Frederic Shapiro

Pediatric Orthopedic Deformities

Volume 1

Pathobiology and Treatment of Dysplasias, Physeal Fractures, Length Discrepancies, and Epiphyseal and Joint Disorders

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To my wife. Carol Ann Satler

Preface

The current edition of *Pediatric Orthopedic Deformities: Basic Science, Diagnosis, and Treatment* has been increased to three volumes, updating and expanding the presentation of skeletal deformation in the growing child. It demonstrates throughout how specific biological and mechanical abnormalities can affect normal bone and cartilage development and lead to problematic deformation. The two main premises of the book remain the same however: (1) current orthopedic treatments of growth deformities of the developing skeleton are most effective when based on an understanding of and relationship to the underlying pathobiology and (2) future treatments can be developed best with deeper understanding of and more specific relationships to the underlying pathobiology.

Volume 1, Chap. 1 (Developmental Bone Biology) describes skeletal development as outlined by several investigational approaches including: histology at the light and electron microscopic levels; molecular biology outlining the wide array of gene and molecular controls for skeletal tissue differentiation, 3- and 4-dimensional limb bud axial differentiation and growth, and synthesis of structural macromolecules; mineralization; mechanical–biophysical effects on the developing skeleton; and radiological parameters of growth such as appearance of secondary ossification centers and times and patterns of physeal fusion.

Chapter 2 (Overview of Deformities) is new and describes: founders of the field of pediatric orthopedics, which essentially began with several individuals specifically addressing the major musculoskeletal deformities of the developing child; 34 basic principles regarding pediatric orthopedic deformity; descriptive terminologies for bone and joint deformities; correction of deformities by spontaneous, non-operative and operative means with an overview of each of the clinical methods and techniques used for the treatment groups; a detailed molecular and histological description of biological tissue repair mechanisms for direct, primary, and endochondral cortical bone repair, metaphyseal cancellous bone repair, articular cartilage (full and partial thickness) repair, and physeal cartilage repair; and derangements of the repair process. Tendon, muscle, and peripheral nerve are also discussed regarding structure and injury and repair mechanisms.

Chapter 3 (Skeletal Dysplasias) discusses the skeletal dysplasias concentrating on clinical and radiographic descriptions of the entities, molecular abnormalities, histopathology, the lethal variants, orthopedic deformities by region and by specific disease entities, and orthopedic treatments.

Chapter 4 (Bone and Joint Deformity in Metabolic, Inflammatory, Neoplastic, Infectious, and Hematologic Disorders) reviews entities known to be associated with pediatric orthopedic deformities from the perspective of: the pathobiology of the disorder, the pathoanatomy of deformation, and the principles of medical and orthopedic management. The epiphyseal, metaphyseal, and joint abnormalities are reviewed for: rickets, juvenile rheumatoid arthritis, benign and malignant neoplasms and pyogenic and tuberculous infections (which concentrate at the epiphyseal-metaphyseal regions), and hematologic disorders such as hemophilia.

Chapter 5 (Epiphyseal Growth Plate Fracture-Separations) describes the relationship of these injuries with the possible growth deformities. These represent the main subset of childhood fractures contributing in a major way to limb deformity.

The cell biology and histopathology of growth plate injuries are reviewed, the commonly used pathoanatomic classifications are explained, and the presence or absence of growth sequelae are outlined. Each major physis is reviewed regarding clinical management and results. A pathophysiologic approach is outlined which provides a more dynamic and biological understanding and classification of these injuries at the cell and the tissue level. Imaging modalities beyond plain radiographs, such as magnetic resonance imaging, computerized tomographic scanning, and ultrasound, provide information on blood supply and tissue injury planes, the main determinants of negative growth sequelae. Management is detailed to eliminate, minimize, or if necessary treat transphyseal bone bridges.

Chapter 6 (Lower Extremity Length Discrepancies) outlines: the natural history of specific disorders leading to discrepancies, negative sequelae of discrepancies, methods projecting eventual discrepancies at skeletal maturity, developmental patterns of discrepancies, and techniques and timing for shortening, lengthening. and bone bridge resection. Femoral overgrowth following diaphyseal fractures is reviewed and updated following immediate casting or intramedullary nailing.

Volume 2 will assess regional deformities of the developing lower extremity. The developmental biology and histopathology at each region are stressed and related to treatment interventions. Chapters are devoted to: Developmental Dysplasia of the Hip; Legg-Calve-Perthes disease; Slipped Capital Femoral Epiphysis (and other causes of coxa vara); Degenerative Joint Disease following childhood hip disorders (femoral-acetabular impingement); Developmental Disorders of the Knee; Club Feet (and other deformities of the foot and ankle); and Rotational and Angular Deformities of Femur and Tibia.

Volume 3 will outline the developmental biology and histopathology of the vertebrae, ribs and spinal cord. Chapters will assess: 1. Spinal Deformities including: (a) congenital and idiopathic scoliosis, (b) kyphosis, (c) spondylolisthesis and d) cervical abnormalities; and 2. Neuromuscular Disorders and their spinal, hip, and limb deformities for: (a) cerebral palsy, (b) the muscular dystrophies and structural myopathies, mitochondrial and metabolic myopathies, myotonic disorders, spinal muscular atrophy, Friedreich Ataxia, and the peripheral neuropathies, (c) spinal cord abnormalities of Meningomyelocele, diastematomyelia, transverse myelitis, tethered cord, and syringomyelia, and (d) brachial plexus injuries.

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1 Developmental Bone Biology

1.1 Overview of Bone Development

During the embryonic period, limb buds filled with undifferentiated mesenchymal cells form from the lateral side walls. The earliest sites of skeletal formation are characterized by condensation or close packing of mesenchymal cells followed by early cartilage differentiation. Each of the long bones is preformed as a cartilage model. Bone tissue is then deposited beginning in the middle of the model on the calcified cartilaginous core using the endochondral ossification mechanism and directly by the surrounding periosteum using the intramembranous ossification mechanism. Bone deposition progresses toward each end with intramembranous bone formation at the periphery slightly in advance spatially of internally or centrally positioned endochondral bone formation. The proportional involvement of each of the three regions of a developing bone—diaphysis, metaphysis, and epiphysis—is established by the early fetal stage and remains more or less unchanged until skeletal maturity. The central part of the bone is the diaphysis or shaft; the furthermost bone extension of the diaphysis is the metaphysis; and the developing cartilaginous end of each bone is the epiphysis.

