bone matrix. The periosteum has a rich supply of nerve endings and blood vessels. *The inner bone surface is lined by a thin layer of osteogenic cells called the* **endosteum**.

Regulation of physeal growth

Many of the systemic hormones and local growth factors that regulate the formation and resorption of bone tissue also influence growth plate function. Their effect may be on a particular zone of the growth plate, and may vary with the age of the animal. **Growth hormone** and its mediators, the **insulin-like growth factors** (IGF-I and IGF-II), act throughout all zones of the growth plate, but **IGF** receptors are most abundant in the proliferative zone, implying a strong influence on cellular proliferation and hence longitudinal growth. **Thyroid hormones** are essential for cartilage growth and the maturation of chondrocytes, and appear to act synergistically with IGF-I. Deficiency of thyroid hormones leads to growth retardation and cretinism. **PTH** acts primarily on the proliferative and upper hypertrophic zones of the growth plate, and has a direct mitogenic effect on chondrocytes, as well as stimulating proteoglycan synthesis. It enhances the mitogenic effect of local growth factors. **Calcitonin** has been shown to accelerate chondrocyte maturation and matrix mineralization. The primary influence of **glucocorticoids** on the growth plate is to inhibit chondrocyte differentiation and proliferation. In young animals, excessive glucocorticoids from either endogenous or exogenous sources result in growth retardation. At physiological concentrations, **androgens** are anabolic factors, stimulating proteoglycan synthesis by chondrocytes in young animals, but they also stimulate mineralization in the growth plate. High doses of androgens actually depress growth and accelerate growth plate closure. **Estrogens**, in general, exhibit an inhibitory effect on longitudinal bone growth, excessive levels leading to premature closure of growth plates.

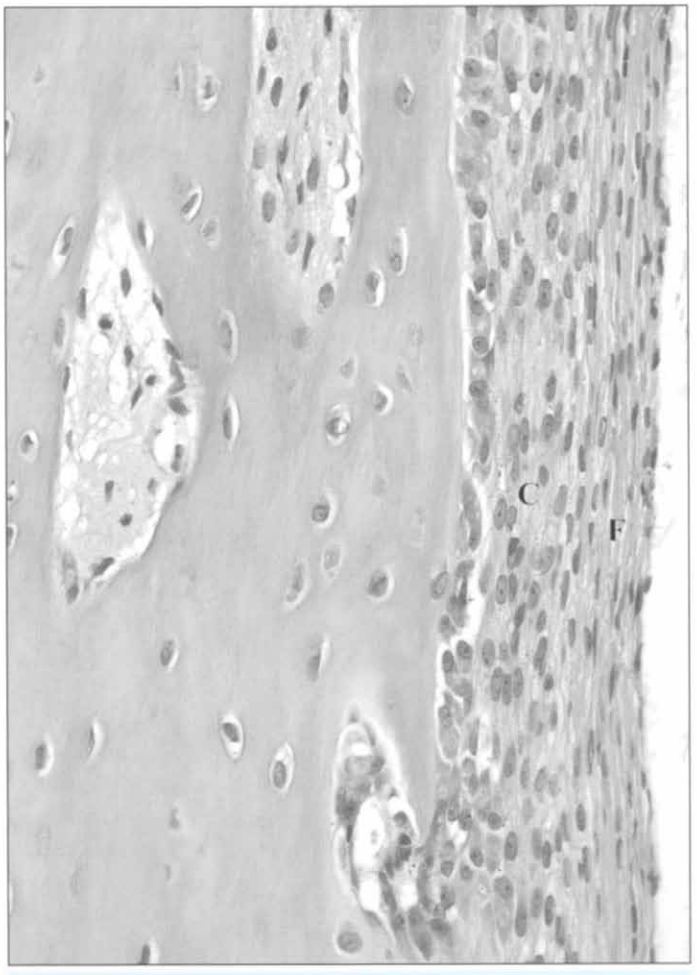

Figure 1.12 Periosteum in an actively growing young animal. Note the outer fibrous layer (F) and the cambium layer (C) containing primitive mesenchymal cells. A single layer of active osteoblasts lines the bone surface.

