Ossification (formation of bone)

- Osteoblasts are responsible for producing the extracellular matrix of the bone and these osteoblasts cannot form bone unless a surface ″scaffold/ base″ or support area is present in order to build up bone.

-During embryonic development we have a tissue that can easily grow (undergoes mitosis) and then bone can be formed within this tissue

-We have two tissues in embryo that will be replaced by bone, the first one is hyaline cartilage (most of the skeleton of the embryo is hyaline cartilage) and the formation of the bone within the cartilage is called *endochondral ossification* (formation of bone within the cartilage, endo: inside or within).

- The second type is *intramembranous ossification,* you've got fibrous membrane and then bone will be formed within this fibrous membrane.

-These two types of ossification start in the prenatal life during embryonic development.
Prenatal: before birth
Postnatal: after birth

Intramembranous ossification: formation of bone on a top or within fibrous membrane.
Endochondral ossification: formation of bone within a cartilage ″hyaline cartilage″.

Endochondral ossification is responsible for the formation of most of our bones (long, short and most irregular bones)
But intramembranous ossification is responsible for the formation of most flat bones (flat bones of the skull and clavicle**)**, this type is also involved in the growth of long and short bone (will be discussed later). Remember that bone is covered by periosteum and lined by endosteum and these are membranes.

Intramembranous ossification

- There is a fibrous membrane which is composed of mesenchymal cells, extracellular matrix (mainly collagen fibers)

- These mesenchymal cells at certain area condense and differentiate to form **osteoblasts**, these osteoblasts start to synthesize extracellular matrix

Remember: Osteoid is the ECM before mineralization

- After few days these osteoblasts will mineralize the extracellular matrix, and these cells will be trapped or encased within the hard extracellular matrix in spaces called lacunae so now they are called osteocytes.

Note: Collagen fibers form a base/scaffold for the formation of bone.

- The starting points for bone formation are called **centers of ossification.**

- Within this membrane more than one center of ossification appears (more than one condensation of mesenchymal cells) and they fuse to form the trabecular bone but in the beginning it is woven bone.

- Centers of ossification will fuse to form trabecular bone around embryonic blood vessels

- Future marrow cavity spaces are filled with embryonic blood vessels and mesenchymal cells forming the future red bone marrow.

- The mesenchymal cells on the edges (outer and inner) differentiate into fibroblasts in order to form periosteum.

- Several centers of ossification appear and fuse to form the trabeculae of spongy bone around the blood vessels of the embryo, however we know that the final appearance of flat bone differs from this

description, Flat bone is a trabecular bone sandwiched between two layers of cortical bone, so by the osteoclastic activity, the bone will be removed from the edges and cortical bone will be deposited.

- Osteoblasts form a row of cells which resembles simple cuboidal epithelium, and remain on the surface of bone, to enable more growth of bone.

- The skull is actually more than one bone connected by joints called "*sutures*"

- At the time of delivery, not all the fibrous membranes were replaced by bone, some areas between flat cranial bones would still be filled with fibrous connective tissue called ***fontanelles*** "soft spots"

- The importance of fontanelles during the delivery of baby:

A. *Facilitate delivery.*
B. *Prevent compression of the brain of baby.*
C. *Give room for the brain to grow*.

- During the first two years of age, the maximum growth of the brain takes place, these fontanelles close around the two years of age in order to allow the brain to grow, as fibrous tissue has the ability to grow but bone does not.

Endochondral ossification

- The shape of the cartilaginous model is exactly like the future bone. This cartilaginous model is composed of hyaline cartilage covered by perichondrium. The perichondrium is composed of two layers; outer fibrous, and inner cellular which contains chondrogenic cells that can differentiate into chondroblasts.

- The chondrogenic cells at the end of the second month of development differentiate into *osteoblasts* **not** chondroblasts.

- These cells are called osteochondrogenic cells, as they can differentiate into osteoblasts or chondroblasts. The resulting osteoblasts will form bone. The bone formed is called bone collar (as it surrounds the diaphysis)

- Keep in mind that the cartilage is avascular, which means that the formation of this bone collar will prevent oxygen and nutrients from reaching cartilage cells in the center, so chondrocytes in the center will die leaving behind spaces

- So again, perichondrium converts to periosteum by the activity of the osteochondrogenic cells of perichondrium, formation of bone collar, cavitation of the center of diaphysis (bone collar prevents oxygen and nutrients from reaching the chondrocytes in the center, cavities will be formed), this is called **primary ossification center.**

- The importance of bone collar is also to provide stability during the cavitation process.