Normal bone development occurs in conjunction with the proliferation and differentiation of cells, the synthesis and interaction of specific molecules, and the generation of intrinsic and extrinsic biophysical forces. Primary genetic blueprints and secondary epigenetic and inductive phenomena lead to the patterns characteristic of each bone throughout the skeleton. A developing long bone consists of the ***epiphyses*** and ***metaphyses*** at each end and the ***diaphysis (shaft)*** in between. These regions are established by the middle of the embryonic stage and go through proportional changes in size until skeletal maturity. The epiphyses are responsible for the transverse and spherical growth of the ends of the bone, the shaping of the articular surfaces, and the longitudinal growth of the metaphyses and the diaphysis. A small amount of longitudinal growth also occurs with interstitial expansion of the epiphyseal cartilage, including the undersurface of the articular cartilage. Each epiphysis, formed initially completely in cartilage, subsequently differentiates into three histologically distinct regions. These are: (i) the cartilage at the outermost boundary of the epiphysis adjacent to the joint space that is the ***articular cartilage***, (ii) the cartilage adjacent to the metaphysis that forms the ***physis (growth plate, epiphyseal growth plate)***, and (iii) the cartilage between the articular cartilage and the physeal cartilage referred to as the ***epiphyseal cartilage***, which will form a ***secondary ossification center*** after vascular and osteoprogenitor cell invasion. The cells and matrices of the ***perichondrial ossification groove of Ranvier*** (the terminal extension of the periosteum) surround the physis and part of the metaphysis and are an integral part of long-bone development.

The growth plate components go through a sequential process of cell proliferation, synthesis of extracellular matrix, cell hypertrophy, mineralization of the matrix, localized vascular invasion accompanied by osteoprogenitor cells, and apoptosis. These highly coordinated activities lead to longitudinal bone growth and bone formation at the physeal–metaphyseal region and encompass the mechanism of ***endochondral ossification***. The growth cartilage replenishes itself through the germinal zone and continually is replaced with bone at the physeal–metaphyseal junction. With these coordinated events, the length of the entire bone increases; the physes at either end are displaced progressively further away from the center of the bone, and the physis itself maintains the same height throughout the growth period. At the same time, there is radial growth of the diaphysis and parts of the metaphysis by ***intramembranous bone formation*** with direct apposition of cortical bone by osteoblasts from the inner cambial layer of the periosteum. This is coordinated closely with resorption of bone by osteoclasts on the inner cortical endosteal surfaces and lateral metaphyseal surfaces to maintain the relative proportions of the marrow cavity to the cortices and the overall shape of the bone as it grows. We have outlined 16 stages with several additional substages of long bone and

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Table 1.1 Early observations on bone growth

1727	Bones grow in length by the addition of new tissue at their ends. (Hales)
1731–1736	There are two mechanisms of bone formation, one occurring directly in a membrane (intramembranous ossification) and one via pre-existing cartilage (endochondral ossification). Vascularization immediately precedes bone formation in cartilage. (Nesbitt)
1736	Growing bone is stained red by madder (alizarin) in diet. (Belchier)
1739–1743	A long bone grows in length from its ends and in thickness by formation of new bone on its outer surface. The osteogenic function of the periosteum thickens the bone on its outer surface, based on madder feeding studies. (Duhamel)
1740	Increased diameter of marrow cavity with growth is due to absorption of pre-existing bone internally. Bone development includes both bone deposition and bone absorption. (Hunter)
1815	Interplay of cartilage and bone formation in long bone development. Cartilage vascular canals in epiphyses. Gross and crudely magnified evidence of physeal cartilage. Vascularization of inner periosteum precedes earliest site of bone formation. Cartilage model determines the shape of the future bone and establishes ossification within it. Mechanical pressure variously modified is the principal agent in effecting progressive changes of structure in growing bone. (Howship)
1841–1847	Bone is formed in the periosteum, grows in thickness by superimposing new layers externally, grows in length by adding new layers at the growth cartilage at each end, and has the marrow cavity formed by resorption of the inner bone layers. There is formation–resorption– reformation of the bone with growth accompanied by constant change in bone substance. (Flourens)
1850	Resorption of bone as part of developmental sequence is mediated by osteoclasts. (Kolliker)
1852–1853	Tissue at epiphyseal–diaphyseal (metaphyseal) junction is characterized by differing layers as determined by careful gross and early lower power microscopic examination. (Broca; Tomes and de Morgan)
1858	Detailed microscopic–histologic structural recognition of physeal layers and endochondral sequence. (Müller)
1860	Bone description as a **tissue** (bone cells plus calcified matrix) and as an **organ** (encompassing bone tissue, marrow, periosteum, articular and epiphyseal cartilage, vessels, and nerves). Detailed cellular description of physeal sequence including hypertrophic chondrocyte fate. (Virchow)
1864	Bone forming cells first referred to as osteoblasts (Gegenbaur)

epiphyseal development to show the timing and coordination of the growth process. The endochondral and intramembranous ossification sequences are involved together not only in long bone formation but also in formation of the pelvis, vertebrae, sternum, ribs, scapula, and clavicle. Skull and facial bone formation and repair are similar but have slight differences owing to origin from differing embryonic lines and are outside the scope of this review. An overview of early observations on bone growth is presented in Table 1.1.

1.1.1 Epiphysis

The term epiphysis refers to the entire developing end of the bone [1, 2]. The epiphyses are responsible for long bone longitudinal growth, for transverse growth at the ends of the bone, and for the shape of the articular surfaces. This region is formed initially completely in cartilage and subsequently subdivides during development into three histologically distinct regions: (a) the cartilage immediately adjacent to the joint, referred to as articular cartilage; (b) the cartilage adjacent to the metaphysis, referred to variously as the growth plate, the epiphyseal growth plate, or the physis. It is the functionally and cytologically specialized region where the bulk of longitudinal growth occurs and encompasses the area from the reserve zone of cells to the end of the hypertrophic cell layer; and (c) the cartilage between the articular cartilage and the growth plate cartilage, referred to as the epiphyseal cartilage. This is eventually transformed entirely into bone and marrow following the appearance and enlargement of what is variously referred to as the secondary ossification center, the bony nucleus, the ossific nucleus, or the bony epiphysis (Fig. 1.1). The epiphysis is sometimes referred to as the chondroepiphysis, but use of this term should be restricted to the time prior to formation of the secondary ossification center.