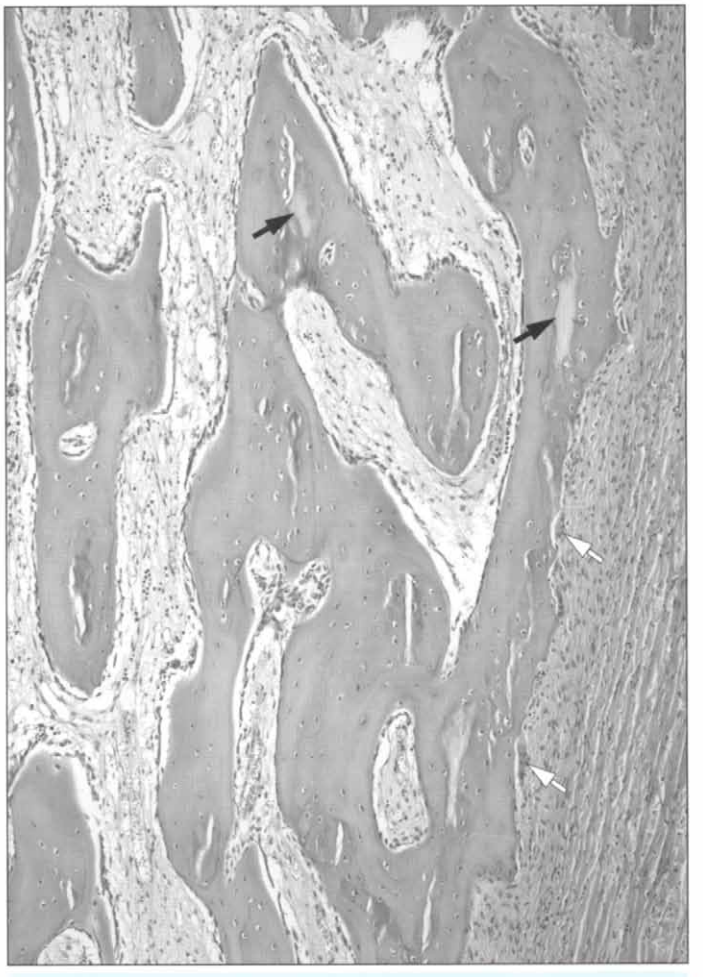

Figure 1.13 Architectural modeling in the metaphysis of a long bone during growth in length. In order to maintain the flare in the metaphyseal region, osteoclasts (white arrows) resorb bone at the periosteal surface, while osteoblasts actively deposit bone along trabeculae of the primary and secondary spongiosa. Trabecular bone of endochondral origin is thus converted into dense cortical bone. The spicules of mineralized cartilage (black arrows) derived from the growth plate persist until the cortex is remodeled.

Replication of chondrocytes is stimulated by several local growth factors, including **TGF-β**, **FGF**, and **epidermal growth factor** (EGF). TGF-β, in addition to its role as a regulator of bone formation, is also involved in the formation of cartilage, and has been shown to be a potent inhibitor of interleukin-1, which induces degradation of growth plate chondrocytes. **Prostaglandins** are present at low levels in the growth plate and although they have been shown to inhibit alkaline phosphatase activity and collagen synthesis while stimulating proteoglycan synthesis, their overall effect on growth plate function in unclear.

Modeling

In order to establish the unique shape of a long bone, extensive architectural **modeling** *occurs throughout the growth phase.* As the bone increases in size, the diameter of the diaphysis increases by deposition of new bone beneath the periosteum and resorption from the endosteal surface. However, growth in length is more complex and involves the coordinated actions of osteoclasts and osteoblasts operating on different bone surfaces. The diameter of most long bones is greatest at the level of the growth plate, then tapers through the metaphyseal region to its narrowest region in the diaphysis. This basic funnel shape is maintained during growth in length by continual osteoclastic resorption beneath the periosteum around the circumference of the metaphysis, thereby reducing its diameter. Meanwhile, osteoblasts rapidly deposit new bone within tunnels between the peripheral trabeculae of the primary and secondary spongiosa, converting it into dense cortical bone (Fig. 1.13). During this process, spicules of mineralized cartilage originating from the growth plate become incorporated into the cortex and will remain there until they are removed by remodeling.

The peripheral metaphysis of a growing long bone is therefore an area of intense osteoclastic and osteoblastic activity. The cortex is relatively porous, consisting of trabecular bone undergoing compaction, and there is extensive peritrabecular fibrosis. This must be borne in mind when examining histological sections from such areas in young animals with suspected metabolic bone diseases, particularly fibrous osteodystrophy.

