<u>HISTOLOGY of CARTILAGE, BONE and SKELETAL MUSCLE</u>

I. CARTILAGE

A. Hyaline Cartilage

Slide 173. Trachea, Homo, H&E
Virtual Slide ID 294

<u>Slide 173</u>. There are several oval pieces of hyaline cartilage on this slide. Examine them with low power first. Each cartilage cell is situated in a space called a **lacuna**, and it is separated from other cartilage cells by the firm matrix material it secretes. Each piece of cartilage is surrounded by a **perichondrium**.

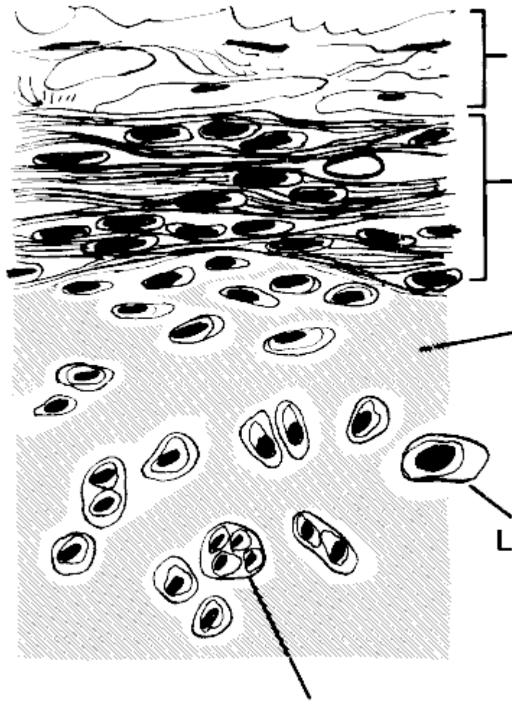
There are no blood vessels internal to the perichondrium. Gases and dissolved nutrients must reach the cartilage cells by diffusion through the matrix.

In places the cells are in groups of 2, 4, or 8, etc. These are **isogenous groups** and the progeny of a single chondrocyte that divided a few times during interstitial growth of the cartilage.

The cells near the periphery of the cartilage appear to be intermediate between fibroblasts and cartilage cells. This should suggest to you that they are in the process of becoming cartilage cells and that cartilage also grows by **appositional growth**.

Note the basophilia of the cartilage matrix. The amount of basophilia group (**capsular or territorial matrix**) and therefore acid mucopolysaccharides is greatest closest to each chondrocyte or isogenous group. Less basophilic and **inter-territorial** matrix lies between the lacunae.

There are collagen fibers within the matrix but they are not visible because they have almost the same refractive index as the ground substance, and because they are small.



B. Elastic Cartilage

Slide 52. Epiglottis, Homo, Elastin and H&E stain
Virtual Slide ID 330

Slide 52 is a section of human epiglottis that illustrates a type of cartilage in which the elastic fiber has become the predominant fiber.

There are two sections on the slide. The elastic fibers are stained black in one of the sections and red in the other. The fibers are so numerous that it is difficult in places to see the ground substance.

The less well differentiated band of tissue at the surface of the cartilage is the **chrondrogenic layer** of the perichondrium.

C. Fibrocartilage

Slide 53. Intervertebral disc, Homo, H&E
Virtual Slide ID 407

Slide 53 is a section of an intervertebral disc. It resembles dense fibrous connective tissue. The features which distinguish it as fibrocartilage rather than dense fibrous connective tissue are that the cells are enclosed within lacunae and they occur often as isogenous groups. The ground substance is less conspicuous than in hyaline cartilage, however, and there is no fibrous perichondrium.