1.1.2 Metaphysis

The metaphysis lies between the lower part of the physis and the outer reaches of the diaphysis. It is the area where the endochondral ossification process leads to bone tissue formation. Cells and capillaries penetrate through the metaphysis, entering into the spaces at the lowermost 3–4 levels of hypertrophic chondrocytes of the physis, forming in older terminology the “osteogenic–degenerative zone of the physis” or currently the osteogenic–apoptotic zone. Undifferentiated mesenchymal cells, preosteoblasts, and osteoblasts accompany the vessels, forming a cellular layer on the remaining calcified longitudinal septae of cartilage, covering them with osteoid tissue as part of the bone synthesis process. The metaphyseal cortical bone is formed by the

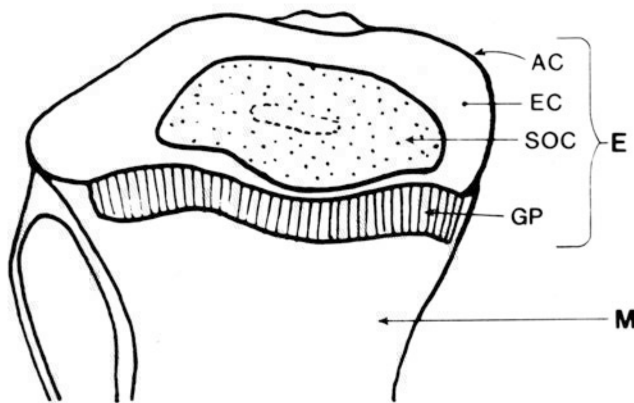

Fig. 1.1 The histologic structure of the epiphysis of the proximal tibia is illustrated. The entire developing end of the bone from the articular cartilage surface to the last cells of the hypertrophic zone of the growth plate is the epiphysis (*E*). This encompasses 3 regions that are initially cartilage: (a) the articular cartilage (*AC*), (b) the growth plate (*GP*), also referred to as the epiphyseal growth plate or the physis and (c) the epiphyseal cartilage (*EC*) which refers to the cartilage mass between the articular cartilage and the growth plate cartilage. It is within the epiphyseal cartilage that the secondary ossification center (*SOC*), also referred to as the bony nucleus, the ossific nucleus, or the bony epiphysis, forms and expands. (Reprinted with permission from Shapiro F, Epiphyseal Disorders. New Engl J Med. 1987;317:1702–1710. Copyright 1987 Massachusetts Medical Society)

coalescence of peripheral endochondral trabecular bone from the physis with intramembranous bone from the inner osteogenic (cambial) layer of the periosteum.

1.1.3 Diaphysis

The diaphysis lies between the two metaphyses and is the shaft of the bone. During the early stages of development it is composed within of endochondral bone but eventually with growth and resorption the marrow space predominates. The outer part of the diaphysis is composed of cortical bone. The cortex is formed and widened by the inner cambial layer of the periosteum and maintains its relative thickness by resorption by osteoclasts on its inner surface.

1.1.4 Bone Tissue Formation

In bone tissue formation osteoblasts synthesize and deposit type I collagen, the main protein constituent of bone matrix, in only two basic conformations, ***woven*** and ***lamellar***. In woven bone the collagen fibrils are randomly oriented while in lamellar bone they are clustered in parallel arrays. The fibrils in lamellar bone are not invariably parallel to each other and to the longitudinal axis; rather they alternate in adjacent layers between longitudinal and circular orientations in an orthogonal pattern. These matrix orientation processes are repeated in normal bone development, in bone repair utilizing any of several mechanisms, and in several pathologic conditions. Bone is formed by two mechanisms, endochondral ossification and intramembranous ossification.

1.1.4.1 Endochondral Ossification

The cartilage models of a developing bone and ultimately the epiphyses form bone by a mechanism referred to as endochondral bone formation. The major characteristics of this mechanism of bone formation involve growth of the cartilage by interstitial expansion involving chondrocyte proliferation, matrix formation, and chondrocyte hypertrophy. At a certain stage of development, the cartilage matrix adjacent to the hypertrophic cells mineralizes, there is vascular invasion of the lacunae in which the hypertrophic cells reside, and this vascular invasion is accompanied by mesenchymal cells which shortly differentiate to osteoblasts and synthesize a bone matrix on the calcified cartilage cores. The calcified cartilage is thus serving as a scaffold on which bone is deposited initially at the center of the developing cartilage model of the bone at the primary center of ossification, eventually at the lower regions of the physis merging into the metaphysis and within the epiphyseal cartilage where the bone formed is referred to as the secondary ossification center. The epiphyseal cartilage immediately surrounding the secondary ossification center and undergoing chondrocytic hypertrophy is referred to as the physis of the secondary ossification center.

1.1.4.2 Intramembranous Ossification

Bone formation is also characterized by a mechanism referred to as intramembranous ossification which occurs in long bone formation from the surrounding periosteum. In this mechanism, bone tissue is formed directly from mesenchymal cells without the mediation of a cartilage scaffold phase. The periosteum has a specific structure with two layers, an outer fibrous layer and an inner osteogenic or cambial layer. The inner cambial layer also displays an organized cellular differentiation pattern although it is not as structurally specific as the physis. The outermost part of the inner layer is composed of undifferentiated mesenchymal cells; these then begin to secrete and surround themselves with an osteoid matrix as they differentiate from pre-osteoblasts to osteoblasts; further toward the cortex, osteoblasts line the surface of the bone to synthesize osteoid preferentially on the bone surface. When the osteoid matrix surrounds a cell completely and then becomes mineralized, that cell is referred to as an osteocyte.

1.1.5 Perichondrial Ossification Groove of Ranvier

In all long and most flat bones both mechanisms of bone formation, endochondral and intramembranous, are present. They relate intimately and specifically to one another at the periphery of the growth plate in a region referred to as the perichondrial ossification groove of Ranvier (Fig. 1.2). The tissues comprising the intramembranous ossification mechanism circumferentially ensheathe and support the physeal cartilage at this area. A circumferential groove indenting the cartilage is present whose deepest part is opposite the epiphyseal cartilage/physeal cartilage junction. It contains three tissue components: (a) an outer fibrous layer which is continuous with the outer fibrous layer of the periosteum and inserts beyond the physeal region into the epiphyseal cartilage; (b) a zone of densely packed cells which is a continuation of the inner cambial layer of the periosteum and is present into the depths of the groove as far as the resting zone of physeal cartilage. This collection of dense cells synthesizes osteoid and intramembranous bone directly; and (c) a collection of loosely packed cells between the outermost reaches of the zone of dense cells and the fibrous tissue layer that adds chondrocytes to the periphery of the epiphysis just beyond the physis itself. The intramembranous bone synthesized by the tissues within the groove region is sometimes referred to as the bony bark of Lacroix. It is frequently discontinuous with the cortical bone of the diaphysis and metaphysis in those areas where the metaphyseal cutback zone is extensive. The perichondrial ossification groove of Ranvier with its fibrous, chondroprogenitor, and osteoprogenitor cells is an integral part of the epiphyseal region [1].

