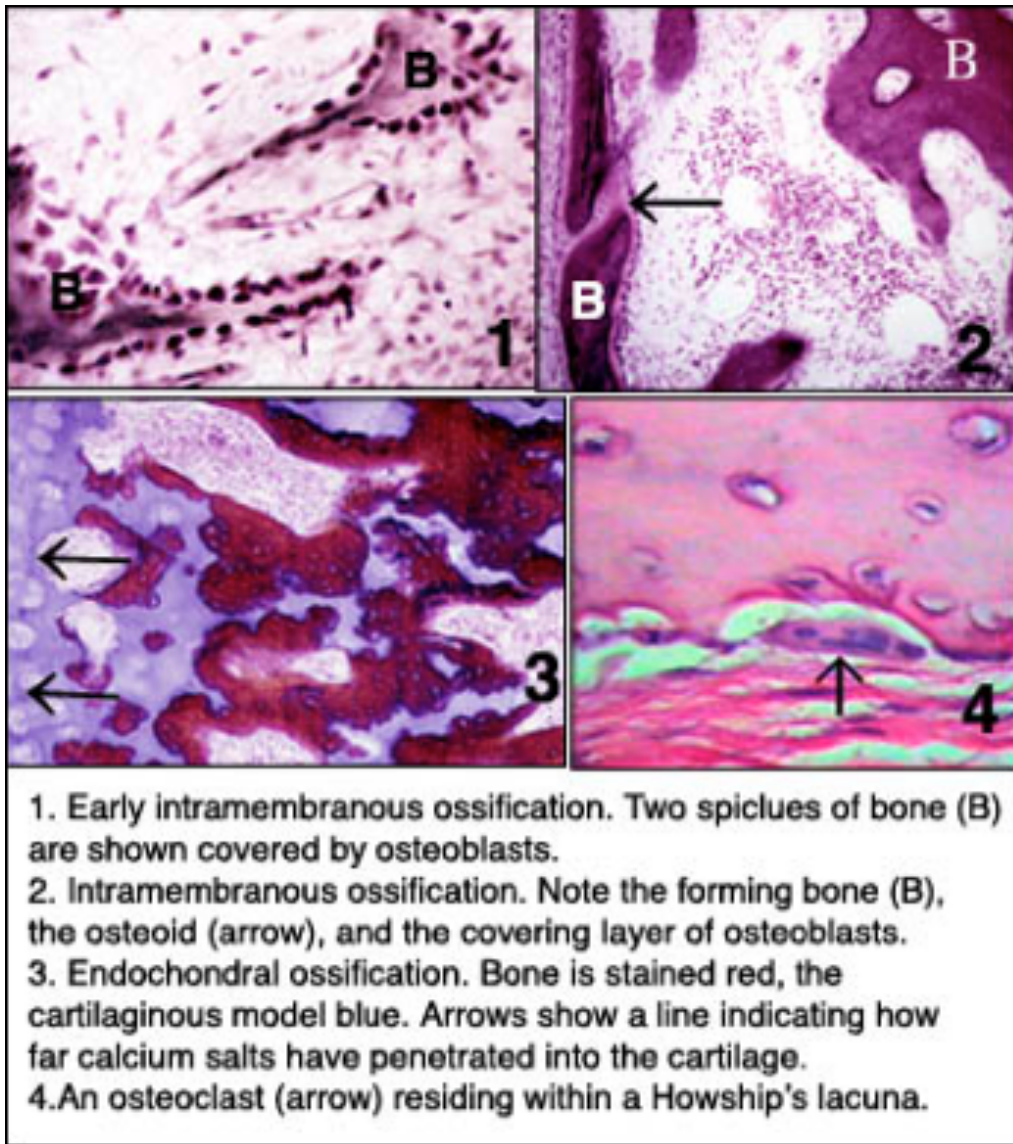


Cartilage and Bone Development



Cartilage

All cartilage arises from mesenchyme, and at sites where cartilage is to form, the mesenchymal cells lose their processes, round up, proliferate, and crowd together in a dense aggregate of chondrogenic cells (a center of chondrification). The cells in the interior of the mass are chondroblasts, which lay down fibers and ground substance. As the amount of intercellular material increases, the cells become isolated in individual compartments (lacunae) and take on the characteristics of chondrocytes. New chondroblasts are recruited from the more peripherally placed immature cells. The perichondrium is derived by condensation of the mesenchyme that surrounds the developing cartilage. Further growth occurs by expansion of the mass of cartilage from within (interstitial growth) and by addition of new cartilage at the surface (appositional