The normal curvature present in some bones is produced during growth by a modeling process referred to as **osseous drift**, whereby the shaft of a bone moves on its long axis. This is accomplished by successive waves of osteoblastic and osteoclastic activity beneath appropriate periosteal and endosteal surfaces of the diaphyseal cortex,

Figure 1.14 Transphyseal blood vessels Cabinet radioangiogram of a 2 mm thick slice of decalcified distal 3rd metacarpal bone from a 13-day-old foal. The physis is between the arrowheads. The arterial blood supply to this area had been injected with radio-opaque dye immediately after death. Numerous transphyseal arteries can be seen crossing from the epiphysis (E) to the metaphysis (M). (Courtesy of EC Firth.)

presumably under the influence of both genetic and mechanical forces, leading to the formation of laminar bone deposits. The same process is involved in efforts to correct shape abnormalities in long bones resulting from malunited fractures, or other acquired defects altering the mechanical forces acting on a bone.

Bones respond to increased usage during the growth phase by an increase in bone mass, particularly in the density and thickness of the cortex. In adults, increased mechanical usage does not increase bone mass, but can decrease remodeling and conserve the amount of bone already present.

Blood supply

The blood supply to bones is derived from arteries entering the medullary cavity through foramina in the cortices of the diaphysis, metaphysis, and epiphysis, as well as periosteal arteries. In young growing animals, **nutrient arteries** supply the diaphyseal marrow and most of the central area of the metaphysis, while **metaphyseal arteries** supply the peripheral regions. Terminal branches from these vessels pass vertically towards the metaphyseal surface of the growth plate, where they end in fenestrated capillary loops immediately below the last intact transverse septum of the mineralized cartilage matrix. At this point they turn back sharply into wide-bore venules characterized by low flow rate. Some terminal branches of the nutrient and metaphyseal arterial systems anastomose with each other but they do not penetrate the growth plate.

Epiphyseal arteries supply the epiphyses or secondary centers of ossification and small branches pass through narrow cartilage canals in the reserve zone of the growth plate to terminate at the start of the proliferative zone. This is the only source of oxygen and nutrients to the growth plate as no blood vessels terminate in the hypertrophic zone. Further branches of the epiphyseal artery pass to the undersurface of the overlying articular cartilage, where they form vascular loops similar to those on the metaphyseal side of growth plates, and participate in endochondral ossification.

Transphyseal blood vessels have been identified in newborn animals of several species (Fig. 1.14), but their function remains obscure. Most evidence suggests that the direction of arterial flow in these vessels is from the epiphysis to the metaphysis, but that venous flow occurs in the opposite direction. Transphyseal blood vessels do not provide nutrients to the growth plate but may enhance the blood supply to the metaphysis during the rapid growth phase in very young animals. At sites where transphyseal vessels enter the metaphysis, they are surrounded by cartilage projections, which might be expected to strengthen the union between the epiphysis and metaphysis at a time when the growth plate is highly susceptible to shear forces. These vessels may also be involved in certain diseases of bones, such as osteomyelitis, where they provide a possible route for spread of infection across the growth plate. The periphery of the growth plate is supplied by perichondrial arteries to the perichondrial ring of LaCroix, and by metaphyseal arteries.

The blood supply to the bone cortex in young animals is predominantly derived from the endosteal surface by way of nutrient arteries, and the flow of blood within the cortex is centrifugal. Arterial blood enters Haversian systems of the cortex through capillaries communicating with medullary sinusoids, but venous drainage occurs through the periosteal surface. With age, the cortex becomes increasingly dependent on **periosteal arteries** for its blood supply.

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POSTMORTEM EXAMINATION OF THE SKELETON

Of all organ systems, the skeleton is perhaps the most neglected during postmortem examination, even by experienced pathologists. Most organs are examined as part of the routine necropsy technique, but examination of the skeleton is more often confined to those occasions where the clinical history clearly indicates a skeletal problem. As a result, many skeletal disorders are likely to be missed. Furthermore, lack of familiarity with the normal appearance of skeletal structures commonly leads to misinterpretation in cases where a skeletal disease is suspected and a detailed examination of the skeleton is performed.

Gross examination

Complete examination of the skeleton is both impractical and unnecessary. A standard procedure for examining the skeleton should include an assessment of the shape, flexibility and breaking strength of readily accessible bones, such as ribs, cranium and key limb bones during routine necropsy. *No skeletal examination is complete without sectioning one or two representative long bones longitudinally* to reveal the growth plates, the thickness of the cortex, and the amount and density of trabecular bone in metaphyseal and epiphyseal regions. When the clinical history suggests the possibility of a skeletal disorder, a more detailed assessment is required. Antemortem radiographs are a valuable component of the gross examination in such cases and may highlight areas requiring special attention. The pathologist should insist on viewing them before commencing the necropsy. Radiographs of lesions identified during necropsy, either in the form of whole bones or sawn slabs, can also provide valuable information on the extent and severity of bone lysis or demineralization, but is an insensitive indicator of diffuse bone loss, as occurs in osteoporosis.

