COURSE CODE:	VBA 204
COURSE TITLE:	BASIC VETERINARY HISTOLOGY
NUMBER OF UNITS:	3 Units
COURSE DURATION:	Three hours per week

COURSE DETAILS:

Course Coordinator: DR. ADEBAYO A.O.
Email: releadebayo@yahoo.com
Office Location: College of Veterinary Medicine, Dept. of Veterinary Anatomy, UNAAB.
Other Lecturers: Drs. AKINLOYE A.K., OLUDE M.A., and MUSTAPHA O.A.

COURSE CONTENT:

Histology of basic tissues of domestic animals, epithelia, connective, muscle and nervous tissues; epithelia: basic characteristics, simple squamous, columnal, cuboidal, stratified, pseudostratified and transitional epithelia; connective tissue: intercellular materials, loose ordinary and dense ordinary connective tissues; cartilage and bone formation: blood and haemopoiesis; lymphatic tissue and lymphopoiesis, thymus, spleen, tonsils, haemal and haemolymph nodes; muscle tissue: striated, non-striated and cardiac muscles; nervous tissue: neuron, neuroglia and neurillema; histology of cerebellum, spinal cord and ganglia, introduction to histology of organs; circulatory system, comparative histology of arteries, veins and lymphatics; the integumentary system: thick and thin skin, hair follicles sebaceous and sweat gland and epidermal modifications.

COURSE REQUIREMENTS:

This is a compulsory course for all students in the University in view of this, students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. Barbara Young and John W. Heath: Wheater's Functional Histology – text and colour atlas. Fifth edition
2. Jo Ann Eurell and Brian L. Frappier: Dellmann's textbook of veterinary histology. Sixth Edition.
3. William J. Bacha, Jr. and Linda M. Bacha: Color Atlas of Veterinary Histology. Second edition.
4. Luiz Carlos Junqueira and Jose Carneiro: Basic Histology text and atlas. Eleventh edition.

LECTURE NOTES

BASIC TECHNIQUES

Preparation of histological sections

In order to prepare thin sections for examination by microscopy, it is necessary to preserve the tissues (fixation) and embed them in a supporting medium (such as paraffin wax or resins) prior to sectioning. Sections are usually stained in order to provide contrast.

1. **Fixation**

In order to preserve tissues and prevent structural change or breakdown of the components of the tissues, it is necessary to stabilize or fix the tissue. The fixative needs to preserve the tissues as close as possible to the living state. The fixatives commonly stabilize or denature proteins. A widely used fixative is formaldehyde, which has the advantage of being cheap and penetrates tissues rapidly. For better fixation, it is necessary use pH buffers in the fixative.

2. **Embedding**

The most commonly used embedding or support medium is paraffin wax, with a melting point of about 56°C. Prior to infiltration of the tissue with molten wax, it is necessary to remove all the water from the tissue (dehydration). Dehydration is achieved using an ascending series of alcohols (70%, 95%, 100%). This is followed by tissue immersion in a wax solvent such as xylene or chloroform. The tissue is then transferred to

molten paraffin wax (in an embedding oven) for a couple of hours. The tissue is then placed in a square or rectangular mold, and orientated in the required position, prior to adding hot wax to form a wax block.

3. **Microtomy**

Sections of the tissue embedded in the wax block are cut on a machine, known as a microtome, using special knives (nowadays these are disposable). Typically series or ribbons of sections are cut at a thickness of 6-8mm. The sections are transferred to the surface of a hot waterbath (where the sections flatten and lose any wrinkles). Sections are collected on glass microscope slides (standard dimensions of 3 x 1 inches). In order for the sections to adhere to the slides they are dried for up to 24 hours in a drying oven (at a temperature of about 40°C). This prevents sections falling off the slides in the later stages of preparation.

4. **Staining**

The most common staining technique is known as Hematoxylin and Eosin (or H&E) staining. In order to stain the sections the wax needs to be removed. This is done using a wax solvent such as xylene. The slide is then hydrated using a series of descending alcohols (100%, 95%, 70%) and then water. The slide is then immersed in Hematoxylin stain, rinsed in running water (preferably alkaline), followed by staining with Eosin, and rinsing in water.

5. **Permanent Mounting**

After staining the sections are again dehydrated with ascending alcohols (95%, 100%) and xylene, prior to covering with a mountant and a glass coverlip. Mountants need to have good optical properties. The slide is left for at least 24 hours for the mountant to dry. The finished (permanent) slide with its stained tissues can then be examined under the microscope.

Frozen sections

Embedding in paraffin wax is a lengthy process and during the embedding many components (such as lipids) are dissolved and lost. Enzymatic activities are also largely destroyed. A rapid alternative to wax embedding is to prepare frozen sections and in a cryostat (a microtome operated in a low temperature cabinet, usually about -30°C. Frozen sections can then be stained or used for enzyme histochemistry, and mounted in a suitable water-soluble mountant.

Total preparations

In some cases the tissue to be examined is a very thin membrane. In such cases the tissue does not need cutting on a microtome, but can be stained, mounted and examined directly. This is known as a total preparation. Total preparations are not as 2-dimensional as histological sections, and adjustment of focus is necessary during examination.

Cell Smears

Cell smears are a form of histological preparation that does not require sectioning. Smears can be made for example of the blood or bone marrow. Smears are also common for swabs or scrapings of epithelial cells (e.g. from the oral cavity, cervix uteri).

STAINING TECHNIQUES

1. **Hematoxylin and Eosin** (**H&E**)

This is the most commonly used staining technique for histological and histopathological sections. The Hematoxylin is a basic dye that stains acidic components of cells a blue color. This characteristic is known as basophilia. Hematoxylin stains the nuclei of cells, and the RER of the cytoplasm. Eosin is an acidic dye that stains the basic components of the cells a reddish-pink color. This characteristic is known as acidophilia. Most of the cytoplasm of cells is stained by eosin. Bone matrix is also stained by eosin.

2. **Periodic acid-Schiff (PAS) staining**

PAS is a widely used staining technique that stains the neutral sugars of glycosaminoglycans a pink color. Common components stained positively with PAS include mucus, the basal lamina, glycogen.

3. **Orcein**

Orcein staining is used to stain elastic fibers a dark brown-purple color. This is used, for example, to show the elastic components in the walls of arteries, or in the matrix of elastic cartilage.

4. **Osmium tetroxide**

Osmium is used to stain lipids a dark black color. It is very useful for demonstrating the myelin of myelinated nerves, or lipid droplets in the liver or steroid-secreting cells.

5. **Oil Red O**

Oil Red O is used to stain lipids a red-orange color in unfixed frozen sections.

6. **Toluidine blue**

Toluidine blue is a so-called metachromatic stain. It is a blue stain that stains specific components of tissues a purple color. This change in staining color is known as metachromasia. Metachromasia is seen in the matrix of hyaline cartilage, or in the granules of mast cells.

7. **Impregnation**

Impregnation is a staining technique in which blocks of tissue are processed in solutions containing metals such as silver or gold, which attach to specific components in tissues. The silver or gold are then further processed (reduced) and develop into dark metallic deposits. The stained blocks are only then sectioned. Silver impregnation is widely used in neurohistology to stain neurons and their processes. Silver impregnation techniques are also widely used to demonstrate reticular fibers.

8. **Vital staining**

Vital staining refers to the uptake of dyes (usually particulate) by cells. If we inject Trypan blue into experimental animals, the dye is rapidly engulfed by specific macrophages. We can use such vital staining to demonstrate the Kupffer cells of the liver.

EPITHELIUM

INTRODUCTION TO THE CONCEPT OF EPITHELIUM

Single celled organisms (Protozoa) have very little control of their internal environment. If a group of single-celled organisms become linked together to form a multicellular colony, then there is the possibility for mutual cooperation of cells involving cellular differentiation and specialization for specific functions. The cells at the exterior of the colony can be modified to assume functions related to their position close to the external environment, whereas the cells deeper in the colony can assume different functions. This specialization of function provides added advantages to the multicellular organism. The layer of cells near the periphery can be modified for protection or defence and can prevent fluid loss or desiccation. These cells can also be modified for metabolic purposes such as control of substances taken up by the organism or excreted and for the collection of information or signals from the external environment. These cells at the border between the external and internal environments, the epithelial cells, are extremely important in many aspects of physiological homeostasis.

FEATURES OF EPITHELIUM

Epithelium lines the surfaces of the body and is mainly located on the borders between the external and internal environments. Epithelium also lines all the internal body spaces that have a connection with the external environment at some stage.

Epithelium plays an important role in homeostasis of the body and in maintaining the physiological parameters of the internal environment different from those outside the body.

Epithelium is a tissue composed of cells, tightly-bound to each other, with no intercellular connective tissue. There are specializations of the cell membranes that play roles in maintaining the integrity of the tissue.

Epithelium is an avascular tissue and has no integral blood supply.

Epithelium develops in the embryo from all the three germ layers (Ectoderm, Mesoderm, Endoderm). For example, the epidermis of the skin is ectodermal in origin, the epithelium lining the serous cavities (peritoneum, pleura, pericardium) is derived from mesoderm (and is often referred to as mesothelium), whereas the epithelium lining most of the intestinal tract is endodermal. The endothelium, lining the blood vessels, is

not a true epithelium, as it is derived from mesenchyme (and should be considered as belonging to connective tissue. Moreover, endothelium has no connection at any stage with the external environment.)

FUNCTIONS OF EPITHELIUM

Owing to the strategic location of epithelium at the border between the internal and external environments, the functions of epithelium are many and varied, but can be conveniently divided into two major categories: **protective** or **metabolic**.

Protective functions of epithelium include protection against :

- mechanical damage
- loss of fluids (desiccation) - waterproofing
- invasion of foreign bodies

Metabolic functions of epithelium include :

- **Exchange of metabolites**, typically described as **ion-transport**. All the substances entering or leaving the body must pass through epithelium and are under its control. The ion-transporting epithelium may become highly specialized for **absorption** or **excretion**.

- The **glandular secretions** of the body by glands (exocrine and endocrine) are mainly a function of specialized epithelium.

- Some epithelia are modified for **sensory reception** including recognition of sensory stimuli such as pain or as chemoreceptors (such as taste buds).

Polarity

Epithelial cells are polarized cells and we can distinguish different areas of the cells (apical, basal, lateral) with specific structural modifications (unlike other tissues, where structural polarity is not found).

Specific structures found on the **apical surface** (the free surface facing the lumen or external environment) include : **microvilli**, **stereocilia**, **cilia** or **flagella**.

The **lateral surfaces** (between adjacent epithelial cells) typically have "junctional complexes" including :

- **tight junctions** (impermeable, enabling the organism to maintain the integrity of its internal environment)
- **adhering junctions (desmosomes)** promoting adhesion and reinforcing the structural integrity and sites for stress fibers
- **communicating junctions (gap junctions or nexuses)**, which allow the exchange of nutrients, ions, signals between adjacent cells).

Epithelial cells are separated from the underlying connective tissue by a **basal lamina**, secreted by the cells themselves. The plasmalemma at the base of epithelial cells, especially those with metabolic function (ion-transporting epithelia) may be modified by having marked invaginations to increase the surface area.

MORPHOLOGICAL CLASSIFICATION OF EPITHELIA

Epithelia are described according to the number of layers they possess and the appearance of the cells at the border adjacent to the external environment.

Simple epithelia are composed of a single layer of epithelial cells.

Stratified epithelia are composed of more than one layer of epithelial cells.

The histological appearance describing the various epithelia is that seen in vertical sections. It is customary when drawing epithelia to show the free surface directed to the top of the page, whereas the basal surface is depicted in the direction of the bottom of the page.

SIMPLE EPITHELIA

Simple epithelia consist of a single layer of cells.

When seen in vertical sections the epithelial cells are described as :

- **squamous** (flattened)
- **cuboidal** (more square or cube-like)
- **columnar** (tall and thin)

If all the cells in the epithelium consist of a single cell type, the epithelium is described as being **homogeneous**. If the epithelium has more than one cell type, the epithelium is described as being **heterogeneous**.

If the apical edge of the epithelium has cilia, the epithelium is described as being **ciliated**.

Epithelial cells that have large numbers of microvilli on the apical or luminal surface (such as the columnar absorptive cells of the small intestine) are described as possessing a **brush border**.

If histological sections of the simple epithelium are cut tangentially, they may give a false impression of being composed of more than one layer (stratified).

EXAMPLES OF SIMPLE EPITHELIA

Simple squamous epithelia line the serous cavities of the body (peritoneum, pleura, pericardium). These epithelia are also known as **Mesothelia** (because of their mesodermal origin). If a small piece of the omentum is removed and examined as a total preparation (thin enough not to need sectioning) it can be seen to be composed of a mosaic of tightly-connected cells. In vertical sections these simple squamous epithelia are seen as very flat, elongated cells.

Simple cuboidal epithelia line many small ducts in the body. Examples include the urinary ducts of the kidney, the bile ductules of the liver, or the cells lining thyroid follicles.

Simple columnar epithelia line the gallbladder, the larger ducts near the urinary papilla of the renal pyramids and the absorptive cells of the small intestine.

Ion-transporting (metabolic) epithelia are typically simple epithelia.

STRATIFIED EPITHELIUM

Stratified epithelia consist of more than one layer of cells. These typically are at sites needing a more defensive, rather than a metabolic function.

Stratified epithelium is described as squamous when the cells nearest to the external environment are flattened. In typical multilayered epithelium, the cells nearest to the base of the epithelium are more columnar or cuboidal, whereas the cells nearer to the surface are flatter. The more basal areas are sites of cell proliferation

(mitosis) and there is a continuous movement of cells in the direction of the free surface, with the flattened surface cells being sloughed off. **Stratified squamous epithelium** is found in the esophagus and epidermis of the skin. The stratified epithelium of the skin (epidermis) has a layer of keratin and is called a **keratinized** (or dry) epithelium.

Pseudostratified columnar epithelium

Some epithelia give the false appearance of being stratified, but in effect consist of a single layer of irregular columnar cells, all in contact with the basal lamina. Pseudostratified columnar epithelium is the typical epithelial type of much of the respiratory tract. This epithelium also is ciliated and is an example of a heterogeneous epithelium owing to an additional cell type (mucus-secreting unicellular goblet cells). One additional characteristic feature of pseudostratified epithelium is that the nuclei of adjacent cells are not orderly arranged but appear at different levels.

Transitional epithelium

Transitional epithelium is a form of stratified epithelium lining the urinary bladder and part of the urinary tract. This epithelium is subjected to large mechanical changes and can adapt accordingly. If the bladder is empty, the epithelium appears to be thicker and have more layers, than when the bladder is full and distended, in which case, the cells appear more stretched. Transitional epithelium can be recognized by the large rounded epithelial cells lining the lumen (in contrast to other stratified epithelia, where such surface cells are squamous).

Basal lamina

All epithelia lie on a basal lamina, separating them from the underlying connective tissue (*lamina propria*). The basal lamina provides structural support and acts in part as a selective barrier for the epithelial layer. The basal laminae are formed by the cells themselves. In some cases the basal laminae are greatly thickened (as in the glomeruli and filtration system of the kidneys). (The older term "basement membrane" should be avoided). The basal laminae, as seen by transmission electron microscopy, are seen to be formed from an electron-dense layer (50-80 nm thick) composed of **non-fibrous type IV collagen** and the proteoglycan, **heparan sulfate**, surrounded on both sides by a less-dense layer containing the glycoprotein, **laminin**.

Basal laminae are not exclusive features of epithelia, but are also found associated with endothelia and some other cell types.

The border between epithelia and the underlying connective tissue is sharp and distinct. In the case of stratified epithelium this border may be ridged or consist of papillae.