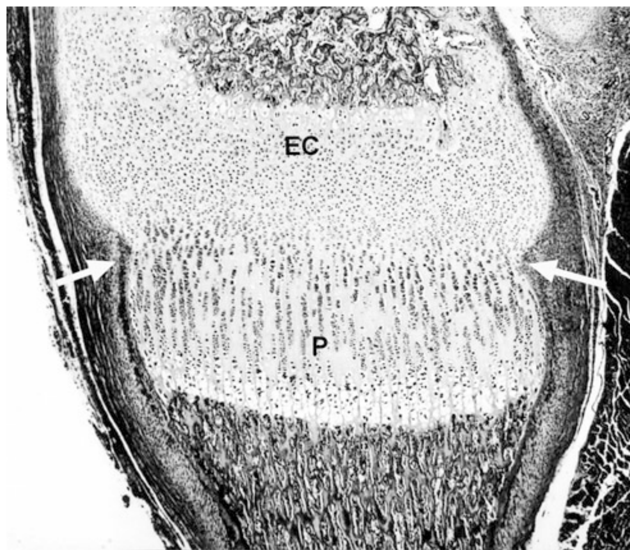

Fig. 1.2 Photomicrograph of the developing end of a rabbit metatarsal is shown. The secondary ossification center is shown centrally at the *top*. EC-epiphyseal cartilage; P-physeal cartilage. The two closed white *arrows mark* the perichondrial ossification groove of Ranvier. Just below the *arrows* at the periphery of the physeal cartilage is the terminal extent/origin of the cortex which is formed by the intramembranous ossification mechanism. The metaphyseal bone just *below* the physis is formed by the endochondral ossification mechanism as is the bone of the secondary ossification center. (Reprinted with permission from Shapiro et al. Organization and cellular biology of the perichondrial ossification groove of Ranvier. J Bone Joint Surg Am. 1977;59A:703–723)

1.1.6 Cellular Components

A brief definition of the cells present in bone follows.

1.1.6.1 Undifferentiated Mesenchymal Cells

The developing tissues of the skeleton form from undifferentiated mesenchymal cells. These are present initially in the limb buds prior to any histologic developmental differentiation. They are uniform appearing cells histologically with a nucleus and cytoplasm not yet surrounded by any specific matrix. They have the potential for differentiation with the appropriate stimulus along various tissue producing lines including becoming chondrocytes to produce cartilage, osteoblasts to produce bone, fibroblasts to produce fibrous tissue, adipocytes to produce fat, or myoblasts to produce muscle.

1.1.6.2 Osteoblasts

Osteoblasts are active bone forming cells. They are characterized by an abundant cytoplasm filled with rough endoplasmic reticulum at the ultrastructural level. Some refer to the cell intermediate between the undifferentiated mesenchymal cell and the osteoblast as a pre-osteoblast. The cells are responsible for synthesizing large amounts of collagen primarily of the type I variety which accumulate to form the matrix of bone. We refer to two types of osteoblasts based on their topography [3]. The ***mesenchymal osteoblast*** is surrounded completely by randomly oriented collagen fibrils and is thus responsible for the synthesis of woven bone. The ***surface osteoblast*** lines up along the surface of pre-existing bone tissue and synthesizes collagen fibrils along the pre-existing surface in parallel array. The surface osteoblast is thus involved in the direct synthesis of lamellar bone. As bone synthesis proceeds the osteoblast becomes completely surrounded by matrix referred to as osteoid and when that matrix becomes mineralized, the encased cell is referred to as an osteocyte. An osteoblast-specific transcription factor has been identified [4–6]. The first osteoblast-specific transcription factor is Runx2 (Cbfa1), one of thethree vertebrate homologs of the drosophila runt and lozenge proteins. Cbfa1 appears to have features specific for early differentiation along the osteoblast line. Cbfa1 expression is initiated in the

mesenchymal condensations of the developing skeleton, is strictly restricted to cells of the osteoblast lineage, and is regulated by BMP7 and vitamin D3. These transcription factors are increasingly referred to as runx2 and runx3.

1.1.6.3 Osteocytes

Osteocytes are mature bone cells [7]. They reside in spaces referred to as lacunae and their cell processes are connected to one another with the processes being present in canals passing through the matrix and referred to as canaliculi. Each osteoblast and osteocyte has numerous cell processes passing from it which serve to relate to cell processes from adjacent osteoblasts and osteocytes. These cell processes link up via the gap junction mechanism.

1.1.6.4 Chondroblasts and Chondrocytes

Chondroblasts and chondrocytes are cells responsible for the synthesis and maintenance of cartilage tissue. The transcription factor Sox9 is required for mesenchymal differentiation into chondrocytes and subsequent maintenance and maturation of chondrocytes. It is considered to regulate the earliest stage of the chondrocyte line. The cells surround themselves with a matrix composed primarily of type II collagen although there are also considerable amounts of type IX, X, and XI collagens and a large array of proteoglycans. Cartilage has a high proportion of water which composes approximately 80 % by volume of the tissue mass. It is difficult to make a histologic differentiation between chondroblasts and chondrocytes since both cell types are surrounded by cartilage tissue which unlike bone does not, except in rare incidences, mineralize. The chondroblast or chondrocyte has an oval shape with an appearance of the cytoplasmic wall by ultrastructure of mild scalloping. The collagen fibrils in cartilage are randomly arrayed and tend to be much thinner than those in bone averaging 10–20 nm in diameter.

1.1.6.5 Fibroblasts and Fibrocytes

These connective tissue cells are found in bone in the outer fibrous layer of the periosteum. They primarily synthesize type I collagen, although this does not mineralize.

1.1.6.6 Osteoclasts

The cell type responsible for resorption of bone and cartilage tissue is the osteoclast. It is not part of the mesenchymal series but has its origin from the hematopoietic system. The osteoclast is derived from hematopoietic cells of the monocyte–macrophage line. The osteoclast is a large, multinucleated cell formed by fusion of circulating monocytes. The same cell type can resorb cartilage or bone. If the cell is relating to cartilage exclusively then the term chondroclast can be used although the tendency is to simply use the term osteoclast in relation to resorption of either bone or cartilage. The osteoclast attaches itself to the underlying tissue by a characteristic structural mechanism evident only by ultrastructural assessment. The cell surface has a circular region free of organelles, which is referred to as the clear zone. This serves to attach the cell in a donut-like fashion to the underlying bone and cartilage. The cell surface within the rim of the clear zone is then thrown into innumerable folds or outpouchings forming what is referred to as the ruffled border. This mechanism serves to allow for increased secretion of lytic enzymes by the cell, with the extreme increase in extent of the cell border being caused by the ruffling phenomenon. The circumferential clear zone seals the environment and allows the lytic enzymes to be present in high concentration. It is these enzymes that are responsible for resorption of the underlying mineral and then matrix of the cartilage and bone.