growth). Interstitial growth results from proliferation of young chondrocytes that divide and lay down a new matrix to produce an internal expansion of cartilage. Such growth occurs only in young cartilage that is soft enough to allow daughter cells to move away from each other after division. Appositional growth occurs as a result of perichondrial chondrogenic activity. The cells called chondrogenic cells are recruited from the inner surface of the perichondrium. These cells form a layer, continue to divide and differentiate into chondroblasts, secrete matrix about themselves, and add new cartilage to the surface. Once entrapped within their own matrix these cells are properly termed chondrocytes. Although adult perichondrium retains some potential to form new cartilage, the capacity for growth and repair is limited by its avascularity. Adult cartilage has the lowest mitotic rate of any skeletal tissue. Where elastic cartilage forms, chondroblasts at first elaborate fibers that are neither elastic nor collagenous in character. These indifferent fibers subsequently mature into elastic fibers. Fibrous cartilage develops much as does ordinary connective tissue, but some fibroblasts transform into chondroblasts and secrete a small amount of cartilage matrix about themselves.

Bone

Bone is mesenchymal in origin and develops by replacement of a preexisting tissue. If bone is formed directly in a primitive connective tissue, the process is called intramembranous ossification; when replacement of a preformed cartilaginous model is involved, the process is known as endochondral ossification. The essential process of bone formation is the same in both, however.

Intramembranous Ossification

The flat bones of the cranium and part of the mandible develop by intramembranous ossification; these bones frequently are called membrane bones. In the areas where bone is to form, the mesenchyme becomes richly vascularized, and the cells proliferate actively. Some cells undergo changes that subsequently lead to bone formation and are regarded as osteoprogenitor cells.

Structurally, they closely resemble the mesenchymal cells from which they arise, and their identification depends mostly on their proximity to forming bone. The osteoprogenitor cells enlarge and transform into osteoblasts that lay down layers of matrix. The plasmalemma of osteoblasts has receptors for parathyroid hormone and 1, 25-dihydroxyvitamin D₃ and can be stimulated by these molecules. The osteoblasts remain in contact with one another by long tapering processes, and as more and more matrix is deposited, the cells and their processes become entrapped in the matrix. The cells then are called osteocytes. Ultimately the cells and cell processes are completely enclosed in the matrix, thus forming the lacunae and canaliculi. The first matrix consists of type I collagen fibers embedded in a glycosaminoglycan gel that contains specific glycoproteins and, being unmineralized, is soft. At this stage, the matrix is called osteoid; after a short lag period, it becomes mineralized to form true bone. Osteoblasts elaborate osteocalcin and alkaline phosphatase which hydrolyzes phosphate- and calcium-containing substrates to release phosphate and calcium. In addition,

osteoblasts release matrix vesicles which concentrate calcium and phosphate during the mineralizing process. Blood alkaline phosphatase levels are good indicators to access not only osteogenesis but also bone repair. As osteoblasts become trapped in the newly deposited matrix and become osteocytes, new osteoblasts are recruited from differentiation of osteoprogenitor cells. Bone development by intramembranous ossification occurs in several foci throughout the mesenchymal field and results in the formation of scattered, irregular trabeculae of bone that increase in size by addition of new bone to their surfaces. The resulting bone is of the spongy type. In the first formed bone, collagen fibers run in every direction; the bone is called woven bone and has randomly scattered osteocytes and lacks a lamellar arrangement. Woven bone is gradually replaced by lamellar bone. In areas that become compact bone, such as the inner and outer tables of the skull, the trabeculae continue to thicken by appositional growth, and the spaces between them are gradually obliterated. As bone encroaches on vascular spaces, the matrix is laid down in irregular, concentric layers that surround blood vessels and come to resemble osteons, except that the collagen fibers in the lamellae are randomly arranged. These are called primary osteons. Between the plates of compact bone, where spongy bone persists, thickening of the trabeculae ceases and the intervening connective tissue becomes the blood-forming marrow. The connective tissue surrounding the developing bone condenses to form the periosteum. The osteoblasts on the outer surface of the bone assume a fibroblast-like appearance and are incorporated into the inner layer (cambium) of the periosteum, where they persist as potential bone-forming cells. Similarly, osteoblasts on the inner surface and those covering the trabeculae of spongy bone are incorporated into the endosteum and also retain their potential for producing bone.