The manifestations of generalized skeletal diseases are likely to be most severe in certain bones. Even within bones, some regions may be affected more severely than others. For example, lesions associated with metabolic bone diseases, such as rickets and fibrous osteodystrophy, will be most marked at sites of rapid bone formation. The growth plates of the distal radius, proximal humerus, distal femur, and proximal tibia should therefore be targeted for gross and histological examination. Costochondral junctions of the largest ribs are also useful sites to examine in such cases. In osteoporosis, the depletion of trabecular bone is more rapid than that of cortical bone, presumably due to the greater surface area available for resorption in trabecular bone tissue.

Histological techniques and stains

Bone specimens for histological processing should be sawn at approximately 5 mm thickness and immersed in neutral buffered formalin. Other than in a few specialist laboratories equipped to prepare undemineralized bone sections, the specimens must then be demineralized prior to sectioning. In most laboratories, this involves the use of *commercial decalcifying agents*, usually consisting of strong acid solutions, which induce decalcification within 24–48 h. In the interests of section quality, the specimen should not be left in the decalcifying fluid any longer than necessary. It is important that bone slabs are no thicker than 5 mm, in order to minimize the time they spend in the fluid. The endpoint for decalcification can be judged by probing the tissue with a needle or by radiography. The decalcified tissue should be immersed in flowing tap water for 2–4 h to remove the acid, which would otherwise interfere with staining procedures. Although strong decalcifying solutions will allow the rapid preparation of sections for diagnostic purposes, they will also cause more tissue damage and may therefore impair interpretation. *Slower decalcification* in a chelating solution such as ethylene-diamine tetra-acetic acid (EDTA), will take approximately 7 days, but enables the preparation of higher-quality histological sections.

The *preparation of undecalcified sections* requires the use of plastic embedding media, such as methyl methacrylate, and a heavy-duty sledge microtome. Several useful staining methods for undemineralized bone sections are available, including hematoxylin and eosin, Von Kossa, and Villanueva's bone stain.

Hematoxylin and eosin is also a good general-purpose stain for routine histological examination of demineralized bone sections, allowing clear differentiation of bone and cartilage matrices, and providing adequate cellular detail. However, it does not reliably allow assessment of the thickness of osteoid seams, which is of diagnostic significance in diseases such as rickets or osteomalacia. These seams generally appear pale orange/pink, in contrast to the slightly more basophilic bone that was previously mineralized, but the distinction is often too subtle or variable to allow confident interpretation. The Masson's trichrome method is another useful general-purpose stain for bone sections, but has similar limitations with regard to identifying osteoid seams. Staining methods that allow identification of unmineralized osteoid in demineralized sections have been published (see Ralis and Ralis, 1975 and Tripp and MacKay, 1972 in bibliography) and, although not used routinely, can be easily performed in laboratories that are unable to cut undemineralized sections.

Preparation artifacts in histological sections

Because of the difficulty in preparing histological sections from bones, artifactual changes are often present and could be misinterpreted as lesions. During the process of sawing bones prior to demineralization, multiple small, irregular-sized fragments of *bone "sawdust"* and soft-tissue debris often become embedded in spaces between bone trabeculae (Fig. 1.15). Such fragments are commonly misinterpreted as necrotic bone. Rinsing the cut surface of the bone under running water, and gently brushing it before fixation, can minimize this artifact. Since the fragments will be most abundant near sawn surfaces, further trimming of the face to be sectioned, after demineralization, will further reduce them. In a section where "sawdust" is a problem, slicing deeper into the paraffin block is likely to yield cleaner sections for examination.

The heat generated by a band saw, or power drill in the case of bone biopsies, may create coagulative changes, resembling early ischemic necrosis, along the edges of the specimen. Overexposure to strong acid solutions during decalcification inhibits the staining of nucleic acids by hematoxylin, and of collagen by eosin, resulting in poor cellular detail and difficulties in interpretation.