Several factors affect osteoclast formation at differing stages of their development [8]. Transcription factors needed are Pu.1, an ETS domain-containing transcription factor expressed in the myeloid and B-lymphoid lineages; c-Fos, a protooncogene; and nuclear factor κB (NF-κB) [9]. Signaling molecules needed are M-CSF (macrophage colony-stimulating factor) and RANKL (receptor for activation of nuclear factor κ-B = RANK, L = ligand). The two major molecules exclusively essential for osteoclast function are macrophage colony-stimulating factor (M-CSF) and receptor for activation of nuclear factor κ-B (RANK) ligand, (RANKL), also known as osteoprotegerin ligand (OPGL) [8, 10]. The latter is a tumor necrosis factor family molecule identified as an osteoclast differentiation factor. Hematopoietic stem cells differentiate to monocyte/macrophage cells with the help of Pu.1 and M-CSF. The macrophages become monocular osteoclasts under the influence of C-Fos, NF-κB, M-CSF, and RANKL. RANKL stimulates both the fusion of mononuclear osteoclasts into multinuclear osteoclasts and their activation to resorption, with the latter activation also aided by microphthalmia transcription factor (MITF). Another molecule involved in the sequence is osteoprotegerin (OPG), a soluble receptor member of the tumor necrosis factor (TNF) receptor family. OPG actually inhibits osteoclast differentiation. Its ligand RANKL is a membrane bound TNF. RANKL binds to OPG allowing for osteoclast activation. RANK, a member of the TNF receptor superfamily, binds to RANKL and induces osteoclast differentiation.

1.1.7 Gap Junctions Linking Bone Cells

Gap junctions serve as areas for direct cell–cell communication either by electrical coupling or as points of passage for small low molecular weight messenger molecules [11]. They are intercellular channels formed by different membrane spanning proteins called connexins. Gap junctions are

observed linking the cell bodies of surface osteoblasts, linking surface osteoblast processes passing through newly synthesized osteoid with osteocyte processes, and linking osteocyte processes of adjacent cells within bone. Gap junctions are arranged in five basic shapes as defined by thin section transmission electron micrographs. These appear as linear, stacked linear, curvilinear, oval, and annular. Light microscopic sections of bone show the osteocytes to be present in round- to oval-shaped spaces in the bone referred to as ***lacunae*** which are linked to adjacent lacunae by multiple small canals referred to as ***canaliculi*** [11–16].The lacunae house the osteocytes and the canaliculi contain the ***osteocyte cell processes***. The lacunar–canalicular intraosseous system plays a key role in the transfer of nutritive fluid from blood vessels to bone cells. The common technique used for assessing bone by light microscopy involves hematoxylin and eosin staining of paraffin embedded sections. This technique does not demonstrate the lacunar–canalicular system well, and frequently scarcely shows it all. Plastic embedded, toluidine blue-stained sections outline well the organized lacunar-canalicular system in cortical bone [16]. The canaliculi link osteocytes and blood vessels extensively. A canaliculus passes into or away from a lacuna by a mean distance of 1.9 μm over the entire osteocyte perimeter [16]. Undifferentiated mesenchymal cells have no processes, as seen by transmission electron microscopy, but soon sprout a florid array of processes as differentiation to early mesenchymal osteoblasts proceeds. Osteoblast and osteocyte cell processes are packed with ***actin*** (7 nm) and ***intermediate filaments*** (10–11 nm) including vimentin that are continuous with those in the cell bodies [17]. Intercellular ***gap junctions*** are seen between surface osteoblasts, between osteoblasts and underlying osteocytes, between osteocyte cell processes in the canaliculi, and between osteocytes and the intracortical blood vessels. The woven bone canaliculi pass into the matrix in irregular, poorly defined pathways, but once cortical lamellar bone is formed, the canaliculi assume a more regular orientation passing between osteocytes either perpendicular or parallel to the longitudinal bone axis.

1.2 Embryology of the Limbs

1.2.1 Timing and Staging of Human Limb Development

The limb buds form as outpouchings of the embryo lateral plate mesoderm mass and are composed initially of undifferentiated mesenchymal cells uniformly packed throughout the entire extent of each bud and continuous with the undifferentiated mesenchyme of those regions which will become the shoulder and pelvis. There is a craniocaudal differential time gradient in development with the upper limb buds appearing in the lower cervical region in the human on day 24 and the lower limb buds in the lower lumbar region on day 28. By 33 days the hand plate is seen and by the end of the 6th week all upper and lower limb segments can be seen. Digital rays appear in the upper limb during the 6th week and in the lower limb during the 7th week. By the end of the 8th week components of each of the upper and lower limb bones are formed in cartilage. The embryonic period comprises the first eight post-ovulatory weeks with limb morphogenesis in the human occurring between the 4th and 8th weeks. At 8 weeks ossification of the humeral diaphysis begins, a time at which embryonic development is arbitrarily considered to be over and fetal development, which involves growth of fully established models, begins [18–23].

Embryonic staging terminology. The human embryonic phase is divided into 23 stages using the Carnegie system. This system, adopted in the early 1970s, is a refinement of what were previously referred to as Streeter's horizons. Much human embryologic and fetal study is categorized on the basis of the crown-rump length expressed in millimeters. The Carnegie staging system incorporates 23 stages and relates them to crown-rump length and age in post-ovulatory days. It is given in Table 1.2 listing some general correlations in the human embryonic periods along with developmental features particularly related to limb development. A more detailed outline of human limb development is listed in Table 1.3.

O'Rahilly [23] makes several points in terms of previous descriptions referable to embryonic staging in the human. These include: (1) The term horizon used by Streeter is no longer used and has been replaced by the term stage; (2) Roman numerals from the old Streeter classification have been replaced by Arabic numerals to denote what are now referred to as the Carnegie stages; (3) The most useful single measurement of an embryo or a fetus is the crown-rump (C-R) length which is expressed in millimeters (4) The crown-rump lengths used by embryologists agree closely with those determined ultrasonically; (5) The length of an embryo is not a stage and when used in a descriptive mode should simply be reported as, for example, 15 mm; (6) Stages within the embryonic period are expressed as post-ovulatory weeks or days; (7) Ages previously described by Streeter are incorrect for the human; and (8) the 23 stages of the Carnegie system refer to the embryonic period only, that is the 1st eight post-ovulatory weeks; no widely accepted staging system has been devised for the fetal period.