Epithelia are avascular. Blood vessels of the underlying connective tissue (*lamina propria*) supply the necessary nutrients and metabolites, which are transported to and from the epithelial cells by diffusion.

Some epithelia, especially the stratified epithelia of the epidermis and the nasal mucosa may have sensory nerve endings. These help transmit information from the external environment.

Proliferation and regeneration

Epithelia are present in vulnerable sites of the body, where they are continually exposed to the hazards of the external environment. Epithelial cells are constantly subjected to mechanical damage, destroyed, or sloughed off. Epithelia have remarkable proliferative properties and typically show many dividing cells (**mitoses**) in order to replace the cells lost and maintain the integrity of the tissue. In cases of trauma or wounds, the epithelia need to cover the lesion as rapidly as possible, repair the lining tissue and prevent damage to the underlying tissues.

Most of the cancers of the body are the result of uncontrolled proliferation of epithelial cells (aden).

GLANDULAR EPITHELIUM

One of the specialized functions of epithelia is **secretion**.

Glands consist of groups of epithelial cells modified to synthesize and secrete.

Glands are classified as:

- **exocrine glands**, which secrete to the external environment via ducts.
- **endocrine glands**, which secrete directly into the blood (**ductless glands**)
- **mixed glands**, which have both exocrine and endocrine secretion.

Secretion of exocrine glands is classified as :

(a) **Merocrine** (or **eccrine**) **secretion**

This is the most common type of exocrine secretion. Secretory granules (packaged in the Golgi bodies) migrate to the apical surface of the cell. The membranes of the secretory granules fuse with the apical membrane and the secretion is released to the external environment by exocytosis.

(b) **Apocrine secretion**

With apocrine secretion the apical portion of the cells, together with the secretory contents, are budded off and released to the lumen or external environment. Examples of such apocrine secretion are found in the apocrine sweat glands of the armpits.

(c) **Holocrine secretion**

Holocrine secretion involves the secretion of whole cells and their contents. This is best seen in the sebaceous glands associated with hairs of thin skin.

CLASSIFICATION OF EXOCRINE GLANDS

Exocrine glands are classified into two groups:

- **unicellular glands**
- **multicellular glands**.

Unicellular exocrine glands

The main example of a unicellular exocrine gland is the **goblet cell**. Goblet cells are found scattered in the heterogeneous epithelium of mucous membranes, for example, in the pseudostratified epithelium of the respiratory tract or in the absorptive epithelium of the small and large intestine. These glands synthesize and secrete mucin (the precursor of mucus) to the epithelial surface. This mucoid secretion helps lubricate and maintain the moistness of the epithelium, and may also be involved in trapping dust or particulate material and in responding to infection. The nuclei of goblet cells are basally situated and usually are very flattened.

The secretory contents of goblet cells are stained weakly acidophilic (pink staining after H&E) and are stained intensely by the PAS technique owing to their proteoglycan (polysaccharide) content.

Multicellular exocrine glands

Multicellular exocrine glands develop by proliferation and invagination of epithelial cells into the underlying connective tissue. The initial portions develop into the **secretory duct**, whereas the terminal portions develop into the **secretory units**. In cases where the gland develops from simple epithelium, the duct and secretory units are also single-layered (e.g. the exocrine glands of the small and large intestine). In cases where the gland develops from stratified epithelium, the duct and secretory units usually have more than one layer of cells.

The cells of the secretory ducts are typically less poorly differentiated than the cells of the secretory units. All the epithelial cells of both the ducts and secretory units show marked polarity.

Multicellular exocrine glands are classified as **simple** or **compound glands**.

- **Simple exocrine glands** have unbranched secretory ducts.
- **Compound exocrine glands** have branched secretory ducts. The branching is often complex and similar to that of branches of a tree.

The morphology of secretory units in both simple and compound glands is very varied and is described as:

- **tubular (e.g. intestinal crypts of Lieberkuhn)**
- **coiled tubular (e.g. sweat glands)**
- **branched tubular (e.g. Brunner's glands of the duodenum)**
- **alveolar**
- **acinar (e.g. exocrine pancreas)**

and various combinations such as **tubulo-alveolar**

The epithelial components of glands are often referred to as **parenchyma**, whereas the connective tissue components are called the **stroma**. The connective tissue of glands provides support and typically many exocrine glands are surrounded by a **capsule** of connective tissue, which may send out septa of connective tissue that divides the gland into **lobes** (larger anatomical structures) and smaller functional units, the **lobules.**

Secretory cells of exocrine glands

The secretory cells (of secretory units) of exocrine glands are classified into two histological categories based on their secretory characteristics.

- **Mucous cells**. These are rounded acidophilic cells (typically with basal flattened nuclei), that are rich in glycoproteins (PAS-positive) and produce a mucoid secretion.

- **Serous cells**. These are basophilic cells that are more cuboidal or columnar. The serous cells synthesize and secrete polypeptides or proteins. Their nuclei are fairly centrally located, and the secretory granules may be visible in the apical portion of the cells. The basal region of the cells have accumulations of rough endoplasmic reticulum (RER) that provide the basophil staining.

The secretory units of some exocrine glands are entirely serous in nature (e.g. pancreas, parotid gland), whereas other glands may be mixed with both mucous and serous cells (e.g. submaxillary gland, glands of the fundus of the stomach).

The process of synthesis, storage and secretion in serous cells illustrates the structural and functional polarity of the cells. The RER in the basal region synthesizes the polypeptide or protein molecules, which are transported to the Golgi bodies and packaged into membrane-bound granules. These granules accumulate in the apical region of the cells and as a result of the necessary secretory signals are discharged at the apical surface to the external environment by exocytosis.

Several types of multicellular exocrine glands (of ectodermal origin) have an additional cell type known as the **myoepithelial cells**. These are contractile cells surrounding the secretory units, and when they receive a signal to contract, result in secretory discharge from the secretory cells. Examples of glands with myoepithelial cells include the salivary glands and the mammary glands.

ENDOCRINE GLANDS

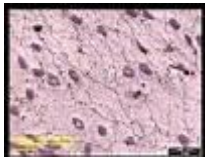
Endocrine glands develop initially in the embryo like the multicellular exocrine glands, however their ducts degenerate and disappear (ductless glands) and the glands secrete directly into the blood capillaries in the surrounding connective tissue. Endocrine secretions are known as hormones and the endocrine glands form part of a major regulatory system, known as the endocrine system. Endocrine glands, which will be considered later, are very variable in histological appearance and owing to their great structural diversity are hard to classify according to morphology, though the secretory cells may be classified into two major groups:

- **polypeptide (or protein)-secreting cells**

- **steroid-secreting cells**.

The endocrine **polypeptide (or protein)-secreting cells** are typically characterized by well-developed RER (rough endoplasmic reticulum), Golgi bodies and membrane-bound secretory granules. These endocrine cells may be isolated or in small groups (the **diffuse endocrine system**) and include many of the endocrine cells of the intestine. The **APUD concept** was originally conceived owing to the fact that most of the polypeptide-secreting endocrine cells have several common ultrastructural and biochemical characteristics and were thought to have a common embryological origin. It is nowadays accepted that APUD cells of the intestinal tract and respiratory tract are derived from endoderm, whereas the other APUD cells are of neural crest origin. The name APUD is derived from ***A***mine ***P***recursor ***U***ptake and ***D***ecarboxylase. This concept is now superceded by the **diffuse neuroendocrine system**, though the term **apudoma** is still used to describe tumors of APUD cells. Polypeptide or protein-secreting endocrine cells are also present in many of the classical endocrine glands.

The **endocrine steroid-secreting cells** (e.g. in the testis, ovary, suprarenal cortex) are characterized by well-developed SER (smooth endoplasmic reticulum) and abundant lipid droplets. The mitochondria of these cells have tubular cristae (unlike the typical lamellar cristae of other cell types).

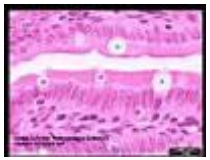


Simple Squamous Epithelium

Simple Cuboidal Ep.

Simple Cuboidal Ep.

Simple Columnar Ep.



S.Columnar Heterogeneous Ep.

Pseudostratified Columnar Ep.

Stratified Squamous Ep.

Stratified Squamous Ep

Transitional Epithelium

Goblet Cells

CONNECTIVE TISSUE

Connective tissue is responsible for providing structural support for the tissues and organs of the body. This mechanfunction is important in maintaining the form of the body, organs and tissues. The tissue derives its name from its function in connecting or binding cells and tissues.

Connective tissue is composed of:

- **cells**
- **extracellular matrix**.

The extracellular material of connective tissue, which plays a major role in the functioning of the tissue, is the dominant component of the tissue. The dominance of the extracellular material is a special feature that distinguishes connective tissue from the other tissues of the body.

The **extracellular matrix** is composed of :

- **protein fibers** (collagen fibers, reticular fibers, elastic fibers)
- **amorphous ground substance**
- **tissue fluid** (not preserved in histological preparations). The amount of tissue fluid is fairly constant and there is an equilibrium between the water entering and leaving the intercellular substance of the connective tissue. In pathological conditions (traumatic injury, inflammation) fluid may accumulate in the connective tissue, a condition known as edema.

Connective tissues are very heterogeneous in structure and function, however all have the three main structural components (cells, fibers and ground substance). The diverse composition and amount of these components in the various connective tissues can be correlated with the specific functional roles of the tissue.

FUNCTIONS OF CONNECTIVE TISSUE

- **Structural support**

The connective tissues serve several functions, of which the most prominent function is **structural support** to enable maintenance of anatomical form of organs and organ systems. Examples include the connective tissue capsules surrounding organs (such as the kidney, lymph nodes). The loose connective tissue acts to fill the

spaces between organs. The tendons (connecting muscles to bone) and the elastic ligaments (connecting bones to bones) are examples of specialized orderly forms of connective tissue. The skeletal tissues (cartilage and bone) are special forms of connective tissue.

- **Metabolic functions**

The connective tissues serve a **nutritive role**. All the metabolites from the blood pass from capillary beds and diffuse through the adjacent connective tissue to cells and tissues. Similarly **waste metabolites** from the cells and tissues diffuse through the loose connective tissue before returning to the blood capillaries.

The **adipose tissue** (especially that of the hypodermis) serves as an **energy store** and also provides **thermal insulation**. Surplus calories can be converted into lipid and stored in adipocytes.

- **Blood components and blood vessels**

The **hematopoietic tissues** (blood-forming tissues) are a further specialized form of connective tissue. These include the **myeloid tissue** (bone marrow) and the **lymphoid (lymphatic) tissue**. The lining of the blood and lymphatic vessels (**endothelial cells**) as well as the peripheral blood, are also specialized forms of connective tissue.

- **Defensive functions**

Various components of the connective tissue play roles in the defense or protection of the body including many of the components of the vascular and immune systems (plasma cells, lymphocytes, neutrophils, eosinophils, basophils, mast cells). The various macrophages of the body are also categorized as connective tissue cells. These all develop from monocytes and are grouped as part of the **Mononuclear Phagocyte System** of the body. Macrophages are important in tissue repair as well as defense against bacterial invasion. The fibroblasts of connective tissue proliferate in response to injury of organs and migrate to and deposit abundant new collagen fibers, resulting in the formation of **fibrous scar tissue**.

Cell type	Chief function
Mesenchyme	Embryonic source of all connective tissue cells

Fibroblasts Chondroblasts Osteoblasts	Structural support
Plasma cells Lymphocytes Neutrophils Eosinophils Basophils Mast cells Macrophages	Defense and immune
Adipocytes	Metabolic Energy storage Thermal insulation

Mesenchyme and the origin of connective tissue cells

All connective tissue cells are derived from mesenchymal cells. **Mesenchyme cells** are found in embryos and are for the most part derived from the middle germ layer of the embryo (mesoderm).

Several of the connective tissues of the head region are derived from the neural crest (ectodermal origin). Endothelial cells lining blood vessels are derived from mesenchyme and therefore are classified as connective tissue rather than epithelium. Epithelium, which can develop from all three embryonic germ layers, never develops from mesenchymal cells.

Mesenchymal cells are typically elongated cells, with relatively little cytoplasm. These cells have regular, oval nuclei with prominent nuclei. The nuclei are often eccentric in position. Mesenchymal cells have several thin cytoplasmic processes. The spaces between the cell processes are filled in ground substance.

Mesenchyme cells are only found in embryos, however some mesenchyme-like cells persist in adult connective tissue. These mesenchyme-like cells retain their capacity to differentiate into other connective tissue cells in response to injury. Examples include the pericytes (perivascular cells) of blood capillaries.

Amorphous Ground Substance

The intercellular ground substance is an amorphous, transparent material composed mainly of glycoproteins and proteoglycans, with a fairly high water content.

The main proteoglycans consist of a core protein associated with sulfated glycosaminoglycans (GAGs). The main GAGs include : chondroitin-4-sulfate, chondroitin-6-sulfate, keratan sulfate, heparan sulfate) and the non-sulfated hyaluronic acid.

All substances passing to and from cells must pass through the ground substance.

CONNECTIVE TISSUE FIBERS

Connective tissue fibers are composed of structural proteins. The three main types of fibers are:

- **collagen fibers**
- **reticular fibers**
- **elastic fibers**

Collagen fibers

Collagen is the most abundant protein in the body (up to 30% dry weight). There are more than 12 different types of collagen, though the most common types are Types I toV.

Collagen type	Main sites	Special features
Type I	Bones, tendons, organ capsules, dentin	Most abundant, Typical collagen fibers (64nm banding)
Type II	Hyaline cartilage Elastic cartilage	Very thin fibrils

Type III	Reticular fibers	Often associated with Type I
Type IV	Basal lamina associated with epithelial and endothelial cells	Amorphous (non-fibrous)
Type V	Basal lamina associated with muscle	Amorphous (non-fibrous)

Collagen is synthesized by a wide number of cell types (including: fibroblasts, osteoblasts, chondroblasts, odontoblasts, reticular cells, epithelial cells, endothelial cells, smooth muscle cells, Schwann cells).

The **main amino acids** of collagen are:

- glycine (33.5%)
- proline (12%)
- hydroxyproline (10%)

The amino acids, hydroxyproline and hydroxylysine are characteristic of collagen. Collagen is the only naturally occurring protein with both these amino-acids.

Tropocollagen molecules (280 nm long, 1.5 nm wide) form the basic unit, which polymerize to form collagen fibrils. The tropocollagen molecule consists of three linear twisted polypeptide chains (left-handed helices), which are further twisted to form a major right-handed helix. Two of the three polypeptide chains have similar amino acid composition, while the third is different.

At the ultrastructural level each collagen fibril shows a 64nm banding (periodicity), which is due to the stepwise overlapping arrangement of the rodlike tropocollagen subunits.

Collagen fibers consist of closely packed orderly fibrils and when seen in bundles (as in tendons, aponeuroses) appear white. In histological preparations after regular staining they are acidophilic (pink staining with eosin). Collagen fibers are flexible, but very inelastic with extremely high tensile strength.

Reticular fibers

Reticular fibers are very thin (diameters between 0.5 - 2□ m) and are not visible in normal histological preparations after regular staining (H & E), however they can be visualized and stained black after impregnation with silver salts. This affinity for silver is called **argyrophilia**. Reticular fibers are also stainedwith the **PAS reaction** due to the high content of glycoproteins associated with the fibers (6-12% hexoses as opposed to 1% in collagen fibers). It is now recognized that reticular fibers are a special form of collagen (Type III).