Another common histological artifact is the presence of empty clefts between bone surfaces and the soft tissues of the marrow cavity. This reflects the much greater shrinkage of the soft tissues, when compared to bone, during

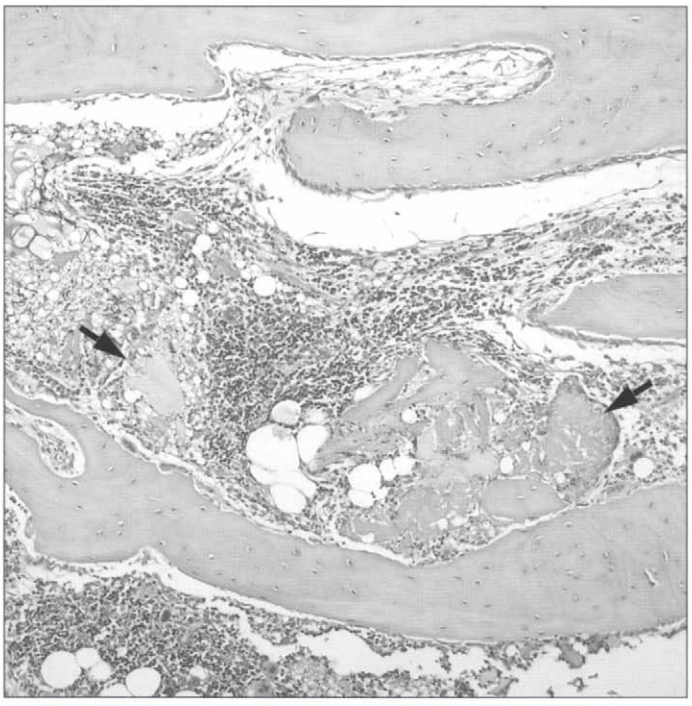

Figure 1.15 The multiple small fragments of bone and cartilage (arrows) embedded in the marrow spaces between trabeculae are **artifacts of sawing** ("sawdust") and should not be misinterpreted as lesions. Such artifacts are commonly present if sections are prepared too close to a sawn surface.

fixation in formalin. Consequently, osteoblasts or osteoclasts lining the bone surface may become separated from their site of activity. However, where bone resorption has been occurring, the surface of the bone will have a characteristic scalloped appearance. Also, bone does not adhere to microscope slides as well as soft tissues of the marrow spaces and may become dislodged, leaving large spaces lined by osteoblasts. These could be misinterpreted as vascular spaces.

Other laboratory techniques

The use of technetium labeling to identify areas of metabolically active bone, detected by scintigraphy, has dramatically improved the ability to detect bone abnormalities in the live animal. This technique greatly assists in locating multifocal lesions, such as the spread of metastatic disease within the skeleton, and although widely used in human medicine, has a relatively limited use for this purpose in animals.

A variety of other techniques may be used in the study of bones, but most are confined to the research laboratory. **Bone ash** measurements have historically been performed in animals with suspected metabolic bone diseases, but are of limited value for routine diagnosis because of the variability between individual bones and the lack of reliable reference ranges for animals of different age groups. More sophisticated and accurate methods for determining **bone density**, such as dual energy X-ray absorptiometry (DEXA scanning), have been developed for assessing bone mineral density in human patients, but are not readily available to most veterinary pathologists.

Microradiography of thick sections provides an indication of the pattern and degree of mineralization within the bone. Sections of bone, 60–100 μm thick, are placed in close contact with X-ray film and exposed. This creates an image of the bone section, which can be examined microscopically in association with histological sections prepared from the same slab.

The periodic administration to growing animals of **fluorescent markers**, which are deposited at sites of active mineralization, allows objective measurement of the rate of bone formation in physiological and disease states. The most commonly used marker is the antibiotic **tetracycline**, which fluoresces bright yellow when examined in undemineralized sections under UV light. Since the marker is only deposited at sites of active mineralization, a thin fluorescent line results from each dose. The distance is measured between lines representing sequential periods of exposure to the marker, and the rate of bone formation estimated.

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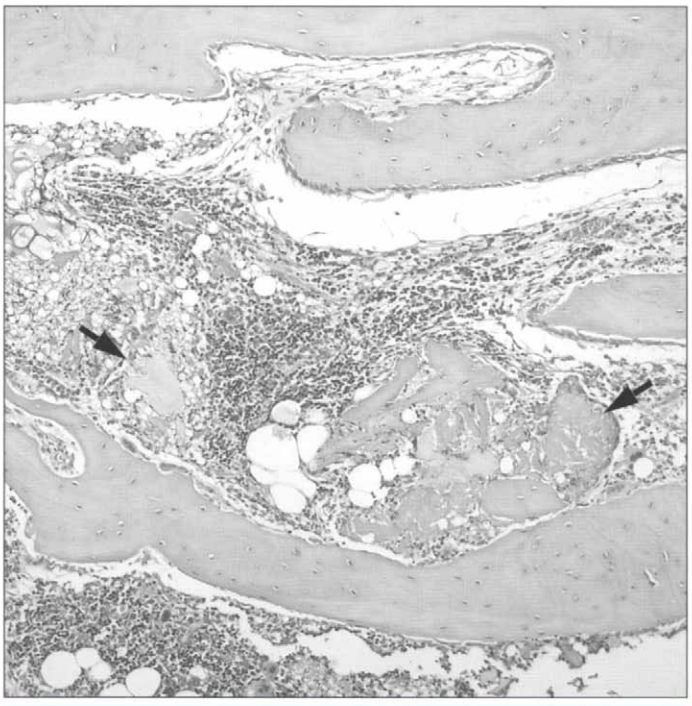
RESPONSE TO MECHANICAL FORCES AND INJURY