Table 1.2 Correlation of timing systems used for human embryos (Weeks 1 through 8)

Week	Day	Length (mm)	Carnegie stage	Features
1	1	0.1–0.15	1	Fertilization
	1-5-3	0.1–0.2	2	First cleavage divisions (2–16 cells)
	4	0.1–0.2	3	Blastocyst free in uterus
	5–6	0.1–0.2	4	Blastocyst hatches, begins implanting
2	7–12	0.1–0.2	5	Blastocyst fully implanted
	13	0.2	6	Primary stem villi appear; primitive streak develops
3	16	0.4	7	Notochordal process forms; gastrulation commences
	18	1–1.5	8	Neural plate and neural folds appear; primitive pit forms; vasculature begins to develop in embryonic disk
	20	1.5–2.5	9	Caudal eminence and first somites form; neuromeres appear in presumptive brain vesicle; primitive heart tube forming
4	22	2–3.5	10	Neural folds begin to fuse; cranial end of embryo undergoes rapid flexion; myocardium forms, and heart begins to pump; first two pharyngeal arches and optic sulci form
	24	2.5–4.5	11	
	26	3–5	12	Primordial germ cells begin to migrate from wall of yolk sac; cranial neuropore closes; optic sulci form
	28	4–6	13	Upper limb buds appear; caudal neuropore closes; urorectal septum begins to form; pharyngeal arches 3 and 4 form
				Dorsal and ventral columns begin to differentiate in mantle layer of spinal cord and brainstem; lower limb buds appear; septum primum and muscular ventricular septum begin to form in heart; spleen appears; ureteric buds appear; otic vesicle and lens placode appear; motor nuclei of cranial nerve appear
5	32	5–7	14	Spinal nerves begin to sprout; semilunar valves begin to form in heart; metanephros begins to develop; lens pit invaginates into optic cup; cerebral hemispheres become visible
	33	7–9	15	
				Hand plate develops; arterio-ventricular valves and definitive pericardial cavity begin to form; lens vesicle forms and invagination of nasal pit creates medial and lateral nasal processes; cranial nerve motor nuclei appear in ventral column of brainstem; sensory and para-sympathetic cranial nerve ganglia begin to form
6	37	8–11	16	Foot plate forms on lower limb bud; major calyces of kidney begin to form and kidneys begin to ascend; genital ridges appear
	41	11–14	17	Finger rays are distinct; bronchopulmonary segment primordia appear; septum intermedium of heart is complete; subcardinal vein system forms; nasolacrimal groove forms; cerebellum begins to form
7	44	13–17	18	Skeletal ossification begins; elbows and toe rays appear; intermaxillary process and eyelids form on face
	47	16–18	19	Trunk elongates and straightens; pericardioperitoneal canals close; septum primum fuses with septum intermedium in heart, minor calyces of kidneys are forming; urogenital membrane ruptures
8	50	18–22	20	Upper limbs bend at elbows
	52	22–24	21	Hands and feet approach each other at the midline
	54	23–28	22	Eyelids and auricles are more developed
	56	27–31	23	Definitive superior vena cava and major branches of the aortic arch established; gut tube lumen almost completely recanalized

Derived from Larsen (Ref. [19]) and O'Rahilly (Ref. [23])

1.2.2 Outline of Embryonic Development of Long Bones

Mesenchymal condensation has outlined the scapula and humerus of the upper limb and the pelvis and femur of the lower limb by the end of the 5th week. By early in the 6th week the developing models of the more distal limb bones are seen and chondrification has begun in humerus, ulna, and radius; by late in the 6th week carpal and metacarpal chondrification has begun; by mid-6th week femur, tibia, and fibula chondrify with tarsals and metatarsals by late 6th week; by the end of the 7th week all upper extremity

Table 1.3 Stages at which developmental features appear and events occur in human limbs

Feature	Stage for upper limb	Stage for lower limb
Limb bud	12	13
Length: width = 1.1	14	15
Apical ectodermal ridge	14–17	15–18
Hand plate/foot plate	15	16
Mesenchymal skeleton	15	16
Mesenchymal scapula/hip	16	15–18
Mesenchymal humerus, radius, ulna/femur, tibia, fibula	16	17
Chondrifying humerus/femur	16–17	17–18
Chondrifying radius/tibia	17	17–18
Chondrifying ulna/fibula	17–18	17–18
Finger rays/toe rays	17–18	18
Chondrifying metacarpus/metatarsus	17–18	18–19
Chondrifying carpus (except pisiform) tarsus	18–19	18–19
Chondrifying scapula/hip	18	19
Chondrifying proximal phalanges	18–19	19–21
Homogeneous shoulder and elbow/hip and knee	19	19
Homogeneous wrist/ankle	?	21
3-layered elbow/knee	?	21
Chondrifying middle phalanges	19–20	21
Chondrifying distal phalanges	20–21	21–23
3-layered wrist/ankle	21	23
Ossifying humerus and radius/femur and tibia	21–23	22–23
Ossifying ulna/fibula	22–23	23
Cavitation in shoulder and elbow/hip and knee	23	23
Cavitation in wrist/ankle	23	?

Derived from O'Rahilly and Gardner (Ref. [22])

bones are chondrifying as are all bones of the lower extremity except the distal phalanges which do so in week 8. Appearance of the diaphyseal primary ossification centers also follows a regular sequence: clavicle, early 7th week, followed by humerus, radius, ulna; femur and tibia, 8th week; scapula and ilium, 9th week; ischium, 15th week; calcaneus, 16th week and pubis, 20th week.

The developing model of each long bone is preformed in cartilage [18, 24, 25]. The undifferentiated mesenchymal cells, which have undergone condensation and started to outline specific bone shapes, then differentiate, surround themselves with a cartilaginous matrix, and take on the conformation of round chondrocytes. Histochemical stains, such as Safranin O-fast green, show the pinkish development of the matrix indicating the presence of glycosaminoglycans [26]. When the cartilage model of each of the long bones has been formed, the region where the joint will eventually be present is still filled with cells and is referred to as the interzone area. Early shaping of the epiphyseal ends of the bone occurs prior to necrosis and resorption of cells in the interzone area. When the latter occur, the joint cavity is formed and the complete model of the developing bone and joint has been formed. The cartilage model of the developing bone then increases in size by both interstitial and appositional growth of the chondrocytes. At a certain stage of development, the primary center of ossification forms. There is some confusion in the literature as to the exact meaning of this term. It can refer to the circumferential mid-diaphyseal rim of periosteum which synthesizes the initial bone of the cortex using the intramembranous mechanism without the mediation of a cartilage phase. It can also refer to mid-diaphyseal endochondral ossification within the cartilage model. Contemporaneous with the periosteal new bone formation, there is hypertrophy of cells in the mid-diaphyseal region of the cartilage model, calcification of the cartilage matrix, and vascular invasion of the hypertrophic cell lacunae accompanied by undifferentiated pre-osteoblast cells which then synthesize bone on the scaffold of the calcified cartilage matrix. The vascular invasion occurs in the areas of hypertrophic cells and serves to remove these and replace them with marrow cells and newly synthesized bone. The zone of hypertrophic cells or ossification front is then extended toward either end of the long bone.