Reticular fibers form fine-meshed netaround cells and cell groups

in diverse organs. They are abundant in lymphatorgans (lymph nodes, spleen), smooth muscle (in the sheath surrounding each myocyte), in endoneurium (connective tissue surrounding peripheral nerve fibers), and supporting epithelial cells of several glands (liver, endocrine glands).

Elastic fibers

Elastic fibers, as the name suggests, are highly elastic and stretch in response to tension. In particular they are formed from the protein **elastin**. The amino acid composition of elastin, similar to collagen, is rich in glycine and proline, but in addition has two unusual amino acids, **desmosine** and **isodesmosine**. Elastic fibers also have a high content of **valine**. Elastic fibers are very prominent in elastic tissues such as the elastic ligaments. When present in high concentration, the elastin imparts a yellow color to the tissue. The **elastic laminae of arterial blood vessel walls** are composed of a non-fibrillar form of elastin. Elastin can be stained in histological preparations using **orcein**.

CONNECTIVE TISSUE CELLS

Fibroblasts

Fibroblasts are the most common cell type found in connective tissue. The term "**fibroblast**" is commonly used to describe the active cell type, whereas the more mature form, which shows less active synthetic activity, is commonly described as the "**fibrocyte**". Fibroblasts are elongated, spindle-shaped cells with many cell processes. They have oval, pale-staining, regular nuclei with prominent nucleoli. Abundant rough endoplasmic reticulum and active Golgi bodies are found in the cytoplasm.

Fibroblasts synthesize collagen, reticular and elastic fibers and the amorphous extracellular substance (including the glycosaminoglycans and glycoproteins).

Macrophages

Macrophages show pronounced **phagocytotic activity**. This can be demonstrated following injection of vital dyes such as trypan blue or Indian ink and the uptake of the particulate matter. Macrophages originate from monocytes (from precursor cells in bone marrow), which migrate to connective tissue and differentiate into tissue macrophages. Today the various macrophages of the body are grouped in a common system called the **Mononuclear Phagocyte System** (MPS). Today a wide range of macrophages are included in the MPS and include : **Kupffer cells** of the liver, **alveolar macrophages** of the lung, **osteoclasts**, **microglia** etc.

The main functions of macrophages are ingestion by **phagocytosis** of microorganisms (bacteria, viruses, fungi), parasites, particulate matter such as dust, and they also participate in the breakdown of aged cells including erythrocytes. The **intracellular digestion** occurs as a result of fusion of **lysosomes** with the **phagosome** (ingested body).

Macrophages are normally long-lived and survive in the tissues for several months. In some cases where a foreign body (such as a small splinter) has penetrated the inner tissues of the body, several macrophages may fuse together to form multinuclear **foreign body giant cells**. These large cells accumulate at sites of invasion of the foreign body and sites of inflammation.

Mast cells

Mast cells are oval or round cells (20-30□m diameter) in connective tissue characterized by cytoplasm packed with large round **basophilic granules** (up to 2□m diameter). The **granules are stained metachromatically** (purple after toluidine blue staining). Two of the main components of mast cell granules are **histamine** and **heparin**. The granules of mast cells are released in inflammatory responses. Mast cells are abundant in loose connective tissue (especially adjacent to blood vessels), in the dermis, and in the lamina propria of the respiratory and digestive tracts.

Plasma cells

Plasma cells are responsible for **antibody production**. These large cells have eccentric nuclei, basophilic cytoplasm (much rough endoplasmic reticulum associated with protein synthesis) and well-developed Golgi bodies. Plasma cells are relatively short-lived (10-20 days) and are found in sites of chronic inflammation or sites of high risk of invasion by bacteria or foreign proteins (such as the lamina propria of the intestinal and respiratory tracts).

Leukocytes

The white blood corpuscles are commonly found in connective tissue. They migrate from the blood vessels to the connective tissue, especially to sites of injury or inflammation.

CLASSIFICATION OF CONNECTIVE TISSUE

The two main categories of connective tissue are:

- **Loose Connective Tissue**
- **Dense Connective Tissue**

Loose Connective Tissue

Loose connective tissue (**areolar tissue**) is the more common type. It fills the spaces between muscle fibers, surrounds blood and lymph vessels, is present in the serosal lining membranes (of the peritoneal, pleural and cardiac cavities), in the papillary layer of the dermis and in the lamina propria of the intestinal and respiratory tracts etc.

Dense Connective Tissue

Dense connective tissue is divided into two sub-categories:

- **dense irregular connective tissue**
- **dense regular connective tissue**

Dense connective tissue contains relatively few cells with much greater numbers of collagen fibers. Dense irregular connective tissue has bundles of collagen fibers that appear to be fairly randomly orientated (as in the dermis).
Dense regular connective tissue has closely-packed densely-arranged fiber bundles with clear orientation (such as in tendons) and relatively few cells.

Tendons

Tendons are the most common type of dense regular connective tissue. Tendons connect skeletal muscles to bone. Owing to the dominance of the collagen fibers, the tendons have a white color (stains acidophilic in regular staining). The collagen bundles in tendons are arranged in bundles (primary bundles). Several primary bundles, each surrounded by loose connective tissue, are grouped into larger bundles (secondary bundles). The loose connective tissue surrounding the primary and secondary bundles contains blood vessels and nerves. The whole tendon is surrounded by a denser connective tissue.

Each primary bundle has orderly-arranged rows of **fibrocytes**, when seen in longitudinal section. These fibrocytes have relatively little cytoplasm. Between the rows of fibrocytes, the collagen bundles are closely packed and arranged also in a longitudinal direction.

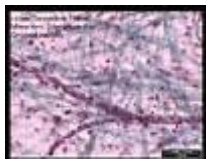
Ligaments

Ligaments are a special type of dense regular connective tissue that connects bones to bones. They have a similar structural arrangement to tendons, but differ in their yellow color, which is due to the abundance of elastic fibers in the tissue. The elastic fibers are stained a dark brown-red with orcein. Elastic fibers provide the ligament with remarkable elasticity (in contrast to tendons).

Mucous tissue

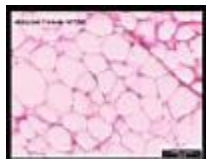
This is found in the umbilical cord (Wharton's jelly). It is a loose connective tissue composed of fibroblasts with several long cytoplasmic processes. The intercellular space is filled with a jelly-like amorphous ground substance, rich in hyaluronic acid and fibers.

Mesenchymal Cells



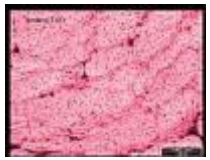
Loose Connective Tissue

Loose Connective Tissue

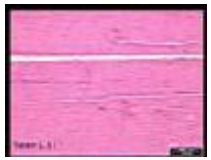


Adipose Tissue-White

Adipose Tissue-Brown



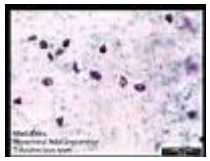
Tendon-TS



Tendon-LS

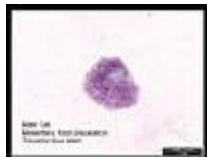
Ligamentum nuchae-TS

Ligamentum nuchae-LS



Mast Cells

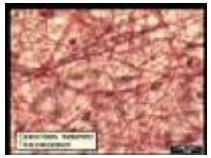
Mast Cells



Mast Cells

Mucous Connective Tissue

Adipose Cell



Elastic Fibers - Mesentery

MUSCLE TISSUE

- Muscle tissue is characterized by its well-developed properties of contraction.
- Muscle is responsible for the movements of the body and the various parts of the body.
- Muscle develops from embryonic mesoderm (with the exception of myoepithelium).

Muscle is classified into 3 categories according to morphology and physiological function:

- **Skeletal Muscle**
- **Cardiac Muscle**
- **Smooth Muscle**

Specific nomenclature associated with muscle commonly involves the prefix **sarco-** or **myo-** .

The cytoplasm of muscle fibers or cells is called **sarcoplasm**.
The endoplasmic reticulum of fibers or cells is called **sarcoplasmic reticulum**.
The plasmalemma of fibers or cells is called the **sarcolemma**.

Individual muscle cells are called **myocytes**.

SKELETAL MUSCLE

Skeletal muscle, also known as **striated** or **voluntary muscle**, comprises some 40-50% of the body mass in adults and constitutes part of the largest organ system of the body.

During embryonic development **mesodermal cells** differentiate into uninuclear **myoblasts**, which elongate and fuse together to form **myotubes**, which further develop into the mature muscle fibers or **myofibers**. These myofibers are the basic units of skeletal muscle and are up to 30 cm in length. Myofibers possess large numbers of elongated or oval nuclei at their periphery, close to the **sarcolemma**. These myofibers are **syncytia** (multinucleated post-mitotic structures in which the nuclei have lost the ability to synthesize DNA). After regular staining myofibers are seen to have periodic cross striations (the source of the name "striated muscle"). A further cell-type, known as **satellite cells,** may be found adjacent to the sarcolemma. These are elongated, poorly-differentiated cells that are very difficult to discern in typical preparations, but become active during repair and regeneration processes after muscle injury.

Connective tissue arrangements of skeletal muscles

In skeletal muscles the myofibers are bound together in a similar manner to wires in a telecommunications cable. The connective tissue in the muscle serves to bind and integrate the action of the various contractile units. A thin and delicate conntissue layer, known as the **endomysium**, surrounds each individual myofiber. Myofibers are grouped together in bundles or **fascicles**, which are also surrounded by connective tissue, known as the **perimysium**. The fascicles are surrounded and bound together by a further connective tissue coating known as the**epimysium**. All these connective tissue coatings (endomysium, perimysium and epimysium) contain collagen fibers, elastic fibers, fibroblasts and are richly vascularized. The ends of skeletal muscles are attached to bones, cartilage or ligaments by means of tendons. The attachment that moves the least is known as the tendon of origin, whereas the other tendon is known as the tendon of insertion. The flattened skeletal muscles have strong flattened sheets of tendon-like tissue at their ends known as aponeuroses.

Light microscopy of myofibers

Longitudinal sections of skeletal muscle fibers show repeated cross-striations after regular staining (H&E). The stained bands are called **A-bands**, and in between these are non-stained **I-bands**. If the same myofiber is examined by polarizing microscopy the A-bands are seen to be **birefringent** or **anisotropic** (bright against a dark background with crossed polars), whereas the I-bands are **non-birefringent** or **isotropic**. (The origin of the nomenclature comes from these polarizing properties: **A** = **A**nisotropic, **I** = **I**sotropic).

At higher magnifications it is possible to see a line in the middle of the I band, known as the **Z line**.

Examination of a myofiber at high magnification shows that that it is composed of many parallel **myofibrils**. The A and I bands and Z lines are visible in the myofibrils. The unit between two Z lines is known as the **sarcomere**. The myofibrils consist of repeating strings of sarcomeres. The sarcomeres in adjacent myofibrils tend to be located in parallel, resulting in the overall cross-striations of the myofibers. It is also possible in some cases to distinguish a less-stained region in the middle of the A-bands, known as the **H-band** (Hensen's band). The sarcomeres form the basic contractile units of the fibers.

Ultrastructure of sarcomeres

Examination of sarcomeres of myofibrils by transmission electron microscopy reveals two sorts of **myofilaments**. The thicker myofilaments belong to the A band and are composed mainly of **myosin**. The thinner myofilaments are mainly found in the I band and are composed mainly of **actin**. These thin myofilaments are connected to the Z-line and partially extend between the thicker myofilaments. This area of

overlap is important in the contraction process. In transverse sections in the area of overlap each thick myofilament is surrounded by six of the thinner myofilaments.

Molecular components of the myofilaments

The myofilaments are composed of four main molecules: **myosin** (thick filaments), **actin**, **tropomyosin**, and **troponin** (thin myofilaments). The actin and myosin constitute about 55% of all the proteins of the fibers.

Thin myofilaments

Two types of actin are found:

- **G-actin** (globular) consists of spherical monomers of about 5.6nm diameter. The monomers are polarized, with one hemisphere having specific binding sites for myosin.
- **F-actin** (fibrous) consists of chains or strings of G-actin molecules.

Tropomyosin is a long polypeptide molecule and to which are attached actin molecules (like a string of pearls).

Periodically **troponin** molecules are located on the tropomyosin molecules. The thin myofilaments are composed of two tropomyosin molecules with attached actin and troponin in a double helix. The troponin molecule is organized into specific regions: TnT, which binds to tropomyosin, TnC, which binds to calcium, and TnI, which in involved in inhibiting the actin-myosin interaction.

Thick myofilaments

The myosin molecules are composed of a rod-like portion (**light meromyosin**) and twin rounded heads (**heavy meromyosin**). These can be separated by brief hydrolysis. The heavy meromyosin portion contains ATP-ase activities, important in the binding of the myosin to actin during contraction process. The thick myofilaments are given structural support and held in place and by a giant protein molecule, **titin**, which connects the myosin molecules to the Z lines. Titin extends from the Z line to the M-band approximately parallel to the long axis of the sarcomere. The part of the titin molecule in the I band extending from the Z line is known as the elastic part of the titin, whereas the part in the A band is less elastic. The most central part of the thick myofilaments are laterally connected by intermediate filaments resulting in the M-band.

The Z-lines contain the proteins □**-actinin** and **desmin**.

Contraction mechanism

The explanation for the contraction process derives from the **Sliding Interdigitating Filament Hypothesis** (of Hanson and Huxley of the early 1960's) based on the changes in sarcomere ultrastructure during contraction as seen by transmission electron microscopy. During muscle fiber contraction sarcomeres become shorter, the Z lines move closer to each other and the I bands become less prominent. The A bands remain the same length in all phases of the contraction. The changes in the length of the sarcomere are the result of the thin myofilaments sliding or interdigitating between the thicker filaments resulting in a greater area of overlap.

T-system of tubules

Tubular invaginations of the sarcolemma penetrate the myofibers in a transverse direction. These are known as **the T-tubules** (transverse tubules) and are found at the area of overlap between the A and I bands of myofibrils. Each sarcomere has two of these tubules. The **sarcoplasmic reticulum** is a network of **sarcotubules** surrounding each myofibril. Swollen **terminal cisternae** or sacs of the sarcoplasmic reticulum are associated with the T-tubules. Two terminal cisternae are associated with each T-tubule to form structures (visible by transmission electron microscopy) known as **triads**. The membranes of the terminal cisternae are separated from the T-tubules by gap junctions. These terminal cisternae are sites of accumulation of calcium ions during muscle relaxation and play an important role in the contraction process.

Mechanism of muscle contraction

- Each myofiber is innervated by efferent nerve impulses from axon terminals of motor end plates.
- The nerve impulse causes depolarization of the sarcolemma and this depolarization continues in the T-tubule.
- On reaching the triad the impulse causes the release of accumulated calcium ions from the terminal sacs of the sarcoplasmic reticulum into the sarcoplasm.
- The calcium ions unite with binding sites of troponin molecules to form a troponin-calcium complex. This results in the exposure of the active-binding sites of the G-actin allowing their interaction with the globular heads of heavy meromysosin.
- The process is energy dependent involving mitochondrial ATP and ATP-ase activity from the heavy meromysoin.

- The angle of the globular meromyosin heads changes repeatedly resulting in their binding with adjacent actin molecules in a rachet-like manner. This results in the filament sliding process and the changes seen in the sarcomeres during fiber contraction.
- At the end of contraction, the calcium ions break their connections with the troponin and accumulate again in the terminal saccules of the triads.

Imbalance in calcium ion homeostasis or a lack of ATP results in a breakdown of the contraction mechanism and may cause stable actin-myosin complexes and **tetany**. A similar muscular rigidity occurs after death (**rigor mortis**).

Other components of the sarcoplasm

- **Glycogen** particles are found and serve as energy stores. (These can be demonstrated by the PAS (periodic acid-Schiff) reaction in histological sections. At the ultrastructural level the spherical glycogen particles (-particles) are seen individually or in small clusters).