The cells of bone tissue are capable of the same basic cellular responses as most other tissues, including *atrophy, hypertrophy, hyperplasia, metaplasia, neoplasia, degeneration, and necrosis*. Bones have an excellent capacity for repair or modification in response to a wide range of injurious stimuli or changes in mechanical demand. Depending on the stimulus, the response may be localized or generalized but, in general, the magnitude of skeletal response is greater in young growing animals than in adults. If the response is generalized, it is likely to be most prominent at sites of rapid bone growth or modeling.

Mechanical forces

Bone adapts or remodels in response to the mechanical demands placed upon it. According to **Wolff's law**, *it is deposited at sites where it is required and resorbed where it is not*. For example, trabeculae in the



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epiphyseal and metaphyseal regions of long bones are aligned in directions which best reflect the compressive forces associated with weight bearing, and the tension associated with muscle insertions. In young individuals, increased mechanical stress on the skeleton increases the density of metaphyseal trabecular bone and the thickness of cortices. Increased mechanical usage in adults does not lead to an increase in bone mass, but reduces remodeling activity and conserves the amount of bone already present. Decreased activity accelerates bone loss by removing the inhibition of remodeling, and reduces formation, leading to a net reduction in bone mass.

Reduced mechanical stress on bones due to partial or complete immobilization, as occurs during fracture repair, leads to increased resorption, resulting in decreased bone strength and stiffness. If an implant, such as a metal plate, remains attached to a bone after a fracture has repaired, it will share the mechanical load with the bone. The bone will then atrophy in proportion to the decreased load and its strength will be greatly reduced. For this reason, *rigid implants should be removed soon after a fracture has healed.* Such implants may also trigger the development of an osteosarcoma at the site (see below), providing further reason for their removal.

Prolonged weightlessness associated with space travel has also been shown to result in decreased bone mass in weight-bearing bones. In contrast, increased mechanical stress associated with strenuous exercise has been associated with increased bone density.

Growth plate damage

In young growing animals, the growth plate is the weakest structure in the ends of long bones and is prone to traumatic injury resulting from shearing forces, compressive forces, or, in the case of traction epiphyses (e.g., lesser trochanter of the femur), excessive tension. In general, *the fastest growing growth plates are the most susceptible to injury*, the distal radial physis being the most commonly affected. Undulations in the growth plates of some bones increase their resistance to separation in response to shearing forces.

The consequences of growth plate injury depend on several factors, including the nature of the lesion, its location, the age of the animal and the status of the blood supply. Growth plates subjected primarily to traction consist at least partly of fibrocartilage, which imparts increased resistance to tensile forces. Such growth plates are sometimes referred to as **apophyses**.

Complete separation through the growth plate, referred to as **epiphyseolysis** (or "slipped epiphysis"), is a relatively common sequel to severe trauma or horizontal shear forces acting in the region of the bone–growth plate interface. The separation almost invariably occurs through the hypertrophic zone, where the cell volume is greatest and the matrix, which provides strength to the physis, is relatively sparse. Providing the epiphyseal vasculature has not been disrupted, the prognosis for this type of fracture is very good as the proliferative zone of the growth plate, and its blood supply, are likely to remain intact. However, epiphyseolysis of the capital femoral epiphysis, which may be associated with birth trauma in calves and occurs with some frequency in growing foals and puppies, may result in avascular necrosis of the femoral head. This reflects the greater risk of vascular damage as the nutrient vessels to the proximal femoral epiphysis travel along the neck of the femur and traverse the rim of the growth plate. The vessels supplying most other long bone epiphyses enter the bone at some distance from the growth plate and are protected by the periosteum or the fibrous layer of the joint capsule. A syndrome characterized by physeal dysplasia and slipped capital femoral epiphysis has been described recently in cats (4.5–24 months of age), most of which were male and obese. The Siamese breed was over-represented. In affected cats, the physeal cartilage was abnormally thick and chondrocytes were in disorganized clusters surrounded by abundant matrix. A similar syndrome is recognized in adolescent, overweight boys. Epiphyseolysis of the femoral head also occurs in pigs and deer as a manifestation of osteochondrosis, where there is likely to be an underlying weakness in the growth plate. Slipped capital femoral epiphysis must be distinguished from Legg–Calvé–Perthes disease and fractures through the femoral neck.