The central replacement of hypertrophic chondrocytes with deposition of bone on the calcified cartilage cores encompasses what is referred to as the endochondral mechanism. In the periosteal region intramembranous bone formation extends the periosteal new bone sleeve. The periosteal development is always spatially somewhat more advanced toward either end of the bone than the central endochondral development. As this developmental sequence works its way toward either end of the bone, the cartilage forms itself into a specifically structured region referred to as the epiphyseal growth plate. This is characterized by specific conformations of the chondrocytes and serves as a functionally specialized region responsible for longitudinal growth.

1.3 Early Scientific Understandings of Bone Growth

Investigations to clarify mechanisms of bone growth began to show definitive advances early in the eighteenth century [27–38] (Table 1.1).

1.3.1 Theories of Embryogenesis. Preformationism and Epigenesis

Prior to the nineteenth century the main theory of embryogenesis was preformationism, the doctrine that the entire adult individual was present in miniature in the egg or sperm and development simply involved an increase in size. With more detailed observation however the concept of development by epigenesis became widely accepted. Epigenesis, as used in an older terminology, refers to the sequential development of morphological complexity in the embryo by the gradual and progressive differentiation of homogeneous material; each stage in development is considered to be dependent on and directed by its immediately preceding stage. The term epigenesis took on more specific meanings in the mid- twentieth century and now itself has evolved into a specific scientific discipline integral to biologic development (as discussed in Sect. 1.12.1 below).

Caspar Friedreich Wolff [39], Pander [40], and Karl Ernst von Baer [41] established the scientific validity of development by epigenesis. Wolff, in 1759, reported his observations on hen's egg development which showed blood vessels appearing where none previously existed and intestine forming from a flat plate. He concluded that epigenesis was real; "each part is first of all an effect of the preceding parts, and itself becomes the cause of the following part."

The structural basis of embryogenesis was more clearly revealed by Pander who defined the three germ layers in 1817 and then by von Baer [41], considered as the founder of embryology. von Baer performed elaborate descriptions of chick embryo development and its similarity to development of several other vertebrate types in 1828 and 1837. He recognized the significance of the three germ layers in development in all vertebrates and the epigenetic mechanism of development from the general to the specific. He recognized that the general path of differentiation proceeds in three sequential stages: the primary formation of the germ layer, histologic differentiation of cell and tissue types within the germ layers, and morphological differentiation to early organ formation. The mode of development is from simple to complex and from undifferentiated masses to new organs. Biological development involved progressive differentiation of the homogeneous, coarsely structured, general to the heterogeneous, finely structured, specific.

It is now recognized that much of development occurs in a self-assembly or "automatic" mode based on chemical and biophysical phenomena. Development is epigenetic in the sense that only early pre-patterns are rigidly determined after which each subsequent step is a combination of gene synthesis and automatic self-assembly based on the physical presence of certain molecules. Genes give approximate direction only. The detailed structure of multicellular organisms occurs on the basis of many intermediate levels of interaction each with its own immediate properties such that the edifice is virtually entirely epigenetic.

1.3.2 Hales, Belchier

Stephen Hales in 1727 showed that the bones grew in length by the addition of new tissue at their ends rather than interstitially [35]. He measured the leg bones of a young chicken twomonths after drilling two holes in the shaft to act as markers. The holes were no further apart although the bone itself had lengthened considerably. John Belchier [27], a London surgeon, observed that the bones of growing pigs and fowl which had been fed on madder were subsequently colored red. It soon became evident that red staining occurred only of the bone added while the animal was on the madder diet. By alternating periods of time on and off the diet (usually of a few weeks' duration) investigators were able to assess regions of bone deposition by studies after sacrifice. [Madder is a red dye (*Rubia tinctorum*) present in the roots of a plant.] The vital staining dye of the madder plant was subsequently recognized as alizarin [42]. The alizarin red stain is still used today in whole mount embryo studies. Staining with alcian blue outlines cartilage structures while alizarin red stains the bone [43, 44]. Subsequent studies by Duhamel, [30–34] Hunter [36], and Flourens [45–49] further demonstrated that bones grew in length by continuous increments at the ends.

1.3.3 Nesbitt

The formation of bone by the two now familiar endochondral and intramembranous mechanisms was noted early. Robert Nesbitt (1736) is recognized as being the first in describing the two methods of ossification in human fetal bone, one occurring directly in a membrane and the other via pre-existing cartilage [29, 38]. In his book Human Osteogeny published in 1736 he indicated that he would "show the ancient and common notion of all bones being originally cartilaginous to be a vulgar error." There were two methods, or species in his terminology, of ossification. "The bony particles in the fetuses begin to be deposited or to shoot either between membranes or within cartilages."

Nesbitt clearly noted that "the periosteum is a delicate fine and strong membrane which is spread on and covers not only all the bones in general but is also continued over the cartilages that have any connection with them; where from its situation it acquires the name of perichondrium". By formation of bone and membrane he was referring to periosteal new bone formation. He also recognized both the

inner and outer layers, or strata as he referred to them, of the periosteum. While bone formation from pre-existing cartilage models had previously been appreciated Nesbitt also showed that "some bones begin and continue to increase until they arrive at maturity without the least appearance of cartilage in or around them." The first species of ossification therefore was intramembranous bone. "The texture of that species of ossification which is produced between membranes by a careful and proper examination may be seen to be of small particles so conjoined together as to form fine bony threads or fibres which are disposed differently according to the particular formation of each bone and its several parts. This is most visible in thin and broad bones, especially in some of those which form the cranium." He noted that "you may observe the bony particles to be gradually multiplied and so conjoined in contact as to produce the appearance of small fine bony threads or fibres which then appear a little like radii shooting from a centre". With time there was an increase in the number of bony fibers which became "pressed so close together to form a single lamina or plate of bone." Membrane bone was that type formed in the cranium and it was also seen in cylindrical bones. He defined well the periosteal intramembranous bone sequence by noting that "their ossifications begin while the circumference of the part is not larger than a small pin in the form of a broad flat ring which surrounds the internal periosteum and is surrounded by the external. As these rings increase in breadth their fibres shoot toward both extremities of the part, not always in straight lines, but according to the particular figure the bone is designed by nature to be." He was aware that "the other species of ossification which first appears within a cartilage begins late …" Bone formation occurred in close association with blood vessels. He described formation of the secondary ossification centers and noted that vascularization immediately preceded bone formation in the epiphyseal cartilage. "The first small corpuscles of bone which become visible are always in that part of the cartilage which has the greatest quantity of red fluid appearing in it and they are not always placed close together but often at small distances from each other." In many diagrams Nesbitt indicated that "there are often 3 or more very considerable vessels going to and penetrating the ossifications" and commented that "near the ossification you will rarely miss feeling by the point of a knife bony particles." In drawings of the formation of the secondary ossification center of the distal femur he notes in one early section that vessels only are seen in the cartilage and in others as ossification increases centrally various additional vessels appear. Thus, even before the development of the cell theory there were clear descriptions involving transformation of cartilage to bone and the close relationship of vascularization to bone formation.