II. BONE

A. Compact and Spongy Bone

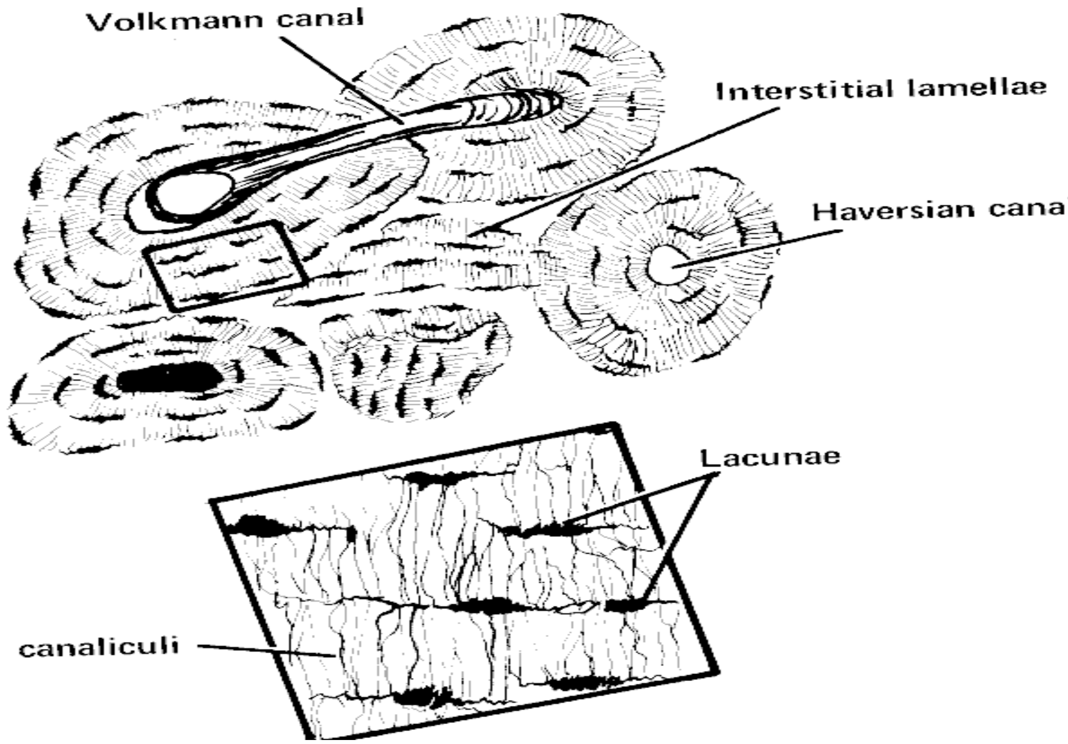
Slide 54. Compact bone, Homo, Ground section
Virtual Slide ID 3382

Slide 56. Spongy Bone, Human rib, H&E
Virtual Slide ID 213

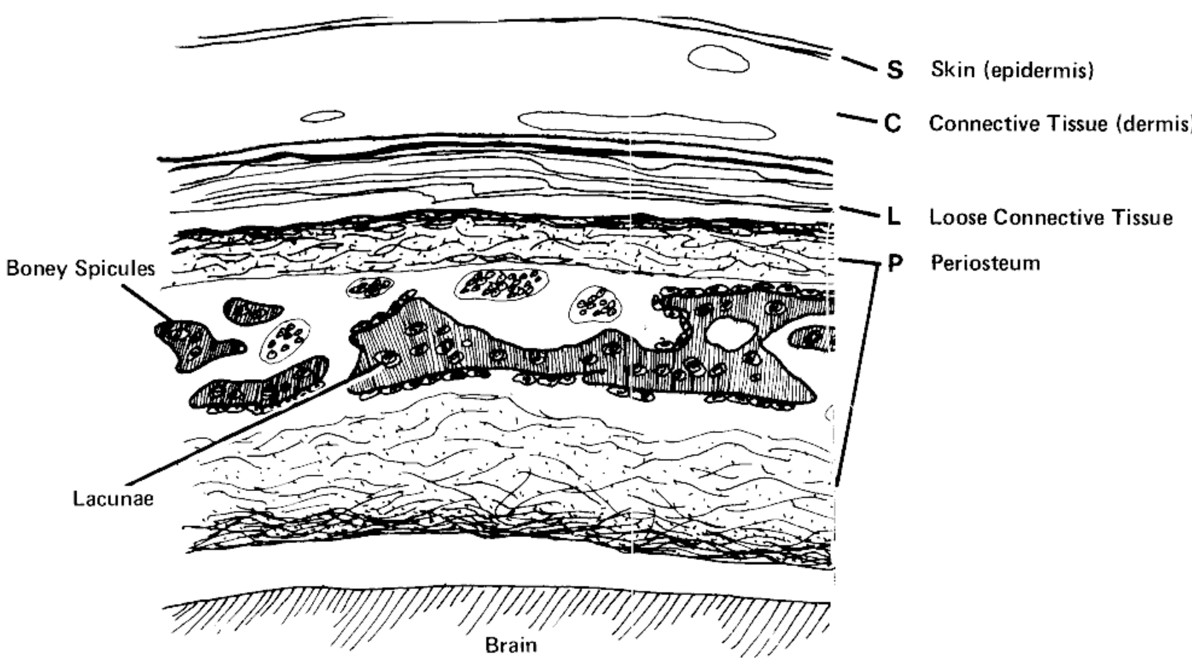
Slide 54 is a sample of compact/dense bone that was prepared in the following way. A clean dried bone was sawed into thin slices and then ground on an abrasive stone to the appropriate thinness. Essentially, nothing is left but the inorganic constituents. The section, after drying, is mounted in thick balsam so that air is trapped in all spaces in the bone matrix, making the spaces appear black.