- Many elongated **mitochondria** are found located between the myofibrils or in accumulations just under the sarcolemma. The numbers and activities of the mitochondria are greater in muscle fibers with high metabolic activity.

- **Myoglobin** is an oxygen-binding protein that gives much of the red color of muscle fibers.

- Relatively little rough endoplasmic reticulum or ribosomes are present in myofibers.

- In aged muscle fibers **lipofuscin** deposits (brown pigment) are common. These are now known to be large secondary lysosomes.

Classification of muscle fibers

Muscle fibers are classified into three main categories:

- **Red fibers (Type I) or slow-twitch high-oxidative fibers**

These have relatively small diameters, much myoglobin, many well-developed mitochondria, a rich blood supply and much ATP-ase. These type I fibers are found in muscles with very high metabolic activity involved in slow sustained contractions. The energy source is from oxidative phosphorylation.

- **White fibers (Type IIa) or fast-twitch glycolytic-anaerobic fibers**

These have larger diameters, less myoglobin and fewer mitochondria, relatively poorer blood supplies and less ATP-ase. These type IIa fibers are involved in rapid contraction (fast twitch) with anaerobic glycolysis.

- **Intermediate fibers (Type IIb)**

These have structural and functional properties in between those of the other two types.

Muscles are characterized according to the predominance of the fiber types. Red muscle ("red meat") is dominated by type I fibers. White muscle ("white meat") is dominated by type IIa fibers. Most muscles are a mosaic of all the muscle types. The gross color reflects the differing proportions of the muscle types. This mosaic of muscle fibers can be demonstrated in frozen transverse sections of muscles subjected to histochemical techniques for enzymatic activities. For example, localization of succinic dehydrogenase activities (localized in mitochondria) or ATP-ase activities, is commonly performed on muscle biopsies to determine the ratio of the various muscle types.

Repair and regeneration after injury

If muscles are used intensively, trained or exercised, they increase in mass as a result of increase in protein synthesis and sarcomere production. This results in **hypertrophy of use** ("Use it or lose it"). On the contrary, limb immobilization (e.g. in plaster casts, or as a result of inactivity due to hospitalization, or lack of gravity) causes loss of muscle mass (**disuse myopathy** or **atrophy**).

Myofibers are syncytial and post-mitotic, with very limited regenerative abilities after trauma. After trauma such as muscle crush, pathological changes occur in muscle and may lead to breakdown of myofibers and release of myoglobin, which can affect renal function and be life-threatening. In the limited repair processes, satellite cells are activated, divide and can form new myotubes and myocytes. In some cases the satellite cells can fuse with existing fibers and contribute to the repair processes.

Atypical Striated Muscle

Some striated muscles of the body with typical histological appearance of striated muscle, are involuntary muscles. An example of such involuntary striated muscle is the cremaster muscle (near the spermatic cord).

In some cases striated muscles are not really "skeletal" as they are not attached to the skeleton (e.g. esophageal striated muscle, external urethral sphincter, external anal sphincter).

CARDIAC MUSCLE

Cardiac muscle is also striated, but differs from the striated skeletal muscle in several respects:

- The muscle **fibers branch** (bifurcate) and are arranged in series to form an anastomosing network.
- Each myocyte has one or two **central nuclei** (unlike the many peripheral nuclei of syncytia of skeletal muscle fibers).
- The fibers have more sarcoplasm.
- The mitochondria are larger and better developed.
- **All the fibers are Type I** (red fibers, with abundant myoglobin, high oxidative slow-twitch).
- **Glycogen** is more common.
- The myocytes have specialized areas of contact - the **intercalated disks**.
- **Contractions are rhythmic, spontaneous and involuntary**.

The cross striations have a similar morphology and staining characteristics to those of skeletal muscle fibers, however the contractile tissue is not organized into discrete myofibrils. At the ultrastructural level sarcomeres are found similar to those of skeletal muscle fibers. The large mitochondria are arranged in rows between the strings of sarcomeres. In histological preparations this gives the impression of longitudinal striations, though these are not myofibrils (Cardiac myocytes lack myofibrils). In aged cardiac muscle, **lipofuscin** is also commonly found.

Cardiac myocytes also possess a system of **T-tubules**. These consist of fairly broad tubular sarcoplasmic invaginations, which terminate in the region of the Z-line of the sarcomeres. Typically these are associated with a single terminal saccule of sarcoplasmic reticulum to form **diads**. In general the sarcoplasmic reticulum of cardiac muscle fiberes is much less well developed than that of myofibers of skeletal muscle.

Intercalated disks

These are step-like areas of interdigitation between adjacent sarcomeres. At the ultrastructural level the intercalated disks are seen to have two main components:

- **transverse regions**, rich in desmosomes and tight junctions. These are important in providing good cell adhesion between adjacent myocytes.
- **longitudinal regions**, parallel to the direction of the myofilaments. These regions have many gap junctions, which are areas of low electrical resistance and permit the spread of excitation from myocyte to myocyte.

Calcium ions play important roles in the areas of intercalated disks. Isolated hearts maintained in a culture medium with reduced calcium ion levels results in a separation of myocytes at the intercalated disks.

Conducting System of the Heart

The contraction of heart muscle is involuntary. The heart has its own system for **impulse generation and conduction**.

- The **impulse generating system** consists of the Sino-Atrial Node (**SA node**), which is composed of modified muscle cells and serves as the "**pacemaker**" of the heart. This SA node is also supplied with fibers of both the sympathetic and parasympathetic nervous system. The SA node cells cause regular waves of depolarization.
- The **conducting system** consists of the Atrio-Ventricular Node (**AV node**) and **Bundle of His**. This system has modified muscle fibers called **Purkinje fibers**, which conduct the impulses. These Purkinje fibers are well seen in cardiac preparations of the endocardia of the ventricles. Each Purkinje fiber consists of rows of connected Purkinje cells. Purkinje fibers are larger than normal cardiac myofibers and each fiber possesses a large central nucleus, surrounded by perinuclear region rich in glycogen. These fibers have well-developed sarcoplasmic reticulum, and relatively little contractile material. Purkinje fibers lack T-tubules.

Cardiac muscle fibers lack motor end plates (unlike skeletal muscle fibers).

Cardiac hormones

Peptide hormones are synthesized and secreted from atrial muscle cells. The hormones are called **atrial natriuretic hormones** and are involved in the homeostasis of sodium in the body. The atrial cells that produce

the hormones possess accumulations of membrane-bound storage granules visible by transmission electron microscopy.

Hypertrophy and regeneration of cardiac tissue

There is virtually no regeneration of cardiac tissue. The coronary arteries supplying blood to the heart are anatomical end arteries and lack collaterals. In the event of blockage of coronary arteries (as a result of a blood clot or atherosclerotic blockage), the cardiac myocytes vascularized by the coronaries cannot receive essential oxygen and the result is infarct. Following infarcts, the remaining heart muscle undergoes compensatory hypertrophy, with subsequent enlargement of the heart. Hypertrophied hearts are commonly an indication of underlying pathological disorders, though they may develop in specific cases of training and overload as in athletes.

SMOOTH MUSCLE

Smooth muscle is also known as "**involuntary muscle**", as contraction is not under conscious control. Smooth muscle is innervated by the **autonomic nervous system**.

Smooth muscle lacks cross-striations (unlike striated and cardiac muscle). Moreover, smooth muscle has the ability to undergo hyperplasia and hypertrophy (as in the uterus of pregnant women). Smooth muscle can also regenerate, and this is important in the repair processes of injured blood vessels.

Location of smooth muscle

- Smooth muscle is found in the **walls of the hollow internal organs** (hollow viscera), where it plays a role in maintaining the patency of the lumen. Smooth muscle forms the contractile layers of the intestinal tract, where it is important in peristaltic contractions involved in the movement of food.
- Smooth muscle is found in the **walls of the respiratory tracts**.
- Smooth muscle is present in the **walls of blood vessels** (vascular smooth muscle, especially in arterial vessels).

- Smooth muscle is found in the **dermis of the skin** (arrector pili).
- Smooth muscle is found in the eye (**iris diaphragm,** controlling the amount of light reaching the retina).
- Smooth muscle is a major component in the wall of the **uterus**.

Smooth muscle is also found in many other sites in the body

Structure of smooth muscle fibers

The smooth muscle fibers (**myocytes**) are **spindle-shaped** (fusiform).

The nucleus is in the widest part of the fiber and is elongated, typically with several nucleoli. In cross section, the nucleus will be evident only when the section cuts through the widest part of the myocyte.

The length of the myocytes is very variable in different organs. In some cases, such as in the uterus during pregnancy, the length can reach 0.5mm. Typically the length of smooth muscle in the various organs is about 0.2mm. In some cases, such as in small arterioles, the length may be only about 20 m. In most cases the thickness of the fibers at their widest part as seen in cross section is typically about 5-10 m.

In most organs, the smooth muscle fibers are orderly arranged in layers, strips or bundles. In cross section, the smooth muscle fibers are seen to form an orderly mosaic of circles of varying diameters, with the nuclei being seen only in fibers sectioned at their widest region. After regular staining (H&E) the sarcoplasm is seen to be acidophilic (stained with eosin). In sections of most of the intestinal tract, it is possible to see the two adjacent, antagonistic bands of smooth muscle (longitudinal and transverse).

Smooth muscle sheath

Each individual fiber is surrounded by a **sheath** (secreted by the fiber itself). The sheath contains proteoglycans, that stain positively with PAS reaction. A network of **reticular fibers** (shown after silver impregnation techniques) is found in the sheath and provides mechanical support for the fibers. In addition the sheath has **collagen fibrils** and **elastin fibers**. The sheath surrounding the individual myocytes is about 40-80 nm thick, except in some locations, where the sheath is absent and the membranes (sarcolemma) of two adjacent myocytes are in contact by means of gap junctions (nexuses). These are important as low resistance pathways permitting cooperation between the cells and in particular play a role as low resistance pathways. In a layer of smooth muscle cells, nerve stimuli only innervate a limited number of cells, but the information

concerning contraction can spread rapidly via the gap junctions to all the myocytes in the layer resulting in integrated contraction.

Smooth muscle cells lack an endomysium. The sheath is not the equivalent of an endomysium as in striated muscle. The sheath lacks connective tissue cells and blood vessels.

The ultrastructure of smooth muscle cells shows that the sheath appears somewhat similar to the basal lamina of epithelial cells. The organelles are located close to the nucleus in two distinct poles. The rest of the sarcoplasm is filled with **myofilaments**, though these are not arranged in ordered sarcomeres as in striated muscle. Three types of myofilaments may be seen:

- **thin myofilaments** (5-7nm thick), which are the most common type
- **thick myofilaments** (about 16nm thick), which are less common
- **intermediate filaments** (about 10nm thick). These may be grouped as "dense bodies" and are also found in contact with the sarcolemma (attachment plaques). It is thought that these intermediate filaments provide some sort of structural support for the cells.

The contraction mechanism of smooth muscle cells is still not very clear. The actin and myosin do not appear to be regularly arranged. Myosin is present in relatively low amounts. A calcium ion target protein, calmodulin, is present. The myocytes lack a T-system, though the sarcolemma has numerous small fixed saccules, known as caveolae. These caveolae may possibly have a role analogous to that of the T-system of striated muscle.

Origin of smooth muscle

Like the other muscle types, smooth muscle is also derived from **mesoderm**. Some researchers believe that smooth muscle has some affiliation to the connective tissue cells derived from mesenchyme, because the fibers synthesize and secrete collagen, elastin and reticulin of the sheath. They consider the smooth muscle fibers as connective tissue cells that have evolved the capacity of contractility.

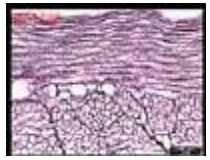
Some glands of ectodermal origin, such as sweat glands or mammary glands, possess smooth muscle cells surrounding their secretory units (**myoepithelial cells**). These myoepithelial cells are ectodermal in origin.

Some sites of the body show an intermingling of smooth muscle fasciculi, with those of skeletal muscle (e.g. part of the esophagus, anal sphincter, tarsi of eyelids).

Smooth muscle

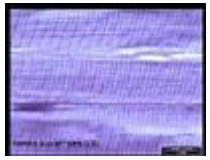
Smooth muscle

Smooth muscle

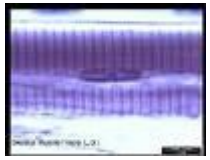


Smooth muscle

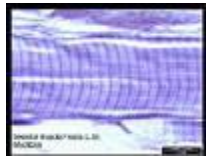
Skeletal Muscle fibers



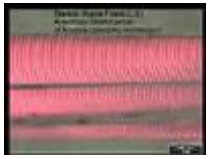
Skeletal Muscle fibers



Skeletal Muscle fibers



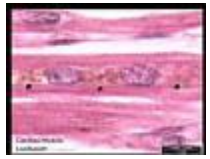
Skeletal Muscle fibers -Myofibrils



Skeletal Muscle fibers

Skeletal Muscle fibers

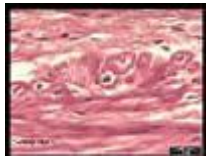
Cardiac Muscle



Cardiac Muscle

Cardiac Muscle-Lipofuscin

Cardiac Muscle



Purkinje Fibers

Cardiac Muscle

Skeletal Muscle Fibers

Skeletal Muscle Fibers

Skeletal Muscle Fibers

NERVOUS TISSUE

There are two basic systems of internal communication and physiological homeostasis in the body: the endocrine system and the nervous system.

The nervous system is derived from embryonic neuroectoderm.

The human nervous system is divided anatomically into:

- **Central Nervous System** (CNS), consisting of the brain and spinal cord.
- **Peripheral Nervous System** (PNS), consisting of nerve fibers, aggregates of nerve cells and glia and ganglia.

It is estimated that the human nervous system consists of at least 10 billion neurons.

Nervous tissue consists of two groups of cell types:

- Nerve cells (Neurons)
- Neuroglia.

Central Nervous System (CNS)

The Central Nervous System consists of the brain and spinal cord.
The nerve cell bodies (perikarya) of the CNS are often found in groups ("nuclei").

The brain and spinal cord are composed of **gray matter** and **white matter**.

Gray matter contains

- nerve cell bodies (perikarya)
- neuroglia
- neuropil (a complicated network of cell processes)

White matter lacks nerve cell bodies (perikarya), but has many processes of neurons. The white appearance is the result of the myelin that envelops many of the neuronal processes. Neuroglia are also found in the white matter and the nuclei seen in white matter belong to neuroglia.

Perikarya in the Peripheral Nervous System (PNS), are found only in ganglia (apart from in some sensory regions such as the retina and olfactory mucosa).

Neurons

Neurons are post-mitotic structures that shortly after birth lose the ability to divide. Further changes involve only reduced number of neurons (neuronal death), or changes in volume or in neuronal connections.

Neurons have two special properties:

- **Irritability** (the ability to respond to a stimulus)
- **Propagation of impulses** (the ability to conduct impulses).

The neuron is the morphofunctional unit of the nervous system. Similar to the Cell Theory, which stipulates the cell as the basic building block of the body, the Neuron Theory describes the neuron as the basic building block of the nervous system, and that the nervous system functions through transmission of information through networks of neurons.

Most neurons have three main parts:

- **Dendrites**
- **Perikarya** (cell bodies)
- **Axon**

The **dendrites** are receptive to stimuli and bring stimuli from the environment (sensory epithelial cells or other neurons) to the cell body. There are usually several dendrites per neuron.