- The cartilage anywhere else will continue to grow.

- Chondrocytes in the middle of diaphysis will not die directly; they will instead go through gradual death.

- As oxygen and nutrients do not reach chondrocytes, they are now under stressful conditions (anaerobic condition), the chondrocytes will respond in two ways

- First they increase their size (swell), they accumulate within their cytoplasm glycogen, we call this condition ***hypertrophy*** **(first response**). When cells get larger, the ECM gets thinner.

  Hyper: increase, trophy: size

- The **second response of the dying cells** is the secretion of osteocalcin and alkaline phosphatase enzyme that increase the local concentration of calcium and phosphate leading to calcification of the matrix of the

cartilage, this condition will further cut off the diffusion of oxygen and nutrients to the chondrocytes so they die leaving behind spaces and calcified matrix.

- These chondrocytes have committed suicide ☹; they have separated themselves from the surrounding environment by calcifying their matrices.

- Not only it is cavitation or degeneration in the middle, it is cavitation with calcification of the relating matrix.

- Now the bone is covered by periosteum and it contains blood vessels. A blood vessel will *penetrate* the diaphysis to enter the center of diaphysis *carrying* with it oxygen, nutrient, osteogenic cells, osteoblasts and osteoclasts. This is called **periosteal bud** (as it originates from periosteum).

- Again, the osteoblasts need a surface to form bone, in case of intramembranous ossification, the osteoblasts used collagen fibers to build up bone, and in this case (endochondral ossification), osteoblasts use the calcified matrix of the cartilage as a scaffold to build up bone.

- The osteoblasts have now found a scaffold (represented by the calcified matrix of the cartilage) and will begin to build up bone. Now we have bone formed in the center of the diaphysis

- Remember that the diaphysis of long bone has a medullary canal (not bone). By the action of osteoclasts, the bone along with the calcified cartilage matrix are resorbed and the medullary canal is formed.

  So bone starts solid, and then becomes hollow later on.

- Around the time of birth, another center of ossification will appear inside the epiphysis, it's called **secondary ossification center**, and we can have more than one secondary ossification center in the epiphysis. For example; in the proximal end of humerus we have three secondary ossification centers:
- One for the head humerus.
- One for the greater tubercle.
- One for the lesser tubercle.

The direction of bone growth:

1. Inside the *diaphysis* is ***proximal*** and ***distal*** (upward &downward)
2. Inside the *epiphysis* is ***radial*** (towards the periphery).

If you have a histological section which has a primary center of ossification but the epiphysis is still cartilage, you know this section is most probably taken from an embryo. Remember that most secondary ossification centers appear after birth.

All cartilage will be replaced by bone except in two areas:
1. Articular cartilage: hyaline cartilage.
2. Epiphyseal plate (growth plate): junction between epiphysis and diaphysis, this plate is responsible for growing taller during childhood. And it closes at late adolescence when growth stops

Note: By the time a fetus is born, most of the cartilage has been replaced with bone. Some additional cartilage will be replaced throughout childhood, and some cartilage remains in the adult skeleton

Note that: Cartilage does not become bone. Instead, cartilage dies first and serves as a template to be completely replaced by new bone

Growth plate

- Upon magnification of the growth plate (here we are talking about the proximal growth plate), it shows 5 distinct regions (zones) of cellular activity **from proximal to distal**:
  (Starting with the cartilage farthest from the ossification center in the diaphysis)

1. Resting zone (zone of reserve cartilage): composed of normal, resting cartilage, which does not undergo any mitosis, it has few chondrocytes with wide ECM
2. Proliferative or growth zone: the cells now begin to form columns, as they undergo mitosis so there is a higher number of cells, as chondrocytes begin to divide rapidly.
3. Hypertrophy zone: there is an increase in the size of chondrocytes, we are getting closer to the bone collar (less oxygen and nutrients).
4. Calcification zone: chondrocytes about to die release matrix vesicles (containing alkaline phosphatase) and osteocalcin to begin matrix calcification (by the formation of hydroxyapatite crystals).
Note: calcification of the matrix leads to different staining reaction resulting in a darker color.
5. Ossification zone: osteoblasts start to build up bone. Osteoblasts settle in a layer over the calcified cartilage matrix and secrete osteoid which becomes woven bone .This woven bone is then remodeled as lamellar bone.