1.3.4 Duhamel

Henri-Louis Duhamel of France demonstrated from 1739 to 1743 that madder colored only those parts of the skeleton which were being formed at the time of its administration [30–34]. When madder feeding had been suspended for several weeks before sacrifice, the bone at the extreme ends of the shafts of long bones was uncolored as was that of the most peripheral cortical bone surrounding the mid-shaft region. By varying the time of madder feeding, he inferred that a long bone grew in length from its ends and in thickness by the progressive development of new bone on its outer surface. The discovery of the osteogenic function of periosteum is credited to Duhamel based on his interpretation of madder feeding and subsequent patterns of bone staining. Duhamel also found that bone grew in length at its extremities by drilling two holes at a measured distance in the shaft of a growing bone, filling them with metal plugs, and finding no difference in their position with time. He was unable to explain the increasing width of the marrow cavity with growth.

1.3.5 Hunter

The biologic mechanism of the increased diameter of the marrow cavity with growth was first understood by John Hunter who from 1740 onwards recognized both the growth of bone in length at its ends and the deposition of new bone by the periosteum on the outer surface of the shaft, but also the absorption of pre-existing bone which must occur within the marrow cavity as well as on the external surface of the expanded metaphyses [36]. He referred to this phenomenon as "modeling absorption" and clearly expressed the dynamic component of bone formation. Hunter used madder staining and diaphyseal hole drilling approaches to assess subsequent bone growth in pigs and fowls. He also discussed the modeling of the head and neck of the femur. Based on his madder studies of the proximal femur in growing pigs he recognized that "the addition of new matter was made to the upper surface, and a proportional quantity of the old removed from the lower, so as to keep the neck of the same form, and relatively in its place." [36] He established the principle that bones grow by two processes going on at the same time and assisting each other; the arteries bring the supplies to the bone for its increase; the absorbents at the same time are employed in removing portions of the old bone, so as to give to the new bone the proper form. "By these means the bone becomes larger, without having any material change produced in its external shape." [36] His work was the first which clearly recognized that absorption of bone was as essential to overall bone growth as deposition of bone.

1.3.6 Howship

Howship demonstrated the interplay of cartilage formation and bone formation in human and animal embryos based on studies with a solar compound microscope [50]. His text and illustrations presented in 1815 defined the embryonic 8 week human hand showing the primary ossification "rings of bone" of the metacarpals and phalanges. He identified cartilage canals in the epiphyseal ends of the bones and was able to outline the laminar structure of cortical and trabecular bone tissue. In a remarkable tissue section of the distal femur of a newborn child the epiphyseal–metaphyseal junction was magnified. The edge of the newly formed bone, by which he refers to the metaphyseal region, "exhibited an appearance of small short pointed villi shooting forward from the surface of the bone into the substance of the cartilage." He noted that "all sections exhibited an apparent alteration in the texture of the cartilage upon the surface connected with the bone. In many instances the cartilage seemed to be more opaque here than elsewhere, this slight opacity forming a line equal to 120th of an inch in breadth." This latter reference is to the physeal cartilage which indeed can be distinguished from the adjacent epiphyseal cartilage at low powers of magnification.

Howship further studied the cartilage–bone junction in a distal femur from a 3-week-old child. He examined the bone from the diaphyseal region toward the growth region. "It was observed that in proceeding from the middle of the cylindrical bones, where the medullary spaces are larger and the cancellated structure stronger towards the more recently formed extremities of the bone, the ossific masses become more numerous, of a lighter substance, and a thinner texture; the same gradation being continued up to the margin of the newly ossified surface, where the structure is most curiously wrought, and so exquisitely fine as scarcely to admit a description." His description clearly relates to the changes at the lower end of the physis and the furthest reaches of the adjacent metaphysis. "It was ascertained that the first and earliest state in which the particles of ossific matter become apparent, after they have formed a mass by their cohesion, may be considered as an assemblage of the finest and thinnest fibers, molded into the form of short tubes, arranged nearly parallel to each other, and opening externally upon the surface connected with the cartilage. These tubes appeared to correspond in number to the villi noticed in the last examination." Similar studies were also performed in animals. Howship clearly defined cartilaginous canals within the cartilage tissue at the ends of the bone. He noted that the principle of bone formation involving the appearance of the cartilage and ossifying bones "was in every respect precisely similar" in many species and that "the same purpose of ossification is accomplished by one and the same means." Howship concluded that the first rudiments of ossification in the long bones were associated with vascularization and occurred "upon the internal surface of the periosteum, which produced a portion of a hollow cylinder; this form of bone having been found antecedent to the evolution of any cartilaginous structure."

Howship mentioned the importance of the circulation both for cellular growth and for providing the means of calcification. He noted the value of the cartilaginous mode of bone formation indicating that "at a certain stage of the process the mode of operating is changed in order that it may proceed more expeditiously. A cartilage is formed, which, by the nature of its organization, and by admitting of a specific provision of cavities and canals lined with vascular membranes, which secrete an abundant store of gelatinous matter, is adapted to this particular purpose; while at the same time it serves to determine the future figure of the extremity of the bone by establishing and conducting the ossification within its own substance". He indicated that "from the period when the ossification proceeds in the mode above described by the medium of cartilage the process is continued in the same uniform manner until it has completed the growth of the bone. The growth of the epiphyses at the ends of the bone is also effected by the same means." He also noted the simultaneous formation of bone peripherally in cylindrical bones being "deposited primarily in the form of fine thin tubular plates: a mode of deposition of all others the most favorable for their being subsequently remodeled and for facilitating all the subsequent changes of structure they are destined to undergo."

He commented on the mechanical aspects of bone development noting that "the principal agent in extending the cylinder and in effecting the subsequent progressive changes of structure which in a growing bone are continually taking place appears to be simply the mechanical pressure exerted by the fluid secretions within the medullary cavities of bone, this power operating successively in different directions according to the particular determination given by the circulation." "The particular simplicity observable in the mode of production of the bones of the skull affords a strong argument in favor of the opinion that pressure variously modified constitutes one of the most efficient instruments in the hand of nature."

1.3.7 Flourens

Flourens also emphasized that longitudinal growth of the long bone took place at the ends (Fig. 1.3a). He outlined six major principles of bone growth based on extensive reference to the work of Duhamel as well as his own experiments