The **perikaryon** (cell body) is also receptive to stimuli, but also serves as the trophic or synthesizing center for the whole nerve.

The **axon** is a long process emerging from the cell body. There is only a single axon for each neuron. The axon transmits impulses to other neurons, or to effectors: muscle or gland cells. The distal portion of the axon is usually branched (terminal arborization).

Neurons and their processes are very variable in form and size. Some neurons are very large (with perikarya of up to 150□m), whereas others are very small (perikarya of only 4-5□m).

Morphological classification of neurons

Neurons are classified according to the size, number and shape of their processes.

- **Unipolar neurons** (pseudounipolar) have a single process (axon). These are found in sensory ganglia of dorsal roots of spinal nerves.
- **Bipolar neurons** have two processes (one dendrite and one axon). These are very rare and have a limited distribution in the body. They are present in special sensory structures including the retina, olfactory epithelium, and vestibular and cochlear nerves).
- **Multipolar neurons** possess several processes (several dendrites and a single axon). Most neurons belong to this category.

Physiological classification of neurons

Neurons may also be classified according to their function.

- **Sensory neurons**. These receive sensory stimuli from the environment (from receptors) and from within the body (e.g. unipolar neurons).
- **Motor neurons**. These control the effector organs (muscles, exocrine glands, endocrine glands)
- **Interneurons** (Intermediate neurons). These are typically found in the CNS and connect between other neurons (often between sensory and motor neurons).
- **Neurosecretory neurons**. These are specialized neurons that synthesize and secrete hormones.

Each neuron has 3 physiological parts or segments:

- **Receptive segment** (dendrites and perikaryon). The perikaryon also has an additional trophic and synthesizing role.
- **Conductive segment** (axon)

- **Transmissive segment** (synapse).

Reflex arcs

The functional roles of various neurons are best illustrated by simple reflex arcs in which peripheral receptors are connected to peripheral effectors in a neuronal network.

- Stimulation of the **receptor** (in skin or skeletal muscle spindle)
- Propagation of an impulse via **afferent sensory nerve** (unipolar neuron), which enters the gray matter of the spinal cord.
- **Interneurons** connect with cell body of motor neuron in the ventral horn.
- **Motor neuron** transmits the efferent impulse to an effector.
- The **effector** e.g. motor end plate of skeletal muscle responds to the impulse.

(An analogy can be made to a cable system of telephone wires. The phone message is sent to a central exchange, that directs the message to the correct connection, resulting in a specific response by the recipient).

MORPHOLOGY OF NEURONS

(1) **Dendrites**

Most nerve cells have several dendrites. These increase the receptive area of the neuron. Dendrites do not maintain a constant diameter (unlike axons) and transmit impulses to the cell body decrementally (unlike axons. The regions of the dendrites closest to the perikaryon are usually larger, than those farther away. Typically dendrites have large numbers of thorny spines, which are now known to be areas of synaptic contact.

(2) Perikaryon

The perikaryon (neuronal cell body or soma) consists of the nucleus and surrounding cytoplasm. (The term perikaryon implies the area surrounding the nucleus, but the term is used freely today to describe the whole cell body including the nucleus). The perikaryon is the trophic center of the neuron involved in protein synthesis. The surface of the perikaryon receives nerve impulses and is the site of many synapses, bringing excitatory or inhibitory stimuli.

Nucleus

The nuclei of perikarya are large, regular, round or oval, typically situated fairly centrally. The nuclei are euchromatic (pale staining with dispersed chromatin). Such large regular nuclei are typical of cells involved in intense synthetic activities. Sex chromatin (**Barr's body**) is commonly seen in the nuclei of females.

Rough Endoplasmic Reticulum (RER) - Nissl bodies

RER is abundant in the cytoplasm and is associated with the protein synthetic activities of the neurons. The RER is basophilic as seen in regular (H&E) staining by light microscopy. This RER is also stained with cresyl violet in the Nissl staining technique (**Nissl bodies**). In the event of injury to axons, Nissl bodies, are displaced to the periphery of the perikaryon.

Golgi bodies

Large well developed Golgi bodies are present in the perikarya.

Mitochondria

Many large mitochondria are found throughout the perikaryon.

Neurofibrils

Neurofibrils are seen in perikarya (and also in the nerve processes) after silver impregnation techniques. At the electron microscope level these are seen to consist of clumped neurofilaments and neurotubules.

Lipofuscin

Lipofuscin is a brown pigment that is common in perikarya of aged neurons. It is now known to be common to post-mitotic cells and to consist of large secondary lysosomes.

(3) Axons

Each neuron has a single axon. The diameter of the axon is fairly constant. The length of axons is fairly variable, and some reach up to 100 cm (the axons innervating the toes have their cell bodies in the spinal cord). All axons originate in a short pyramid-like structure called the **axon hillock**, which lacks Nissl substance. The plasma membrane of the axon is termed the **axolemma**, and the cytoplasm of the axon is termed the **axoplasm**.

In myelinated axons the initial portion, between the axon hillock and the start of the myelin sheath, is called the **initial segment**.
Axons sometimes have right-angled branches known as **axon collaterals**.
The nerve impulse travels down the axon non-decrementally.

Myelinated fibers

Nerve fibers consist of axons enveloped by special sheaths. In peripheral nerves the sheath cell is the **Schwann cell**, whereas in the CNS, the sheath-forming cells are the **oligodendrocytes**.

The axons of small diameter are usually non-myelinated fibers, whereas the thicker axons have concentric wrappings of the enveloping cell to form the myelinated sheath. The fibers with myelinated sheaths are called **myelinated fibers**. Myelinated nerves, composed mainly of myelinated axons, appear white in the fresh state. The sheath of myelinated fibers is formed by concentric layers of membranes of the Schwann cell (or oligodendrocyte in the CNS) around the axon, which unite to form a lipoprotein complex. This stains black with osmium tetroxide. The whorled structure of the myelin sheathe when examined by transmission electron microscopy is seen as a repeating dark line (**major dense line**) and and a thinner repeating **intraperiod line**. The major dense line is formed by the fusion of two of the inner layers of sheath cell membrane, whereas the intraperiod line is formed by the fusion of the outer layers of sheath cell membrane when they come in contact as a result of the concentric arrangement. The myelin sheath is essentially an accumulation of closely packed whorls of lipoprotein rich membranes surrounding the axon.

If a single fiber of a myelinated peripheral nerve is teased, stained with osmium tetroxide and examined by light microscopy, the myelin sheath surrounding the axon is seen as a series of myelinated **internodes** (0.08-1.00 mm) separated by **nodes of Ranvier**. (The myelinated axon is somewhat similar to a long string of sausages). The myelin of each internode is formed by a single Schwann cell, whose nucleus is seen at the periphery. Tangential non-stained areas (similar to arrow heads) are seen in the myelin of the internodes (**Schmidt-Lantermann clefts**). These are areas of cytoplasm of the Schwann cells, where the membranes are not closely apposed. An **endoneurial connective tissue sheath** surrounds each fiber.

In wax sections stained with H & E, the lipid of the myelin is dissolved by the xylene or chloroform during processing and the site of the myelin sheath appears empty apart from a fine network stained by the eosin. This is known as **neurokeratin**.

Myelinated axons of the CNS have myelin sheaths, similar to those of the peripheral nerves. However, a single oligodendrocyte produces the myelin sheaths of several axons. No endoneurial connective tissue sheath is present. The nodes of Ranvier are larger and exposed to the extracellular space.

Nodes of Ranvier

The nodes of Ranvier have several important features:

- sites of axon collaterals
- large concentrations of mitochondria in the axon at these sites (high localmetabolic activity)
- site of saltatoryconduction (non-decremental)
- sites of paranodal loops (important in the saltatory conduction).

Axonal transport

Transport of molecules along the axon (**axonal transport**) is in two directions: anterograde (from the cell body to the terminal synapse) or retrograde (in the direction of the cell body). The axonal transport involves neurotubules and neurofilaments.

Two different systems of axonal transport occur:

- **Slow axonal transport** system, from the cell body in a single direction at a rate of about 1mm per day. This system conveys components needed for growth and regeneratioof the axon.
- **Fast axonal transport** system, which occurs in both directions, at a rate of about 100-200 mm per day. This system involves transport of enzymes needed for synthesis of neurotransmitters within the terminal synapse.

NEUROGLIA

Glia or **neuroglia** get their name from the Greek word for "glue". There is very little connective tissue in the CNS, and the structural support for neurons comes from neuroglia and their processes.

It is estimated that for every neuron there are at least 10 neuroglia, however, as the neuroglia are much smaller than the neurons they only occupy about 50% of the total volume of nerve tissue. Neurons cannot exist or develop without neuroglia.

There are **4 basic types of neuroglia**, based on morphological and functional features.

- **Astrocytes (Astroglia)**
- **Oligodendrocytes (Oligodendroglia)**
- **Microglia**

- **Ependymal cells**

The astrocytes and oligodendroglia are large cells and are collectively known as **Macroglia**.

Neuroglia differ from neurons:

- Neuroglia have **no action potentials** and cannot transmit nerve impulses
- Neuroglia **are able to divide** (and are the source of tumors of the nervous system)
- Neuroglia **do not form synapses**
- Neuroglia **form the myelin sheathes** of axons.

Astrocytes (Astroglia)

These are present only in the CNS and are the largest of the neuroglia. They have many long processes, which often terminate in "**pedicels**" on blood capillaries and contribute to the **blood-brain-barrier**.

There are two categories of astrocytes:

- **Protoplasmic astrocytes**. These are present in the gray matter of the brain and spinal cord. Their processes are relatively thick.
- **Fibrous astrocytes**. These are present in the white matter of the CNS. Their processes are much thinner than those of the protoplasmic astrocytes.

Because of their number and their long processes, the astrocytes appear to be the most important supporting elements in the CNS.

Oligodendrocytes

These are smaller than the astrocytes, with fewer and shorter processes. They are found in both the gray and white matter of the CNS and are responsible for the formation of the myelin sheath surrounding axons. The **Schwann cells** of the PNS belong to the oligodendrocytes and form the myelin sheath around peripheral axons.

Microglia

These are small cells, with elongated bodies, elongated nuclei with dense chromatin and relatively few processes. They are found in both the gray and white matter of the CNS and are thought to function as

macrophages. There is some evidence that they are in fact of mesenchymal origin and derived from blood-borne monocytes.

Ependymal cells

The ependyma is composed of neuroglia that line the internal cavities (ventricles) of the brain and spinal cord (central canal). They are similar in appearance to a stratified columnar epithelium. The ependymal cells are bathed in **cerebrospinal fluid** (CSF). Modified ependymal cells of the **choroid plexuses** of the brain ventricles are the main source of the CSF.

FUNCTIONS OF NEUROGLIA

- Structural support (especially the astrocytes in the CNS)
- Participation in the blood-brain-barrier (astrocytes)
- Formation of the myelin sheath of axons (oligodendrocytes)
- Isolation of junctional surfaces of synapses
- Repair processes following damage or injury to nerves.

NERVE STAINING TECHNIQUES

- **Silver impregnation**

These techniques (developed in particular by the Italian ***Camillo Golgi*** and the Spaniard ***Ramon y Cajal***) stain whole neurons, but selected ones only).

- **Nissl staining**

Cresyl violet is used in the Nissl staining technique to demonstrate the Nissl bodies (RER) of the perikarya. The processes are not stained.

- **Myelin stains**

In these techniques the lipid of the myelin of myelinated fibers is stained.

Connective Tissue of Peripheral Nerves

Examination of a peripheral nerve shows a thin connective tissue layer surrounding each individual fiber. This is the **endoneurium** (also known as the sheath of Key and Retzius).

Fibers are grouped in bundles, which are also surrounded by a connective tissue layer, known as the **perineurium**.

The **epineurium** is a more extensive connective tissue layer between the bundles and extending to the most peripheral parts of the nerve.

Nerves possessing only sensory fibers are called **sensory nerves**, whereas nerves possessing only motor fibers are called **motor nerves**. Most nerves are **mixed nerves** in that they possess both sensory and motor fibers.

SYNAPSES

Synapses are specialized areas of contact between neurons. Various categories of synapses are found including:

- **axo-dendritic**
- **axo-somatic**
- **dendro-dendritic**
- **axo-axonic**

Morphologically the axon terminal is seen as a club-shaped bulb (terminal buttons or "***boutons terminaux***"). If the synapse is not at the end of the axon, but at a site along the length of the axon, it is known as a "***bouton en passage***". Many of the synapses occur on swellings of the dendrite (**dendritic spines**).

The synapses in the human body are chemical synapses. They involve the release of **neurotransmitters**, which combine with receptors on the post-synaptic membrane and result in the transmission of the impulse.

Synapses examined by transmission electron microscopy the terminal bulb are seen to contain membrane-bound **synaptic vesicles** (25-65□m), which store **neurotransmitters**. Mitochondria are also common in this **presynaptic region**. When the impulse reaches the presynaptic area, the synaptic vesicles migrate and fuse with the **presynaptic membrane** and release their contents into the **synaptic cleft** (20nm). The neurotransmitters combine with specific **receptors** on the postsynaptic membrane leading to the transmission of the impulse. Specific enzymes act on the receptors. For example, the neurotransmitter acetycholine (of

cholinergic nerves), when it combines with the postsynaptic receptor is affected by the enzyme, acetylcholinesterase.

Some synapses are **excitatory**, whereas others are **inhibitory**.

MOTOR END PLATES

Motor end plates (**neuromuscular junctions**) are specialized structures at the ends of motor axons and are the sites of innervation of skeletal muscle fibers. In order to contract each individual muscle fiber needs to receive an impulse from a motor nerve. A single motor nerve may innervate a single fiber or may have several neuromuscular junctions. A single motor nerve and all the muscle fibers it innervates is called a **motor unit**.

Motor nerves and the fibers they innervate can be demonstrated by silver impregnation techniques. At the ultrastructural level the axon terminal has several features similar to those of synapses. Neurotransmitters are present in synaptic vesicles and mitochondria are common. There is also a synaptic cleft. The **postsynaptic membrane** is modified sarcolemma of the muscle fiber. This has many **folds** (to increase the surface area). When the nerve impusle reaches the motor end plate, the synaptic vesicles release acetylcholine into the cleft. These bind to specific receptors on the postsynaptic membrane. This results in the transfer of the impulse to the sarcolemma and on to the T-tubules.

NERVE GANGLIA

Ganglia are groups of nerve cell bodies (perikarya) outside the CNS. Two types of nerve ganglia can be distinguished based on their morphology and function:

- **Sensory ganglia** (Dorsal root ganglia)
- **Autonomic ganglia**

Spinal ganglia are found in the dorsal roots of spinal nerves and carry afferent sensory impulses. The ganglia are surrounded by a fairly thick connective tissue capsule. The perikarya belong to the unipolar (pseudounipolar) neurons. Each perikaryon has a T-shaped process continuous with the afferent axon. The cell bodies have a purely trophic function and are not involved with the nerve transmission. Each perikaryon is surrounded by a layer of glial cells (satellite cells). The perikarya are not evenly distributed in the ganglia, but are found in groups, mainly fairly close to the periphery.

Autonomic ganglia are associated with nerves of the autonomic nervous system. They are found as dilatations of autonomic nerves and may be encapsulated. In many cases the ganglia are seen in the walls of organs (intramural) and lack a capsule. They differ from spinal ganglia in that the neurons are multipolar. The perikarya are smaller,have fewer satellite cells and are more evenly distributed.

NON-MYELINATED NERVE FIBERS

Non-myelinated nerves are found in both the CNS and PNS. Postganglionic fibers of the autonomic nervous system are non-myelinated. The axons are enclosed in simple clefts of oligodendrocytes or Schwann cells. Each Schwann cell may enclose several non-myelinated axons.