Endochondral Bone Formation

The bones of the vertebral column, pelvis, extremities, face, and base of the skull develop by formation of bone in a cartilage model or precursor that must be removed before bone can be laid down. Endochondral bone formation involves simultaneous formation of the bone matrix and removal of the cartilage model. The cartilage does not contribute directly to the formation of bone, and much of the complex process involved in endochondral bone formation is concerned with removal of cartilage. The first indications of ossification of a long bone appear in the center of the cartilage model in the region destined to become the shaft or diaphysis. In this area, called the primary ossification center, the chondrocytes hypertrophy and their cytoplasm becomes vacuolated. Lacunae enlarge at the expense of the surrounding matrix, and the cartilage between adjacent lacunae is reduced to thin, fenestrated partitions. The matrix in the vicinity of the hypertrophied chondrocytes becomes calcified by deposition of calcium phosphate. Diffusion of nutrients through the calcified matrix is reduced, and the cells undergo degenerative changes leading to their death. While these events take place in the cartilage, the vascular perichondrium in this area assumes an osteogenic function. A layer of bone, the periosteal collar, forms around the altered cartilage and provides a splint that helps maintain the strength of the shaft. The osteoblasts responsible for development of the bone are derived from perichondrial cells immediately adjacent to the cartilage. In the region of the boney collar, the perichondrium now has become a

periosteum. Blood vessels from the periosteum invade the altered cartilage forming the periosteal bud. Contained within the connective tissue sheath that accompanies the blood vessels are cells with osteogenic properties. With death of the chondrocytes there is no longer a means of maintaining the matrix, which softens. With the aid of the erosive action of invading blood vessels, the thin plates of matrix between lacunae break down. As the adjoining lacunar spaces are opened up, narrow tunnels are formed that are separated by thin bars of calcified cartilage. Blood vessels grow into the tunnels, bringing in osteogenic cells that align themselves on the surface of the cartilaginous trabeculae, differentiate into osteoblasts, and begin to elaborate bone matrix. At first the matrix is laid down as osteoid, but it soon becomes mineralized to form true bone. The early trabeculae thus consist of a core of calcified cartilage covered by a shell of bone. These primitive trabeculae soon are removed both by continued resorption of the calcified cartilage and through the activity of osteoclasts that appear on the bony shell. An expanding cavity is opened up within the developing shaft. Support for the bone is provided by extension of the periosteal collar, which also becomes thicker as the periosteum lays down new bone at the surface. This entire process continues as an orderly progression of activity that extends toward both ends of the developing bone. Several areas or zones of activity can be distinguished in the cartilage. Beginning at the ends farthest from the primary ossification center, these are the following:

Zone of reserve cartilage. This area consists of hyaline cartilage and initially is a long zone but shortens as the process of ossification encroaches on it. The cells and their lacunae are randomly arranged throughout the matrix, and there is slow growth in all directions.

Zone of proliferation. In this region the chondrocytes actively proliferate, and as the cells divide, the daughter cells become aligned in rows separated by a small amount of matrix. Within the rows, the cells are separated only by thin plates of matrix. Growth occurs mainly in the regions farthest from the reserve cartilage, and the length is increased more than the diameter.

Zone of maturation and hypertrophy. Cell division ceases and the cells mature and enlarge, further increasing the length of the cartilage. Lacunae expand at the expense of the intervening matrix, and the cartilage between adjacent cells in the rows becomes even thinner.

Zone of calcification and cell death. The matrix between the rows of chondrocytes becomes calcified and the cells die, degenerate, and leave empty spaces. The thin plates between the cells break down, and the lacunar spaces are opened up to form irregular tunnels bounded by the remains of calcified cartilage that had been present between the rows of cells.