How to differentiate between the calcified region of cartilage and bone?
Calcified matrix of the cartilage: no cells, **basophilic.**
Calcified matrix of the bone: contains cells, **eosinophilic.**

- When does the lengthening of bone stop?

Lengthening stops as closure of the growth plate occurs at the end adolescence, in males 20-21 while in female at 18 years of age.

- How does growth plate close?

Chondrocytes in proliferative zone undergo mitosis under the influence of growth hormones (and other hormones), at the end of adolescence, growth hormone level decreases so mitosis of these chondrocytes will cease, it will be replaced completely by bone then diaphysis and epiphysis meet together at the ***epiphyseal line*** not plate.

Appositional Bone Growth (Bone growth in width)

Remember that the lining of medullary canal is endosteum and the cover of bone is periosteum. As the periosteum contains osteogenic cells (that differentiate into osteoblasts), the diaphysis can grow in width, but at the

same time, to maintain proportionality of the bone, the medullary canal needs to also increase in size. The osteoclasts which line the endosteum resorb bone, so bone increases in thickness from the outside, but it is also being resorbed from inside, in order to increase the diameter of the medullary canal.

Appositional growth (growth in width/ growth in the circumference of long bones) is a combination of two activities:

1- Osteoblastic activity in the periosteum.
2- Osteoclastic activity in the endosteum.

Bone remodeling

Continuous process of bone deposition and resorption.
Resorption of old bone matrix and formation of new bone matrix (cellular activities for bone resorption and bone formation)

Importance of bone remodeling:

1. Reshaping of the skeleton especially during the growth
2. Repair of micro-fractures that do not need medical attention which are caused by everyday stresses, so the bone corrects itself through this mechanism.
3. Maintenance of calcium levels inside the blood. Calcium levels in the blood are always kept within certain limits through bone deposition and resorption.

1-Reshaping of the bone

The bone grows in length and at the same time the bone is resorbed in order to reshape the bone to keep the exact proportions between epiphysis and diaphysis

⇒ We always say that diaphysis is the constricted portion of the bone but the epiphysis is the swollen portion of the bone

⇒ Now, let's compare the original shape of the bone with the future shape of the bone, notice that the growth is accompanied with reshaping

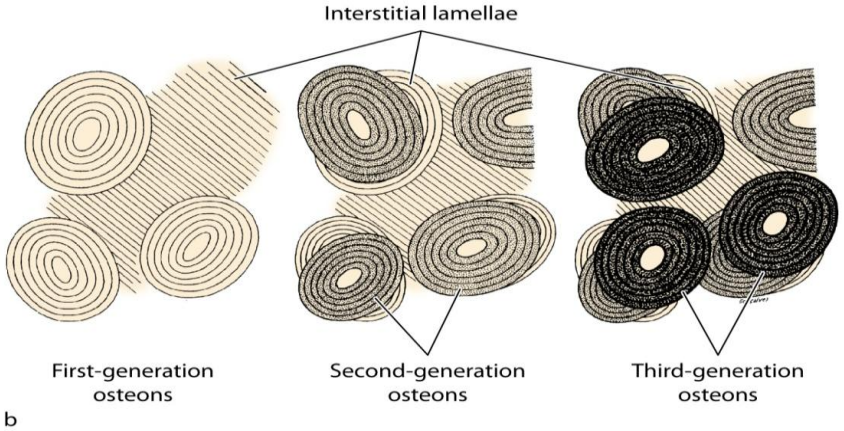
⇒ Let's assume there is no osteoclast activity (only the deposition occurs) so no bone resorption occurs and the result is too thick bone, the proportion is very wrong.