At areas of transmission, the axon lies in a "naked" groove on the surface of the Schwann cell. This is the autonomic neuromuscular junction and is the site of innervation of smooth muscle bundles. The release of neurotransmitter (acetylcholine in cholinergic fibers, nor-epinephrine in nor-adrenergic fibers) causes depolarization of the sarcolemma of the muscle fiber and contraction. The impulse can be transferred to other adjacent smooth muscle cells via the gap junctions in the sheaths. As a result only one muscle fiber needs to be innervated, though the message to contract is rapidly spread to the adjacent fibers, so that they can contract in unison.

DEGENERATION AND REGENERATION OF NERVE FIBERS

Neurons do not divide, though neuroglia can divide. Tumors of the nervous system result from uncontrolled growth of glia. If neurons are damaged in the CNS, there is permanent loss and no regeneration. If, for example the optic nerve is severed, permanent blindness results. In contrast peripheral nerves if crushed or even severed may regenerate provided the perikaryon is not injured.

If a peripheral nerve is severed, the distal segment degenerates. Axonal injury causes morphological changes in the perikaryon including:

- chromatolysis (loss of Nissl bodies) with the remaining Nissl moving to the periphery of the perikaryon
- swelling of the perikaryon
- movement of the nucleus from its central position to the periphery.

Regeneration is possible if the proximal segment of the axon grows, sends out "sprouts" and these penetrate the correct column of Schwann cells.

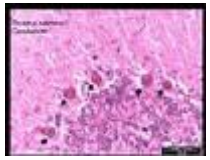
Pyramidal neuron

Fibrous Astrocytes

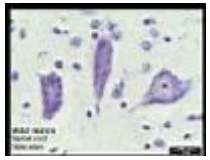
Fibrous Astrocytes

Protoplasmic astrocytes

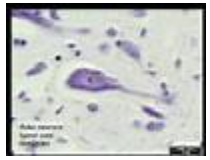
Purkinje neuron



Purkinje neurons - Cerebellum



Motor Neurons



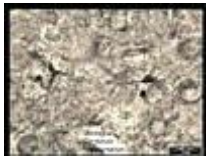
Motor Neurons

Motor Neurons

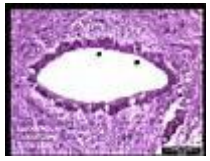
Motor Neuron - neuropil

Fibrous astrocytes

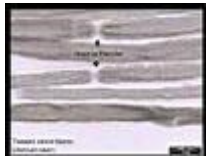
Microglia - Cerebrum



Microglia - Cerebrum



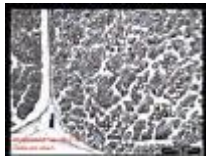
Ependyma - Central Canal



Teased nerve fibers

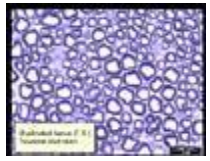
Schmidt-Lantermann Clefts

Node of Ranvie



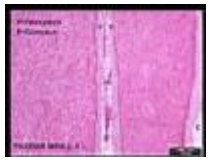
Myelinated Nerve

Myelinated Nerve



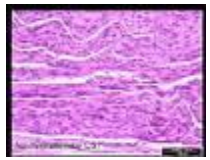
Myelinated Nerve

Myelinated Nerve

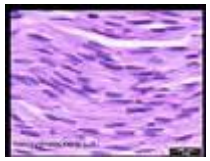


Peripheral Nerve

Myelinated fiber



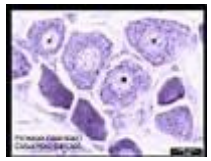
Non-myelinated nerve



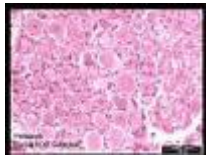
Non-myelinated nerve

Motor Unit

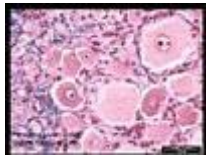
Motor end plates



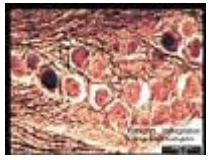
Perikarya (Nissl stain)



Perikarya- Dorsal Root Ganglion

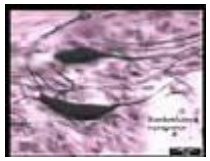


Perikarya (Azan stain)

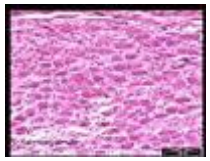


Perikarya - Impregnation

Myenteric Plexus



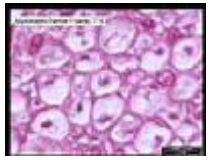
Myenteric Plexus



Autonomic Ganglion

Autonomic Ganglion

Myelinated nerve

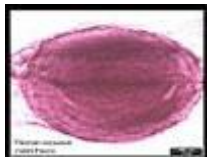


Myelinated Nerve Fibers

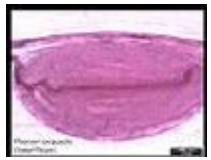
RECEPTORS:

Neuromuscular Spindle

Neuromuscular Spindles



Pacinian corpuscle



Pacinian corpuscle

Meissner corpuscles

Meissner corpuscles

CARTILAGE

Cartilage belongs to the skeletal tissues and is a specialized form of connective tissue. Cartilage is composed of cells, **chondrocytes** (2-5% of the tissue volume only) located in **lacunae** surrounded by an intercellular **matrix**.

Cartilage is an **avascular tissue** with has no blood vessels of its own, though in some cases, such as in epiphyses of long bones, blood vessels traverse the tissue in cartilage canals to supply nutrients to other tissues. Cartilage is a tissue of very **low metabolic activity** and cell turnover (except in the embryo). Cartilage receives its nutrients from blood vessels from a surrounding dense connective tissue, the **perichondrium**. Nutrients and metabolites pass to and from the cells via the matrix by diffusion.

Nerves are not present in cartilage, but nerves and nerve ending are present in the perichondrium.

Cartilage is classified as:

- **Hyaline cartilage**
- **Elastic cartilage**
- **Fibrocartilage**

The difference between the different cartilage types depend on the different properties of the intercellular matrix, and in particular on the amount and type of the fibers embedded in the matrix.

HYALINE CARTILAGE

Hyaline cartilage is the most common form of cartilage. Its name is derived from the Greek "*hyalos*" = glass. Fresh hyaline cartilage is a semi-transparent (translucent), milky-white tissue, that is both flexible and resilient to mechanical forces. In adults hyaline cartilage is found in the respiratory tract (nose, larynx, trachea, bronchi), the ventral part of ribs, and on articulating surfaces of long bones and joints (**articular cartilage**). Hyaline cartilage is much more common in the embryo, where it plays an important role in long bone development.

Chondrogenesis

Like all connective tissue, cartilage is derived in the embryo from mesenchyme.

Mesenchyme cells grow and differentiate into young cartilage cells or **chondroblasts**, that are very active in secreting the surrounding matrix. The chondroblasts grow and develop in lacunae. These chondroblasts further differentiate into mature cartilage cells or **chondrocytes**.

There are two different types of chondrogenesis:

- **appositional growth**
- **interstitial growth**

Appositional growth of cartilage

Appositional growth describes the addition of new cartilage cells from the surrounding perichondrium. Flattened cells (**chondroprogenitor cells**) of the perichondrium divide and differentiate into elliptical **chondroblasts**. These chondroblasts, are active in secreting the intercellular matrix. The chondroblasts typically have basophilic cytoplasm. By transmission electron microscopy the cells are seen to have well-developed RER and Golgi bodies, typical of active secretory cells. The chondroblasts further differentiate into **chondrocytes**. The chondrocytes are less basophilic, more rounded and occupy more rounded lacunae. The amount of matrix surrounding the chondrocytes is greater than that of the chondroblasts.

Interstitial growth of cartilage

Interstitial growth involves the addition of cartilage cells by the division of chondrocytes within specific lacunae deep in the tissue. As a consequence of this mitotic activity, lacunae may possess two, four, eight daughter chondrocytes. These are known as **isogenous** or **nest cells.** Interstitial growth is seen in histological preparations deeper in the older cartilage.

Chondrocytes may shrink during histological preparation and may not occupy the normal dimensions of the lacunae. This is a preparational artefact.

Cartilage matrix

The most important component of cartilage and which provides the biomechanical characteristics of the tissue is the extracellular matrix. The matrix is composed of amorphous substance in which are embedded fibers.

The main components of hyaline cartilage (wet weight) are approximately:

- **Water 72-75%**
- **Proteoglycans 10%**
- **Collagen (type II) 16%**
- Other **glycoproteins** (e.g. chondronectin) 1.6%
- **Minerals 0.5%**

Hyaline cartilage matrix stains basophilic in regular H&E staining. It also stains positively with the PAS technique. The matrix is also **metachromatic** (after staining with e.g. toluidine blue, it is stained a purple color). The staining properties are the result of the molecular composition of the matrix.

The proteoglycans represent a complex of **protein** and **sulfated glycosaminoglycans** (GAG's), and in particular:

- **chondroitin-4-sulfate**
- **chondroitin-6-sulfate**
- **keratan sulfate**

These sulfated GAG's provide the basophilic staining characteristics.
In addition there are molecules of a **non-sulfated GAG**: **hyaluronic acid**.
The hyaluronic acid is a long thread-like molecule, to which are attached **link proteins** periodically along its length. **Proteoglycan monomers** (which resemble a test-tube brush) are attached to the link-proteins and consist of **core proteins** to which are connected the sulfated GAG's.

The network of macromolecules are held in place by the water, known as "**solvation water**". The solvation water is important for the diffusion of nutrients in the matrix of the tissue. The proteoglycans are important for maintaining the high osmotic pressure of the matrix and the resilient characteristics of the cartilage. **Type II collagen fibers** are embedded in the matrix and provide structural support (similar to that found in building

materials such as the fibers embedded in resin in fiberglass). The type II collagen fibers constitute about 40% of the dry weight of cartilage. In normal histological preparations the collagen fibers are not seen as they have submicroscopic dimensions and their refractive index is similar to that of the amorphous matrix. By transmission electron microscopy, the collagen fibers are seen to have a 64nm banding and to be composed of regularly overlapping collagen fibrils.

The biomechanical properties of the cartilage allow it to function as a biomechanical spring or shock-absorber, to spread the load at joints and prevent too great pressures on bones. The capacity of the tissue for water-retention under load and resilience is an extremely important property of cartilage.

In histological sections the matrix surrounding the lacunae (nearest to the cells) is stained more intensely and is more basophilic (**capsular** or **territorial matrix**). Here the relative concentration of GAG's is greater than in the mass of the matrix, where the staining is less intense (**inter-territorial matrix**).

ELASTIC CARTILAGE

Elastic cartilage is characterized by its great flexibility and elasticity owing to the large quantities of elastic fibers in the matrix. These elastic fibers provide the yellowish color in the fresh tissue. The elastic fibers in histological sections can be stained (e.g. with orcein). The elastic fibers are branched. The elastic fibers in the matrix near the perichondrium are less-densely packed (and easier to see) than those deeper in the tissue.

Elastic cartilage is found in the external ear, in the walls of the external auditory meatus and Eustachian tube and also in the epiglottis.

FIBROCARTILAGE

Fibrocartilage is found in areas of the body subject to high mechanical stress or weightbearing. It lacks the flexibility of the other cartilage types.

Fibrocartilage is present in:

- intervertebral disks
- pubic symphysis

- temporo-mandibular joints
- at sites of connection of many ligaments to bones (e.g. *Ligamentum teres femoris*)
- tendon insertions.

Fibrocartilage is characterized by large numbers and concentrations of **collagen fibers** in the matrix. These collagen fibers are the dominant feature of the matrix and with relatively little amorphous matrix. The large amounts of collagen fibers result in the matrix appearing **acidophilic** in histological sections after H&E staining. Fibrocartilage is not surrounded by perichondrium.

The **intervertebral disks** consist of fibrocartilage plates between the vertebrae and act as mechanical shock absorbers. In sections they are seen to be formed of two components:

- **annulus fibrosus**, which is the outer region consisting of orderly concentric arrangements of cells and matrix dominated by type I collagen (as in tendons)
- **nucleus pulposus** (large vacuolated cells, that are vestiges of the embryonic notochord.

FUNCTIONS OF CARTILAGE

Cartilage is important for:

- skeletal support in the embryo prior to the development of the bony skeleton.
- elongation of developing long bones (endochondral ossification).
- articulating joints (articular cartilage).
- flexible support in the ear and eartubes, and in the larger tubes of the respiratory tract (trachea, bronchi).

Regeneration and repair

Despite the fact that cartilage is found in relatively few sites in the adult body, its functions are important for our well-being. Cartilage in adults has very little regenerative ability if damaged and is subject to tear and wear with aging. This is due to the dearth of cartilage cells, minimal mitosis, absence of an integral blood supply and overall low metabolic activity of the tissue. The clinical problems of damage or aging of the tissue

(osteoarthritis) are substantial. With aging the cartilage matrix may develop calcified deposits (calcified cartilage).

Secondary cartilage

This refers to cartilage that develops in association with specific bones formed by intramembranous ossification after the bones are already formed. This is the opposite of cartilage associated with endochondral ossification, where the cartilage precedes the bone formation. The cartilage of the temporomandibular joint is an example of a secondary cartilage.

BONE TISSUE

Bone tissue is a specialized form of connective tissue and is the main element of the skeletal tissues. It is composed of cells and an extracellular matrix in which fibers are embedded. Bone tissue is unlike other connective tissues in that the extracellular matrix becomes calcified.

FUNCTIONS OF BONE TISSUE

- The skeleton is built of bone tissue. Bone provides the internal support of the body and provides sites of attachment of tendons and muscles, essential for locomotion.
- Bone provides protection for the vital organs of the body: the skull protects the brain; the ribs protect the heart and lungs.
- The hematopoietic bone marrow is protected by the surrounding bony tissue.
- The main store of calcium and phosphate is in bone. Bone has several metabolic functions especially in calcium homeostasis.

Bone is a hard, but brittle, tissue and is relatively light per unit volume. Bone is a dynamic tissue, which throughout life bone tissue is continually being formed and resorbed. This **remodelling and reorganization** of bone tissue is the result of many factors including:

- mechanical stimuli
- metabolic causes (lack of dietary calcium, illness, aging)
- endocrine changes
- effects of drugs.

MACROSCOPIC STRUCTURE OF BONE

There are two main categories of bone :

- **Spongy bone (trabecular bone, cancellous bone)**
- **Compact bone (cortical bone)**

Spongy bone

Spongy bone is composed of a lattice or network of branching bone spicules or trabeculae. The spaces between the bone spicules contain bone marrow.

Compact bone

Compact bone appears as a mass of bony tissue lacking spaces visible to the unaided eye.

Anatomical classification of bones

Bones are characterized anatomically as:

- **long bones** (e.g. humerus, femur)
- **flat bones** (membrane bones)
- **irregular bones** (such as the vertebrae)

All these bone types, regardless of their anatomical form, are composed of both spongy and compact bone.

Macroscopic structure of long bones

The main shaft of long bones is called the **diaphysis**. At the extremities of the long bone are the **epiphyses** (in articulating joints). The region involved in bone elongation between the diaphysis and epiphysis in growing bones is called the **metaphysis**. The shaft (or diaphysis) is composed of compact (cortical or diaphyseal) bone. The epiphyses are mainly composed of trabeculae of spongy bone. The articulating surface of the epiphyses of synovial joints is covered with articular cartilage.