Osteocytes (mature bone cells) are similar to chondrocytes in that they are encapsulated within **lacunae** by the mineralized matrix. The cells are not present in a ground section, but the lacunae are easily seen. The **Haversian canals**, occupied by blood vessels, are large central longitudinal passages surrounded by the concentric lamellae of the bone matrix. Very thin black lines can be seen to extend from each lacuna across the bony lamellae. These are called canaliculi.

Channels called **Volkmann's canals** cut across the lamellae or are oblique to the lamellae and carry vessels which communicate between the endosteal or periosteal vessels and the Haversian vessels. The Volkmann's canals are often larger than the Haversian canals and they are not surrounded by concentric lamellae.



The Haversian canal and its surrounding bony lamellae containing osteocytes in lacunae constitute an "Haversian system" or an osteon. Irregular fragments of lamellar bone called **interstitial lamellae** fill the spaces between adjacent Haversian systems. See the diagram above.



Slide 56 contains all the characteristic components of a long bone. The outer portion of the section is composed of compact bone exactly like the compact bone on slide 54. The sample. i.e., slide 56, however, is not a ground section. The organic components are present, and the section is stained with hematoxylin and eosin. The lamellae are difficult to distinguish, but the Haversian canals and the lacunae can be easily. Examine the compact bone on this slide for all the features you identified on slide 54. Covering the surface of the compact bone is a layer of dense fibrous connective tissue called the **periosteum**.

Between the compact outer portions of the bone you will see interlacing trabeculae of bone forming a network of bony lamellae. This arrangement of bony lamellae is called **cancellous bone or spongy bone**. Note the trabeculae are composed of **lamellae**, they have **osteoctyes** in **lacunae**, and **canaliculi** are visible. But, there are no Haversian systems in cancellous bone because the lamellae are not arranged concentrically around blood vessels. The large spaces between the spicules of spongy bone are filled with bone marrow cells and adipose cells.

C. Histogenesis of Bone

Slide 57. Intramembranous Bone, Fetal pig skull, H&E
Virtual Slide ID 214

Three terms used in describing the dynamic aspects of bone – histogenesis, growth and organogenesis or bone formation - can be confusing.

Bone may be **formed** either within or without a cartilage precursor. Certain bones such as those of the cranium develop without a cartilage precursor (**intramembranous**) while the long bones such as the femur develop by replacement of a cartilage model (**endochondral**).

The differentiation of osteocytes from osteoprogenitor cells, i.e., the **histogenesis of bone** however is the same in either case.

Slide 57 is a piece of developing parietal bone of the skull. (See diagram above). Four functional stages of bone cells are involved in bone histogenesis. Three of them are identifiable in this section.

1. **Osteoprogenitor cells**: The developing bone on this slide is an early stage of histogenesis. The scalp is on the convex side of the section. Scan the area with low power for spicules of bone containing identifiable lacunae. This is the place to begin your study.

   Identify a thin layer of dense fibrous connective tissue both above and below the developing bone spicules. These are called the **fibrous layers of the periosteum**. Between the fibrous layers of the periosteum and the developing spicules, the connective tissue is looser and mesenchymal in appearance **(osteogenic layer of the periosteum**). The **osteoprogenitor cells** are found here. They have pale, oval or elongated nuclei and inconspicuous or poorly stained cytoplasm.