Zone of ossification. Vascular connective tissue invades the tunnel-like spaces and provides osteogenic cells that differentiate into osteoblasts. These gather on the surface of the calcified matrix and lay down bone.

Zone of resorption. Osteoclasts appear on the trabeculae and begin to resorb the bone. Ultimately all the calcified cartilages and their bony coverings are resorbed, and the marrow cavity increases in size.

As these changes occur within the cartilage, the periosteal collar increases in length and thickness, gradually extending toward the ends of the developing bone to provide a

continuous splint around the area of changing and weakening cartilage. At about the time of birth, new centers of ossification, epiphyseal, or secondary ossification centers, appear in the ends of long bones. The cartilage of the epiphyses passes through the same sequences as in the diaphysis, but cartilage growth and subsequent ossification spread in all directions and result in a mass of spongy bone. Ultimately, all the cartilage of the epiphysis is replaced by bone except for that at the free end, which remains as articular cartilage. Cartilage also persists between the epiphysis and diaphysis and forms the epiphyseal plate, whose continued growth permits further elongation of the bone. Ossification occurs at the diaphyseal side and for a time formation of new cartilage and its replacement by bone take place at the same rate so that the thickness of the epiphyseal plate remains constant. Eventually, growth in the plate ceases, the cartilage is replaced by bone (closure of the epiphysis), and further increase in length of the bone is no longer possible. Increase in diameter of the bone results from deposition of bone at the outer surface as a result of the activity of the periosteum, while at the same time osteoclasts resorb lesser amounts of bone at the endosteal surface.

Thus, not only is the gross diameter increased, the thickness of the compact bone and the size of the marrow cavity also increase. Bone formed by the periosteum represents intramembranous bone and results in a lattice of irregular, bony trabeculae. This is converted to compact bone by a gradual filling of the spaces between trabeculae. Bone is laid down in ill-defined layers, and where they surround and enclose blood vessels, the first Haversian systems or primitive osteons are formed, in the same way that they form during development of membranous bone. In the primitive osteons the collagen is laid down at random in each lamella. Constant remodeling and reconstruction of bone occurs throughout life, with the primitive osteons being replaced by definitive osteons; these in turn are replaced by successive generations of osteons. Replacement of osteons begins with formation of tunnels within the compact bone as a result of the osteolytic activity of osteocytes and osteoclasts. The Haversian canals widen and enlarge to reach considerable lengths. When osteolytic activity ceases, osteoblasts become active recruited from osteoprogenitor cells within the endosteum, and bone is deposited on the walls of the formed cavity until it is refilled as a definitive osteon. The process continues throughout life; resorption cavities continue to form and to be filled with third, fourth, and higher generations of osteons. The resorption cavities do not always coincide with the osteon being replaced, and fragments of older osteons remain as interstitial lamellae.

Cartilage, because of its capacity for rapid growth as compared with bone, is the ideal rigid, lightweight tissue for the embryonic skeleton because it can keep pace with the rapidly growing fetus. Likewise, it is growth of the hyaline cartilage in the epiphyseal plate that allows for the rapid growth of long bones during adolescence. Ultimately, cartilage of the provisional skeleton, as well as that of the epiphyseal plate, is replaced by the slower-growing bone.

Longitudinal growth of a developing bone depends on the interstitial growth of cartilage in the zone of proliferation of the epiphyseal plate and on the enlargement of chondrocytes in the zone of maturation and hypertrophy nearby. Cartilage of the epiphyseal plate is under the influence of growth hormone secreted by cells in the anterior pituitary gland. Thyroid hormone modulates the activity of growth hormone, and at puberty, androgens and estrogens contribute to masculinization or feminization of the

skeleton, respectively. Increased width of the bone is achieved by appositional growth and the deposition of bone at the boney collar. The first primitive trabeculae observed during endochondral bone formation consist of cores of cartilage covered by bone. They are formed in the region where the boney collar is thinnest and serve as internal struts to support the developing bone until the collar is thick enough to provide support. As the collar increases in thickness, osteoclasts remove bone at the endosteal surface, thereby enlarging the marrow cavity.

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