2-Correction of microfractures of the bone

⇒ When there is a microfracture , the osteoclasts eat up the area of bone fracture and the osteoblasts synthesize new bone

Throughout our life, our bones are in a continuous process of bone deposition and bone resorption. Remember: the compact bone is composed of osteons, and the bone in between these osteons is called interstitial lamellae and these interstitial lamellae represent the old Haversian systems. Osteoclasts drill through the bone (forming tunnels) , then oseoblasts build bone toward the center of these tunnels

⇒ Because of bone remodeling, osteoclast will resorb bone and the osteoblast will build new bone (new osteon), so we have more than one generation of Haversian systems



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- 5% of our bone is recycled weekly
- All the spongy is replaced every 3 or 4 years
- But the compact bone is completely replaced every 10 years
- The bone growth is regulated by hormones such as growth hormone, thyroid hormone and by sex hormone (male hormones produce wider shoulder and larger bones in general but females have wider hips and in general smaller bones)
- Any disturbances in these hormones will result in over- growth of the bone or undergrowth of the bone. Examples: gigantism: excessive growth hormone production results in overgrowth of the bone, while the opposite is called dwarfism
- In general, our bones respond to mechanical stresses applied to our body and also to gravity
- Bones respond to muscles pulling on them (mechanical stress) and to gravity by keeping the bones strong where they are being stressed.
- To understand the gravity effect, lets see this example:
  Astronauts lose an average of more than 1% bone mass per month spent in space (zero gravity).
- High rate of bone deposition in specific areas (for example: tuberosity, ridge, crest) where muscles are attached
- Weight bearing activities → stronger projections where muscles/ligaments attach
- Immobile patients (bedridden) lose bone density because they don't exercise muscles that would otherwise build skeletal strength through motion.

The Joints

A joint is where two or more bones meet. Also known as an articulation

- ❖ The joints in our body are divided according to the range of movement and the type of tissue presents in the space between the articulating bones

Structurally, joints are classified into three main types, depending on their general morphology:

1- Fibrous joints are connected by dense connective tissue and have no joint cavity.
2- Cartilaginous joints are connected by hyaline/ fibrocartilage and have no joint cavity.
3- Synovial joints have a synovial, fluid-filled cavity that surrounds the articulating bones.

Functionally, joints are classified into three main types, depending on their function:

1- Synarthrosis: Joints that do not provide any movement.
2- Amphiarthrosis: Joints that only provide a small degree of movement.
3- Diarthrosis: Joints that allow free movement.

Fibrous joints

In a fibrous joint, the two bones are connected by dense fibrous connective tissue. These joints can be either synarthrotic or amphiarthrotic (no movement or slight movement). Examples: sutures, gomphoses and Syndesmoses

Sutures occur only in the skull where adjacent bones are linked by a thin layer of connective tissue termed a sutural ligament.

Gomphoses occur only between the tooth and surrounding bone. In these joints, short collagen tissue fibers run between the root of the tooth and the bony socket. Some consider Gomphoses as synarthrotic (no movement) and some consider them as amphiarthrotic (slight movement)

Syndesmoses are joints in which two adjacent bones are linked by a ligament/membrane. Example is the interosseous membrane between the radius and ulna in the forearm.

Cartilaginous joints:

There are two types of cartilaginous joints, synchondroses and symphyses

Synchondroses between the two articulating bones there is only hyaline cartilage such as growth plate between the epiphysis and diaphysis of developing long bones. These joints allow bone growth and eventually become completely ossified. (synchondrosis allows no movement, synarthrotic)

Symphyses occur where two separate bones are interconnected by fibrocartilage. Most of these types of joints occur in the midline and include the pubic symphysis between the two pelvic bones, and intervertebral discs between adjacent vertebrae. (symphysis allows slight movement, amphiarthrotic)

Synovial joints

- ⇒ The synovial joints are considered as a diarthrosis
- ⇒ They permit movement inside the joints, these joints permit great mobility, Freely movable joints
- ⇒ Type of tissue between the articulating surfaces: synovial fluid
- ⇒ Ex: shoulder and elbow, etc.
- ⇒ Arthrosis mean articulation
- ⇒ Synovial joints are surrounded by capsules, reinforced by ligaments. The capsule is lined by synovial membrane, synovial membrane secretes synovial fluid inside the joint cavity. The articular surface is covered by articular cartilage
- ⇒ Synovial joints are further sub-classified according to the shapes of the articulating surfaces into: ball and socket, hinge, pivot, plane, saddle, and ellipsoid (condyloid) joints