The *most common type of physeal fracture* reported in dogs, cats, horses, and humans, is characterized by extension of the fracture along the growth plate for a variable distance, then out through the metaphysis, leaving a triangular fragment of metaphyseal bone still attached to the growth plate. As with complete epiphyseolysis, the prognosis for further growth is very good. In contrast, fractures that cross the growth plate, with displacement of the fragments, will lead to the formation of a bony bridge between the metaphysis and epiphysis, precluding further growth in length at that site.

It is relatively common for epiphyseal separations, similar to those described above, to be induced during postmortem examination of young animals when limb joints are disarticulated forcefully. Such "fractures" are not accompanied by hemorrhage and are therefore easily distinguished from antemortem epiphyseolysis.

Growth plates of major limb bones, particularly the distal radius and ulna, are also susceptible to crushing injuries caused by compressive forces transmitted through the epiphysis. Such injuries, if severe enough, damage the epiphyseal blood supply as well as chondrocytes in the proliferating zone, leading to cessation of growth. *When the lesion is confined to one side of the growth plate, as it often is, continued growth on the other side leads to* **angular limb deformity**.

In dogs, *premature closure of the distal ulnar growth plate* is a common cause of limb deformity. Shearing forces acting on this growth plate result in crushing injury rather than epiphyseolysis, because of its conical shape, and are therefore more likely to result in retarded growth. If the growth plate of the adjacent radius escapes injury, the required synchrony between the two bones during development will be disturbed. Shortening of the limb will be accompanied by cranial bowing of the radius, valgus deformity, and outward rotation of the carpal and metacarpal bones.

Angular limb deformities ("bent leg") have been associated with a range of disease syndromes in several species, and are not always due to growth plate lesions. These will be discussed separately in a later section of this chapter.

Detachment of the ischial tuberosity from the pelvis is a well-recognized entity in young breeding sows, resulting in acute lameness. The separation, which may be bilateral, usually occurs between 8 and 14 months of age, prior to the closure of the apophyseal growth plate between the tuber ischiadicum and the rest of the ischium. The tuber ischiadicum serves as the origin for the semitendinosus and semimembranosus muscles, and as an attachment for the sacrotuberous ligament. As such, it is subject to considerable traction force and any weakness in the growth plate, as may occur in osteochondrosis, predisposes it to fracture.

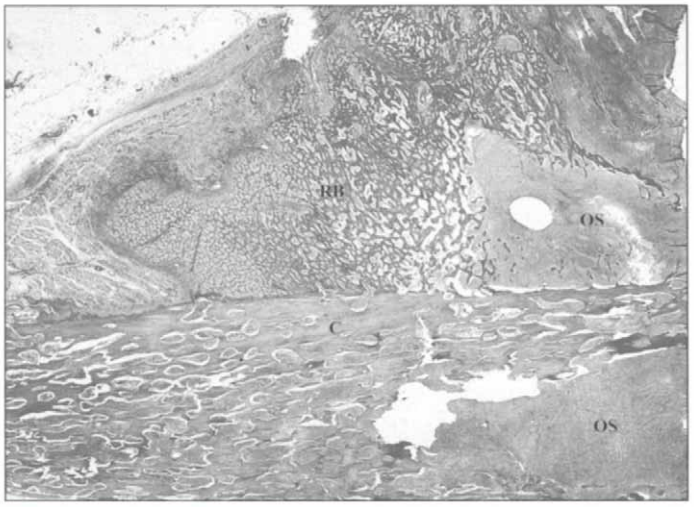

Figure 1.16 Reactive bone (RB) forming a so-called **"Codman's triangle"** beneath an elevated periosteum in a dog with osteosarcoma. The cortex (C) is porous due to tumor invasion. Sheets of tumor cells (OS) fill the medullary cavity and are replacing the sub-periosteal new bone in some areas.

Periosteal damage

Periosteal damage due to trauma stimulates rapid formation of new or **reactive bone** following activation and proliferation of osteoblast progenitors in the cambium layer. Trabeculae of woven bone extend from the underlying bone surface at acute angles, and can be readily distinguished histologically from the mature lamellar bone of the cortex (see Fig. 1.5). Separation of the periosteum from the bone surface by hemorrhage, inflammatory exudate, or neoplasia, or following surgical intervention, is also followed by *sub-periosteal new bone formation.* A pyramid-shaped region of new bone, referred to as **Codman's triangle**, may form beneath the periosteum in association with osteosarcoma (Fig. 1.16), but can also occur in association with other bone lesions, such as osteomyelitis. The mechanism of periosteal new bone formation is not clear, but it often precedes actual involvement of the periosteum by an underlying osteosarcoma or inflammatory process, suggesting that it may involve either local circulatory disturbances or the release of growth factors in response to bone resorption.