2. **Osteoblasts**: Examine the edges of the bony spicules. Many of the edges are lined by rounded cells with eccentrically located nuclei. The cytoplasm of the osteoblasts should be basophilic (it is not in the slide). Their intense basophilia is due to the active synthesis and extrusion of bony matrix in which these cells are involved.

3. **Osteocytes**: Within the bony spicules identify lacunae containing enclosed bone cells. In many places, the cytoplasm of the osteocyte fills the lacuna. These provide a more accurate picture of the living situation of the osteocyte.

4. **Osteoclasts**: These cells responsible for the reabsorption of bone are not present at this early stage of bone development. They will be seen in the next section.

D. Bone Formation

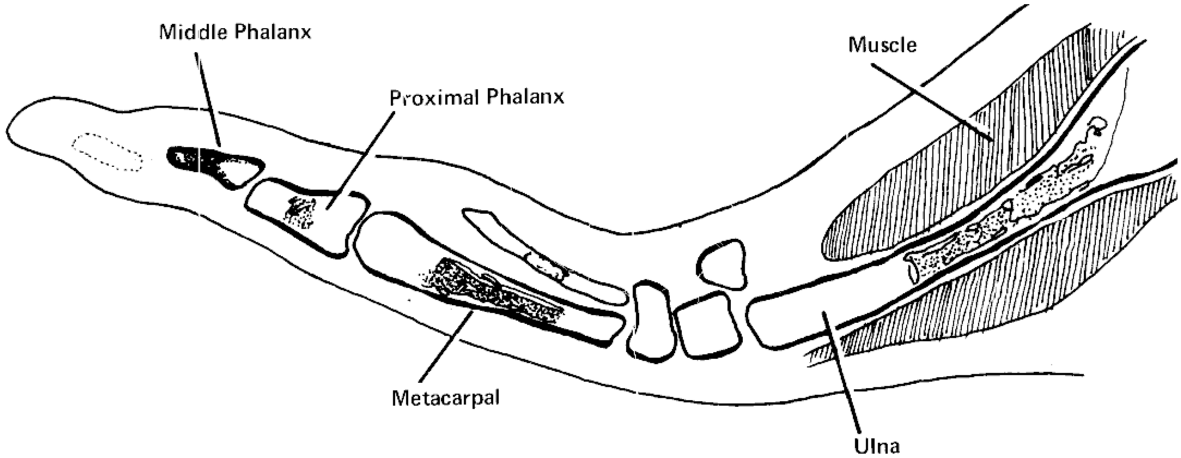
Slide 57. Bone, Fetal pig, skull, H&E
Virtual Slide ID 214

Slide 58. Forelimb, Fetal pig, H&E
Virtual Slide ID 215

The histogenesis of bone tissue always involves the activities of osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts. But the organogenesis of a particular bone will differ depending whether it develops directly within dense fibrous connective tissue as does the parietal bone of the skull (**intramembranous bone formation**) or whether it develops by replacement of a cartilage model (**endochondral bone formation**).

Slide 57 was considered above and is the only stage of intramembranous organogenesis that you will study. Later stages involve thickening of the bony spicules to form inner and outer layers of compact bone with cancellous bone and marrow spaces between them. In this early stage of bone development the osteoprogenitor cells differentiate into osteoblasts which secrete the bony matrix material directly within a dense fibrous connective tissue covering. The osteoblasts become encapsulated by their own activity and become mature osteocytes within the growing spicules.