Bones are covered with a connective tissue called the **periosteum** (absent from the articular cartilage surfaces). A thinner layer of connective tissue, known as the endosteum, surrounds the bone marrow spaces and trabeculae of spongy bone. The periosteum and endosteum are a source of new bone-forming cells (**osteoprogenitor cells**) and are described as possessing **osteogenic potential**. The periosteum and endosteum are also involved in bone repair after injury. Blood vessels of the periosteum and endosteum are involved in nutrition of the bone.

Macroscopic structure of flat bones

The flat bones or "membrane" bones of the skull are composed in a sandwich-like fashion of an outer layer of compact bone (**outer table**), a middle layer of spongy bone (**diploe**), and an inner layer of compact bone (**inner table**). Periosteum covers the flat bone on the outer side (near the scalp) and on the inner side the periosteum is thicker and continuous with the duramater (outer meningeal layer of the brain).

PREPARATION OF HISTOLOGICAL SECTIONS OF BONE

Because bone tissue is hard and calcified, special histological techniques are used to prepare sections.

- **Decalcification**. The most common techniques involve calcium removal from the tissue (decalcification) after fixation and prior to wax embedding. Acids, such as formic acid or nitric acid, can be used as decalcifying agents. After decalcification the tissue is soft and can be embedded and processed as in standard histology. It is also possible to use chelating agents, such as EDTA, which specifically bind calcium. These chelating agents are less damaging to the tissue than acids, but the decalcification process may be quite long (several weeks or more).
- **Ground sections**. It is possible to grind the bone until the sample is sufficiently thin for histological observation. The cells and organic tissue are destroyed in such preparations, though the canaliculi and cell lacunae are well seen. (Similar techniques are used by geologists to prepare thin sections of rock samples).
- **Sections of non-decalcified bone**. It is possible to embed bone tissue in a hard resin and section it with special knives (tungsten-carbide). Small samples for electron microscopy can be cut with diamond knives.

MICROSCOPIC STRUCTURE OF BONE

This is best seen in compact bone, for example, in transverse sections of the diaphysis of a long bone. The cells constitute only a very small percentage of the bone tissue, whereas the bulk of the tissue is occupied by the intercellular, calcified, bone matrix.

The bone matrix has two main components :

- **Organic matrix**
- **Inorganic salts.**

Organic matrix

The organic matrix is composed of **type I collagen fibers** (about 95%) embedded in an **amorphous ground substance** consisting of:

- **sulfated glycosaminoglycans**

  (chondroitin-4-sulfate, chondroitin-6-sulfate, keratan sulfate)

- various **bone proteins** (bone sialoprotein, osteocalcin).

The relative amounts of sulfated GAG's are far less than in hyaline cartilage, and bone matrix appears acidophilic after regular staining (H&E).

Inorganic salts

The main calcium deposits in the bone matrix are in the form of crystals of **hydroxyapatite** $Ca_{10}(PO_4)_6.(OH)_2$

BONE CELLS

4 different cell types are found in developing bone:

- **Osteoprogenitor cells**
- **Osteoblasts**
- **Osteocytes**
- **Osteoclasts**

Osteoprogenitor cells

Bone, like other connective tissue in the embryo, is derived from mesenchyme cells. After birth, flattened, poorly-differentiated, mesenchyme-like cells, are found in the periosteum and endosteum. These cells can divide (mitosis) and differentiate into bone cells (osteogenic potential) and as a result are known as osteoprogenitor cells.

Osteoblasts

The first cells to develop from the osteoprogenitor cells are the osteoblasts. Osteoblasts are involved in the formation of bone and are found on the boundaries of developing and growing bone. The cells are typically oval, with a large eccentric nucleus, and the cytoplasm is fairly basophilic. These cells are very active in synthesizing and secreting the components of the bone matrix and have well-developed rough endoplasmic reticulum (RER), Golgi bodies and granules. Osteoblasts are rich in the enzyme **alkaline phosphatase**, which plays a major role in the formation of the mineral deposits in the matrix. The collagen fibers are synthesized and secreted by the osteoblasts.

The matrix closest to the osteoblasts is not yet calcified and is known as **osteoid** or **prebone**. This osteoid is rich in collagen fibers. Small membrane-bound **matrix vesicles** (not visible by light microscopy) are budded off processes of the osteoblast cell membrane and secreted to the matrix. These play an important role in the calcification process of the matrix.

Osteocytes

Osteocytes are mature bone cells that develop from osteoblasts and are located in lacunae within the bony matrix. Osteocytes have cytoplasmic processes located in **canaliculi**, which penetrate the bony matrix. Cytoplasmic processes from one osteocyte make contact with the processes from neighboring osteocytes and can communicate via gap junctions. Because the bony matrix is calcified there is no possibility of diffusion except via the network of canaliculi.

Osteoclasts

Osteoclasts are the largest of the bone cells (20-100□m diameter) and are multinuclear (with up to 50 nuclei). Osteoclasts are involvedin **bone resorption** and can be found on the eroding surfaces of bone, often in cavities known as **Howship's lacunae**. The osteocytic cell membrane closest to the bone undergoing resorption has multiple invaginations and is known as the "**ruffled border**". Thcells are metabolically very active, possess large numbers of mitochondria (resulting in the acidophilia of regular staining) and have well-developed Golgi bodies. Osteocytes synand secrete the enzyme **acid phosphatase**, which is involved in the erosion of the bony matrix. (More specifically the enzyme is known as Tartrate-resistant Acid Phosphatase or TRAP and histochemical localization of TRAP enzymatic activity is a useful marker for identifying osteoclasts in sections).

Osteoclasts originate from monocytes and are included in the **mononuclear phagocyte system**.

OSTEOGENESIS

Woven bone (Immature bone, Primary bone)

Osteogenesis is the name given to the development of bone tissue. The first bone to develop is a form of spongy bone known as woven bone (immature bone or primary bone). This is a primitive form of bone tissue that can be identified by the lack of order of the lacunae (of osteocytes) and the thick, irregular "woven" network of collagen fibers in the matrix. Woven bone is found temporarily in the developing embryo, before undergoing rearrangement (remodeling) resulting in the development of lamellar bone.

Woven bone is not usually found in people aged over 14 except for some specific locations including the vicinity of sutures of flat bones of the skull, in tooth sockets, and some tendon insertions. Woven bone also develops temporarily in cases of bone fracture and repair.

Lamellar bone (Mature bone, Secondary bone)

Most bone tissue is lamellar bone in which the tissue is well organized and regular. The lacunae (of osteocytes) are regularly arranged as are the collagen fibers of the matrix. The term **lamella** ("leaf") refers to the layer of matrix between two rows of lacunae. The lamellar arrangements are best illustrated in the cortical (compact) bone of the diaphysis of long bones.

Mature compact bone is composed of three **lamellar arrangements** :

- **Osteons (Haversian Systems)**
- **Circumferential Systems**
- **Interstitial Systems**

Osteons (Haversian Systems)

Osteons (or Haversian Systems) are cylindrical structures of compact bone, which in transverse section are seen to be formed of 4-20 regular concentric lamella surrounding a central vascular channel (**Haversian canal**). The diameter of each osteon typically ranges from 20-110 m. The collagen fibers in each lamella are regularly arranged and display anisotropy (birefringence) when examined by polarizing microscopy. The direction of the

collagen fibers alternates from lamella to lamella, so that at any one time the anisotropy is visible only in every alternate lamella. At the periphery of each osteon, and separating it from adjacent osteons or interstitial systems, is a **cement line**. The cement lines do not calcify, have relatively little collagen, but are rich in glycoproteins and stain differently from the matrix of lamella. The antibiotic, tetracycline, if injected, is incorporated into the matrix of developing lamella and can be seen by fluorescence microscopy. If a second injection of tetracycline is injected, it is possible to measure the distance between the two fluorescent lines and to determine the rate of osteon development (about 4-5 weeks).

Each osteon comprises a single trophic unit. Each Haversian canal contains a blood vessel involved in the common nutrition of the osteon, consequently osteons represent the main morphofunctional unit of compact bone. The blood vessels of the Haversian canals are supplied with blood from vessels from the periosteum. These blood vessels penetrate the osteons in a transverse direction and are known as **Volkmann's canals**. Volkmann's canals can be identified as they do not have concentric lamella surrounding them.

Circumferential Systems

Immediately below the periosteum, at the periphery of compact bone of the diaphysis, the lamellae surround the bone in a continuous manner. These are known as the **outer circumferential lamellae**. A similar system of continuous lamellae adjacent to the endosteum is also found and is known as the **inner circumferential lamellae**. Bundles of collagen fibers, known as **Sharpey's fibers** or **perforating fibers**, anchor the periosteum to the outer circumferential lamellae, especially in sites of tendon insertions.

Interstitial Systems

Remodeling of bone is a continuous process involving resorption of osteons and the rebuilding of new osteons. Interstitial systems of compact bone represent the remnants of osteons after remodeling. They are present between regular osteons and can be identified as irregular lamellar structures that lack a central Haversian canal.

Remodelling

The resorption of osteons involves osteoclasts from the Haversian canals eroding parts of lamella leading to the formation of **resorption cavities**. These may connect with resorption cavities from adjacent osteons. When sufficient resorption has occurred, osteoblasts appear in the resorption cavity and start building a new

generation of osteons. When the new osteon is completed, the remnants of the previous osteon result in an interstitial system. This process of remodeling continues throughout life.

Trabecular bone

The spicules or trabeculae of spongy bone are also formed of lamellae, however, these are not arranged into systems as in compact bone. The trabeculae of spongy bone are not penetrated by blood vessels, but receive their nutrition via diffusion from the endosteum lining the bone marrow spaces.

Osteogenesis

There are two different types of bone formation (osteogenesis):

- **Intramembranous ossification**
- **Endochondral ossification**

In both cases the first bone tissue to be formed is primary (woven or immature) bone, which is temporary only, prior to its replacement by secondary (lamellar or mature) bone.

Intramembranous ossification involves the direct formation of bone within primitive connective tissue, whereas with endochondral ossification there is a cartilage model prior to the development of the bone.

Intramembranous ossification

Intramembranous ossification occurs during the embryonic development of many flat bones of the skull ("membrane bones") and jaw. During the initial stages of the process there is a proliferation and aggregation of **mesenchyme cells**, and simultaneously in the area one finds the development of many small blood vessels. The long processes of the mesenchyme cells are in contact with those of neighboring mesenchyme cells. The mesenchyme cells begin to synthesize and secrete fine collagen fibrils and an amorphous gel-like substance into the intercellular spaces. This is followed by the differentiation of the mesenchyme cells into **osteoblasts** (identified by their basophilia and eccentric nuclei). The osteoblasts synthesize and secrete the components of the **osteoid** (prebone) which, at a later stage, becomes calcified resulting in the development of bone spicules or trabeculae.

The process of intramembranous ossification is well seen in histological preparations of the embryonic calvaria. The newly formed bone matrix of developing trabeculae is stained acidophilic (pink) after regular staining. A layer of osteoblasts is present on the surface of the developing trabeculae, whereas osteocytes occupy lacunae in the bone matrix. Even at this early stage osteoclasts are present on the surface of the trabeculae and are active in bone resorption. Primitive blood vessels are seen in the connective tissue located between the trabeculae. At a later stage the connective tissue surrounding the developing flat bone forms the **periosteum**.

Endochondral ossification

Endochondral ossification is best illustrated in the developing long bones.

- The first stages involve the development of a **hyaline cartilage model** with surrounding **perichondrium**. A layer of woven bone (the **periosteal collar**) develops around the central shaft of the cartilage as a result of **intramembranous ossification**.

- **Primary (diaphyseal) center of ossification**.

  The chondrocytes in the developing central sha(primary center of ossification) hypertrophy (enlarge with swollen cytoplasm) and their lacunae also become enlarged. The intercellular matrix becomes calcified. As a result, there is no diffusion via tmatrix and the chondrocytes degenerate and die, leaving a network of calcified cartilage. At the same time, blood vessels and mesenchyme-like cells from the periosteum penetrate this region of the diaphysis. Osteoblasts differentiate from the mecells and begin forming primary bone tissue on the calcified cartilage framework.

- A **bone marrow cavity** forms in the developing diaphysis as a result of osteoclastic activity eroding the primary spongy bone trabeculae. The bone cavity enlarges accompanied by further vascularization. The further elongation of long bones occurs in the growth plates of the metaphysis.

- Examination of the **growth plates** reveals an orderly columnar arrangement of chondrocytes involved in the process of endochondral ossification.

  Several zones can be identified according to the arrangement and appearance of the chondrocytes:

- **resting zone** (small flattened lacunae)
- **zone of proliferation** (site of mitoses, and larger elliptical lacunae)

- **zone of hypertrophy** (greatly enlarged and rounded chrondrocytes in enlarged lacunae)
- **zone of calcification** of the matrix and degeneration of the chondrocytes
- **zone of ossification**. Osteoblasts are involved in forming bone trabeculae on the remains of the calcified cartilage.
- **primary spongiosa** (primary spongy bone) where the newly-formed trabeculae are continuously being eroded by osteoclastic activity and remodelled.

During bone elongation there is a continuous addition of new cartilage cells and subsequent endochondral ossification, accompanied by the enlargement of the diaphyseal bone marrow cavity and erosion of the primary spongy bone.

- **Secondary (epiphyseal) center of ossification**

At a later stage of development blood vessels penetrate the epiphyses accompanied by hypertrophy of the more central cartilage cells and calcification of the matrix and degeneration of the chondrocytes. Osteoblasts start building trabecular bone on the skeleton of the calcified cartilage. The trabecula are radially arranged.

- **Closure of the epiphyses**. At ages 14-17, the bone cavities of the diaphysis and epiphyses unite, with the loss of the growth plates. This closure of the epiphyses prevents the further elongation of the long bones.

Long bones have two sources of bone trabeculae: the trabeculae formed by endochondral ossification at the growth plates and the trabeculae of the diaphysis formed by intramembranous ossification. During developmental stages the trabeculae formed by endochondral ossification can be recognized by the more basophilic staining of their calcified cartilage (lacking in trabeculae formed from the diaphyseal collar).

Long bones grow in width by the addition of bone tissue by osteoblastic activity in the region of the periosteum, whereas in parallel there is erosion of bone tissue by osteoclastic activity from the inner regions of the bone. As a consequence the bone marrow cavity is enlarged.

Bone is continuously being remodelled throughout life. The bone mass is constantly changing and with aging there is a net loss of bone and the quality of bone becomes impaired. Osteoporosis is common in the elderly.

Bone fracture and repair

Although bone is hard, it is also brittle and liable to fracture. The fracture is accompanied by hemorrhage. Cells of the periosteum and endosteum respond to the injury. There is a rapid proliferation of fibroblasts, which are involved in the formation of cartilage and fibrocartilage (**fibrocartilaginous callus**) that fills the injured gap. On the basis of the fibrocartilaginous callus, osteoclasts begin forming bone matrix, resulting in a **bony callus** of primary (woven, immature bone). Subsequently the primary bone is remodelled into a secondary (lamellar) bone.

Synovial joints

Bones are connected to each other by joints. The most common joint type is the **diarthrosis** of articulating joints, which has a fibrous connective tissue capsule (**ligament**), continuous with the periosteum of the two bones and which permits a degree of freedom of movement between the two bones. The inner part of the capsule consists of the **synovial membrane**, which may extend as a fold (**synovial fold**). The synovial membrane is well vascularised with both blood and lymph vessels. The main cell type present in the synovial membrane, are fibroblast-like cells, and involved in the formation of the **synovial fluid**. This fluid is rich in **hyaluronic acid** and fills the joint cavity. Macrophage-like cells are also found in the synovial membrane and are responsible for keeping the synovial fluid clean and free of cell fragments. The synovial fluid plays an important role in the lubrication of the joint and in providing nutrition for the articular cartilage of the epiphyses. **Fat deposits** or pads, found between the synovial membrane and the ligament, function as mechanical shock absorbers.