Localized outgrowths of new bone beneath the periosteum are referred to as **exostoses**. Depending on their size, and the inciting cause, they may either persist or gradually be removed by remodeling.

Fracture repair

Bone fractures are very common in animals and occur either when a bone is subjected to a mechanical force beyond that to which it is designed to withstand, or when there is an *underlying disease process that has reduced its normal breaking strength.* The latter is referred to as a **pathological fracture** and unless the predisposing disorder is corrected then the repair process is unlikely to be successful. The possibility of a localized bone disease (e.g., neoplasia or osteomyelitis) or a generalized disorder (e.g., fibrous osteodystrophy or osteoporosis) should always be considered if bone fracture has occurred without evidence of trauma.

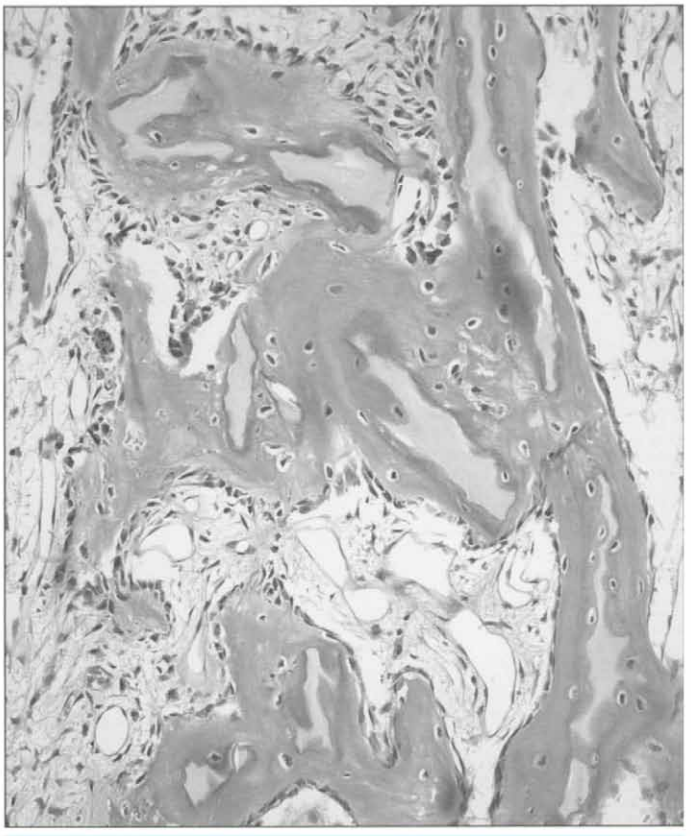

Figure 1.17 Trabecular microfractures in a calf with **osteogenesis imperfecta**. Note the abnormal alignment of cartilage cores in adjacent trabeculae that have been incorporated in a microcallus.

Types of fractures

Fractures are classified as **simple,** if there is a clean break separating the bone into two parts, or **comminuted**, if several fragments of bone exist at the fracture site. When one segment of bone is driven into another the fracture is referred to as an **impacted** fracture, and when there is a break in the overlying skin, usually due to penetration by a sharp fragment of bone, the fracture is referred to as **compound**. If there has been minimal separation between the fractured bone ends, and the periosteum remains intact, the lesion is classified as a **greenstick** fracture. An **avulsion** fracture occurs when there is excessive trauma at sites of ligamentous or tendinous insertions and a fragment of bone is torn away.

Microscopic fractures of individual trabeculae, or localized segments of cortical bone, also occur and are referred to as **microfractures**. Trabecular microfractures can sometimes be detected in histological sections by the abnormal alignment of their cartilage cores, which are normally situated at right angles to the growth plate, and parallel to the cartilage cores of adjacent trabeculae (Fig. 1.17). Such microfractures must however be differentiated from artifactual alterations in trabecular alignment that may occur when a bone is being sawn during processing. Once trabeculae have lost their cartilage core through remodeling this does not apply, and since the direction of remodeled trabeculae is less predictable, detection of microfractures is more difficult. *Multiple microfractures involving several adjacent trabeculae without gross displacement of the bone ends are referred to as* **infractions**. These are sometimes seen