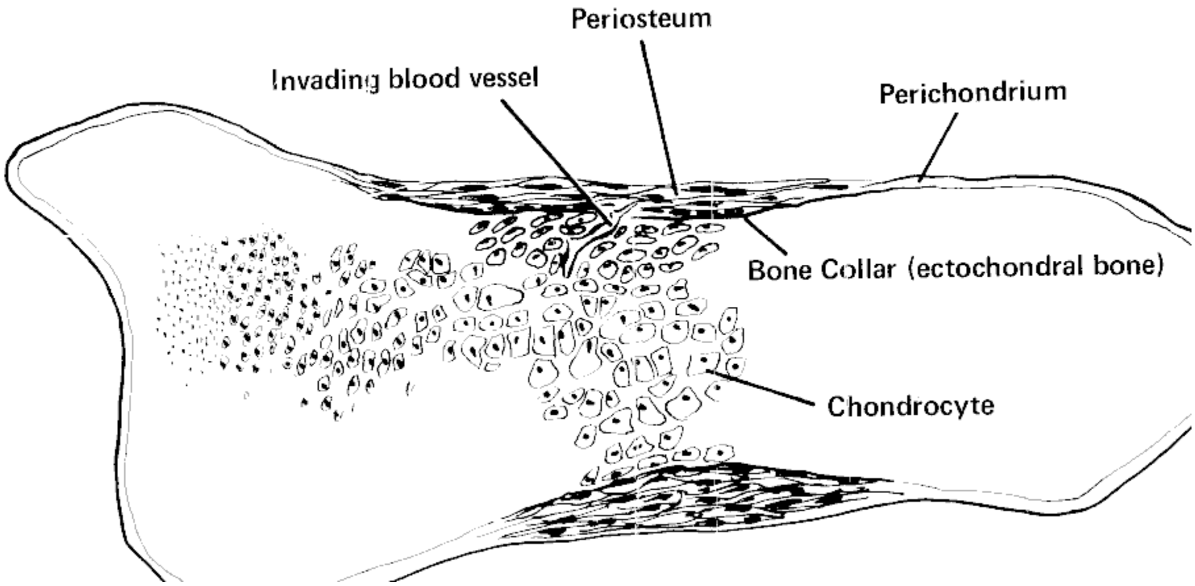
Slide 58 shows early stages of endochondral formation of bone, which is similar to intramembranous organogenesis except that it must also ultimately involve the removal of the cartilage model. This slide is a section of the forelimb of a fetal pig which is about 9 cm in length.



Find and study the following sequential stages:

1. A cartilage "model" of a bone showing only central calcification of the cartilage matrix and enlargement of the cartilage cell lacunae. There may be a bony collar forming around the middle of the shaft.

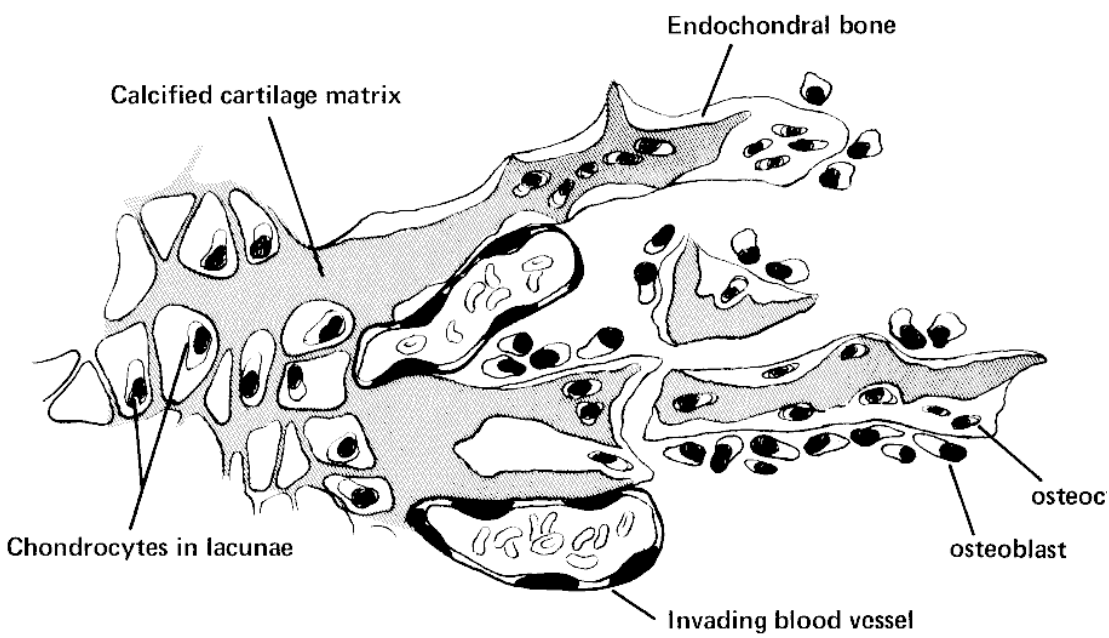
2. Now look at the proximal phalanx for the beginning of cartilage degeneration.



Note that the cartilage is being broken down in the center of the cartilage model (an endochondral location). This area of cartilage breakdown has been invaded by osteogenic cells and blood vessels from the inner layers of the perichondrium. (Recall that normal cartilage never has blood vessels within the matrix). Note also the definite bony collar which is forming around the shaft of the bone. This bony collar is also derived from osteogenic cells in the perichondrium (an **ectochondral** location) making it a type of intramembranous bone. Where the fibrous connective tissue sheath around the developing phalanx covers bone, e.g. the bony collar around the shaft, we refer to it as periosteum, where it covers cartilage, we refer to it as **perichondrium**.

3. Now examine the metacarpal in which a definite primary marrow cavity is present. Identify the zones of cartilage: 1. Reserve; 2. Proliferation; 3. Maturation; 4. Hypertrophy (and calcification); 5. Degeneration; and 6. Ossification.

   Spicules of bone are being laid down within the cartilage (endochondral bone formation). The bony matrix which is acidophilic in its staining properties is initially deposited on top of the calcified cartilage matrix which is basophilic. You can see that the centers of the bony spicules stain more deeply than their periphery (see the B&W drawing below). Even as the bony matrix is being deposited by osteoblasts, portions of it are resorbed by large mutlinucleated **osteoclasts** in order to enlarge the marrow cavity. Identify the large multinucleated osteoclasts which are usually seen near the boney spicules.



4. Study the more advanced stages of bone formation and marrow enlargement which are seen in the bones of the forearm.

III. Skeletal Muscle

Slide 65.1 Skeletal Muscle, cat, H&E.
Virtual Slide ID 188

A number of tissues and structures can be identified on slide 65.1.

Look for cartilage along the edge of the sample. **What kind is it?** ANS. Fibrocartilage.

The fibrocartilage is continuous with connective tissue covering the tendon (epitendineum). Connective tissue layers associated with fascicles of tendon (peritendineum) and muscle (perimysium) are much more subtle.

Dense regular connective tissue (tendon) is mixed in amongst the muscle fibers. In some places the connective fibers of the tendon join with the fibers of the muscle to form a **musculotendinous junction.**

Scattered along the muscle fibers are structures that resemble a bouquet of flowers. These are the nuclei of Schwann (neurolemma) cells, also known as **teloglia.** The Schwann cells cluster over the fine terminal branches of the axon (not seen) that innervates a muscle fiber.

A **muscle spindle** is located at the far left hand side of the slide. A muscle spindle is enclosed in connective tissue and composed of smaller muscle fibers.

IV. Skeletal Muscle Fiber Typing

Muscle: Palmaris Brevis (Small Hand Muscle)
Slides PB 1897R, Control, Skeletal Muscle, Human, *Hematoxylin only*
Slides PB 1897R, Type I, , Skeletal Muscle, Human, *MHC Type I Stain*
Slides PB 1897R, Type II, Skeletal Muscle, Human, *MHC Type II Stain*

Although skeletal muscle is considered a fundamental tissue of the human body, there are many subtypes of constituent muscle fibers found within a given skeletal muscle.

Slides PB 1897R, Control is stained with hematoxylin only, which gives an overall impression of the muscle and nuclei; however, the identity of the individual fiber types cannot be appreciated without a special stain.

Slides PB 1897R, Type I has been stained using immunohistochemistry using antibodies that bind to Myosin Heavy Chain (MHC) Type I, which is found in muscle fibers that have slow twitch fiber properties and an oxidative muscle metabolism. They are also fatigue resistant. The mosaic staining pattern of the muscle fibers can be appreciated with this stain. The unstained fibers are Type II fibers. Note the **Muscle Spindles** found throughout the tissue specimen.

Slides PB 1897R, Type II is a serial section of the previous slide but has been stained using myosin heavy chain (MHC) type II antibodies. The type II stained muscle fibers are fast twitch fibers, and have a glycolytic metabolism and a prone to fatigue. In this section, we can see a reciprocal staining pattern relative to the previous slide. The unstained fibers in this section are Type I fibers.

The proportion of Type I: Type II muscle fibers reflects the overall function of the muscle.

Examples:

Orbicularis Oculi > 80% Fast Twitch Muscle fibers
Soleus > 70% Slow Twitch Muscle fibers

Some muscle fibers express both type I and type II myosin isoforms and stain positive for both Type I and Type II myosin heavy chains. These fibers are called **Hybrid fibers** are have intermediate contractile and metabolic properties compared to pure type I and type II fibers.