Aging changes to joints, in particular pathological changes of the articular cartilage (**osteoarthritis**), are very common in the elderly.

Physiology of bone

Most of the calcium stored in the body is in bone tissue and can be released to the blood according to physiological demands or alternatively can be used to produce new bone. Calcium levels in the extracellular fluid of the body are very closely regulated. Three hormones, in particular, are involved in **calcium homeostasis**:

- **Parathyroid hormone**
- **Calcitonin**
- **Vitamin D3**

The main organ systems involved in calcium homeostasis are the bony skeleton, the kidney and the intestine.

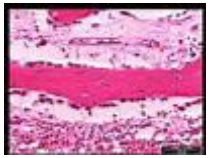
Parathyroid hormone is involved in increasing blood calcium levels by stimulating osteoclastic activity and bone resorption. **Calcitonin** has an opposite effect and is involved in reducing blood calcium levels. Calcitonin encourages bone tissue formation and can be used in clinical treatment of osteoporosis. The active metabolites of **Vitamin D3** are involved in particular in stimulating dietary calcium absorption through the small intestine. Lack of vitamin D_3 can result in improper calcification of bone tissue and the development of **rickets**.

Hyaline Cartilage - Trachea

Elastic Cartilage - External ear

Elastic Cartilage

Intramembranous Ossification



Intramembranous Ossification

Osteoclasts

Endochondral Ossification

Endochondral Ossification

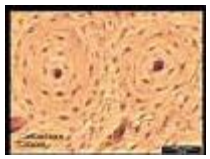
Endochondral Ossification

Endochondral Ossification

Growth Plate

Synovial Joint

Compact bone



Compact bone

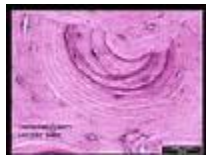
Compact bone

Lamellar bone

Lamellar bone

Lamellar bone

Interstitial System



Interstitial System

Flat bone

Intervertebral disk

Intervertebral disk

Compact Bone

Trabecular bone lamellae

INTRODUCTION TO ORGANOLOGY

Organology means the study of organs. It is the knowledge of how organs are packaged or the **"basic architectural plan of organs"**. All organs of the body are formed by a combination of one or more of the 4 basic tissues.
Organs are characterized by specific cells but apart from this cellular characteristic, amount, distribution and types of the cells and of the various tissues all help to typify the organ.
For purposes of efficient discussion, it is important to become familiar with certain terms used to classify and categorize components of tissues and organs.
The term **PARENCHYMA** is used to denote collectively the cells of the organ which carry out the main function of the organ.
The terms **INTERSTITIAL TISSUE** or **STROMA** are used to denote the supporting tissues in an organ. LOOSE AREOLAR connective tissue (C.T) is by far the most prevalent stromal tissue.
TERMINOLOGIES: The following terms need be noted:

Hypertrophy: increase in cell size
Hypotrophy: decrease in cell size
Hyperplaisia: increase in cell number
Hypoplaisia: decrease in cell number
Trabeculae, Septa, Muralia are all descriptive terms for structures which help to compartmentalize parenchmatous organs. They are composed of C.T cells, fibres, membranes and at times bone.
Follicles is like a bag, normally without an outlet, lined by epithelium. This time, the epithelium is peripheral.
Anastomoses are communicating links between two hollow passages.
Plexus (Plexi) (e.g. brachial plexus) is a network of tubes or fibre bundles.
Organs can be subdivided into **lobes**. Lobes are well visible, relatively large subdivisions of an organ. **Lobules are further subdivisions.**

TUBULAR ORGANS

Most tubular organs consist of 4 concentric layers called Tunics. These are from lumen to outside:

Tunica mucosa,
Tunica Submucosa,
Tunica muscularis
Tunica Serosa (adventitia)

All may be present. One or more may be reduced or absent or modified to meet specific local needs.
These variations together with other factors permit identification of an organ.
The tunica mucosa is the innermost or luminal coat and has 4 layers:

- lamina epithelialis (epithelium)
- lamina membrana propria (b.m)
- lamina propria mucosae
- lamina muscularis mucosae

The lamina epithelialis is the epithelial layer, it consists of one or more types of epithelial cells depending on function. It is a constant lamina of the tunica mucosa. You cannot speak of mucosa without mentioning epithelium.
The lamina propria – the basement membrane serves as a constant separation between CT and epithelial tissues.
Lamina propria mucosae – This is the CT which underlies the epithelial layer. It is usually Areolar/Reticular C.T. Small vessels, nerves and in-folding of the epithelia may be found here (e.g skin and sweat glands). This area may contain large number of protective cells

either free or as lymph nodules. Apart from defensive function, this layer is the means by which the epithelium is nourished and controlled.

The Lamina muscularis mucosa is one or more layers of smooth muscle cells. An inner circular and an outer longitudinal layer may be present. Its presence is of variable occurrence, but when present, it serves as a means whereby local mobility is achieved. It also serves to express secretory products from the glands which may invaginate into the lamina propria mucosa. It serves as a line of demarcation between mucosa and sub mucosa. When absent, mucosa and sub-mucosa blend insensibly.

The tunica sub-mucosa – consists of areolar C.T which is more coarsely arranged than that of the lamina propria mucosae. Larger blood vessels, nerves, nerve plexi and autonomic ganglia are present. In some organs glands may be present too. In the absence of a lamina muscularis mucosa the lamina propria mucosa and tunica submucosa are usually referred to simply as lamina propria mucosa or lamina propria submucosa.

The tunica muscularis – is usually well developed and consists of 2 layers of muscle. In some organs, however, it may be absent. It usually consists of smooth muscle but in some cases, skeletal muscle may be present. It is commonly arranged into inner circular and outer longitudinal fibers. Vessels and nerves, autonomic ganglia, usually separate the two layers. This tunic is responsible for the tone of the organ, size of lumen and movement of materials through the hollow organ.

The tunica Adventitia- is a collection of loose C.T over the periphery of an organ. Blood vessels, nerves, ganglia and adisposa may occur in this tunic. Organs that are intimately associated with the coelomic cavities are surrounded by a layer of mesothelium in which case the most peripheral unit is called tunica serosa. It is composed therefore of mesothelium and C.T. It is through the adventitia or serosa that nerves, blood vessels, lymphatics gain access to the organ. Also this tunic is responsible for suspending the organ within its environment either in the coelum or in the surrounding C.T. In this case it is called **mesenteries.**

Although there are numerous variations, this arrangement is basic scheme for tubular organs.

In blood vessels and lymph vessels the following terminologies are used

Tunica intima,
Tunica media,
Tunica adventitia.

MUCOUS AND SEROUS MEMBRANES

Mucous membranes include some or all of the components of the tunica mucosa. These membranes are kept moist by secretions from cells within the lamina epithelialis and or from glands located within the lamina propria mucosae. The epithelia may consist of stratified squamous as in esophagus, columnar or cuboidal as in G.I.T. columnar or pseudostratified columnar as in respiratory epithelia. Those lined by transitional epithelium occur in the urinary system.

Serous membranes consist of layer of mesothelium and associated C.T. They line the coelomic spaces and are moistened by fluids contained within these spaces.

PARENCHYMATOUS ORGANS

These are also composed of one or more of the basic tissues. The organizational pattern is different from that described for tubular or hollow organs.

The components of solid organs may be divided into two different subgroups.

The parenchyma – the specific functional component of an organ.

The stroma – this includes those tissues (C.T, vasculature nerves, lymphatics) that metabolically and or structurally support as well as control the parenchyma.

In simple form, the following scheme is applicable to these organs whether they are muscle, nerve trunks or glands.

Small groups of parenchyma are surrounded by a fine meshwork of areolar or reticular C.T in which vessels and nerves are located. Small groups of parenchyma may be grouped as a unit and surrounded by a more coarse areolar C.T or the C.T of these small groups

may be continuous with coarse areolar C.T trabeculae. In either case, the C.T is progressively more dense and is continuous with the dense white fibrous CT of the capsule. This type of C.T. continuity affords structural support and facilitates the entry and or exit of vessels and nerves. This scheme or minor variations of it typify the organization of solid organs.

PRACTICALS

(i) Examine slides 97, 98, 99 for the different tissues of tubular organ.
(ii) Examine slide 112 for L.S of tubular organ noting what is observable from lumen outwards.
(iii) Examine slide 113 (Peyers patch) for protective cells that can be found in sub mucosa.
(iv) Examine slide 101 as related in your handout
(v) Examine slide 141 (bladder) and compare with slide 98.

BLOOD VASCULAR SYSTEM

The blood and lymphatic vascular systems are classified as specialized connective tissue.

The **main functions** of the blood are to transport oxygen, nutrients and hormones to the tissues and to collect the waste products (carbon dioxide and waste metabolites) for removal from the body via the excretory system.

The cardiovascular system consists of the:

- **Heart** (muscular pump)
- **Pulmonary circulation** (system of blood vessels to and from the lungs)
- **Systemic circulation** (system of blood vessels bringing blood to and from all the other organs of the body).

Arteries are classified into two main groups:

- **Conducting** (**Elastic Arteries)**.

These are large arteries closest to the heart (aorta, renal artery) with very high blood pressure and flow (320mm/sec in the aorta).

- **Distributing** (**Muscular Arteries)**.

These are smaller diameter arteries with a slower blood flow.

The arteries lead to smaller vessels, the **arterioles**, which lead to the **capillaries**. The capillaries are present in the form of microcirculation networks (**capillary beds**) in the organs and tissues. Exchange of metabolites and transport through the vessel wall is only possible in the capillaries, as only here the blood flow is sufficiently reduced (about 0.3mm/sec) and the vessel wall sufficiently thin.

On the return route to the heart the blood flows in **venules**, **small veins** and **large veins**.

Arterial blood in the Systemic Circulation is richly oxygenated, whereas the venous blood has little oxygen. In the Pulmonary Circulation the arterial blood is poorly oxygenated, whereas the venous blood, are highly oxygenated (having replenished its oxygen supplies in the lungs).

Endothelial cells

The endothelial cells are derived from embryonic mesenchyme and should not be regarded as epithelial, but as connective tissue cells. Endothelial cells line the lumina of all the vessels of the blood vascular and lymphatic vascular systems. Endothelial cells lining the blood vessels are very flattened, elongated cells, with elongated nuclei that protrude into the lumina. The total number of endothelial cells in the body is enormous (estimated as $6x10^{23}$ cells) and cover a very large surface area (700-1000m^2) and in total weigh about 1.5kg.

Blood capillaries

Blood capillaries have a diameter of about 7-9□m, which is close to the dimensions of erythrocytes (about 7.2□m). The diameter of the capillaries varies according to the functional status of the tissue or organ. When functional demands rise the diameter of the capillaries enlarges, allowing increased exchange of oxygen and metabolites.

There are three different types of capillaries, however the differences are only visible at the ultrastructural level (by light microscopy these differences are not detectable) :

- **Continuous capillaries**
- **Fenestrated capillaries**
- **Sinusoids**

Continuous capillaries

These have **continuous endothelial cells** located on a **continuous basal lamina**. In cross sections they are seen to be composed of 2-3 endothelial cells, connected by **tight junctions**. In the region of the contact between the ends of two endothelial cells there is a **marginal fold**, a small area in which the edge of one of the cells protrudes into the lumen. Continuous capillaries are characterized by abundant small invaginations of the cell surfaces (**caveolae**) and numerous **micropinocytotic vesicles** in the cytoplasm. All the materials crossing the cell (**transcellular transport**), in both directions do so via these micropinocytotic vesicles.

Continuous capillaries are found in those organs that need strict control on access of the substances from the blood. These include all the organs with a "blood-barrier" such as the "blood-brain-barrier" of the Central Nervous System or the "blood-thymus barrier".

Fenestrated capillaries

These possess endothelial cells with groups of very small "**pores**" or "**fenestrae**", about 80-100nm diameter. These are seen in transmission electron micrographs and in particular after freeze-etching techniques. Fenestrated capillaries are common in most of the endocrine glands. One prominent site for fenestrated capillaries is in the renal glomeruli. The fenestrated capillaries lie on a continuous basal lamina.

Sinusoids

Sinusoids are irregular vessels with large diameters (30-40nm). In most cases the sinusoids are not cylindrical. Sinusoids are found in the liver, endocrine glands and in the hematopoietic organs (bone marrow, spleen). In many cases the sinusoids are also fenestrated. This is the case in those organs which need a very rich blood supply including most of the endocrine glands (hypophysis, suprarenal cortex, pancreas). Phagocytes are commonly associated with the walls of the sinusoids.

The exchange of materials through capillary walls can be:

transcellular via :

- micropinocytotic vesicles in the endothelium (as in continuous capillaries)
- fenestrations (as in fenestrated endothelium or sinusoids)

or **intercellular** via :

- gap junctions
- spaces between endothelial cells (as in sinusoids of spleen, liver).

Pericytes (Perivascular cells)

Many capillaries have inconspicuous, elongated cells, similar in appearance to embryonic mesenchymal cells, associated with them. These cells, known as **pericytes**, or **perivascular cells**, are quite difficult to see in most histological preparations. These pericytes appear to have important roles in repair of blood vessels and connective tissue after injury. They have the potential to develop into fibroblasts, smooth musccells and may even be phagocytic.

Endothelial cells are known to produce a variety of local factors that are important in the functioning of the cardiovasystem. These include nitric oxide.

Morphology of muscular (distributing) arteries

These vessels bring blood from the heart to the tissues in a high-pressure, fast-flow, system and consequently the arterial wall needs to be able to withstand the biomechanical stresses.

The arterial wall is composed of three main layers or tunics.

- ***Tunica intima*** (internal tunic) consisting of :

    - **endothelium** (single lining layer of endothelial cells)
    - **sub-endothelial layer**
    - **inner elastic limiting membrane** (elastic lamina, which after fixation appears undulating).

- ***Tunica media*** (middle tunic) consisting of :

    - **circular smooth muscle** (or spiral)
    - **concentric elastic lamina** (formed by the smooth muscle cells).

- ***Adventitia*** (outer layer) composed of :

    - **connective tissue** surrounding the vessel
    - **outer elastic limiting membrane** (on the border between the *Tunica media* and the *Adventitia*
    - ***Vasa vasorum***. These are small blood vessels supplying oxygen and nutrients to the wall of the artery. The blood flow in the arterial lumen is too great for exchange of oxygen or nutrients.

Morphology of Elastic Arteries

These are arteries closest to the heart and need to withstand stresses of extremely high blood flow. Their structure is best seen in the aorta. They are called elastic arteries as their Tunica media possesses 50-75 **well-developed elastic lamina** in between the thick smooth muscle bands. These have a similar appearance to the inner elastic limiting membrane. The elastic lamina prevent the excessive expansion of the vessel diameter and when they spring back they push the blood onwards i.e. they provide a shock-absorber effect permitting a more

continuous blood flow despite the intermittent action of the heart. The elastic lamina absorb the intermittent impact of the cardiac pulse. During diastole (inactive heart), the large elastic arteries return to normal size impelling the blood forward. This contributes to a more constant arterial pressure and blood flow. The function of the elastic arteries can be considered as an auxiliary pump, when no forward pressure is exerted by the heart.

Smooth muscle in arteries

The smooth muscle in arteries is important in maintaining the vessel diameter during blood flow and also plays a role in blood pressure levels. The vascular smooth muscle is in a state of **tonus** (partial contraction). The degree of tonus of the smooth muscle cells in the wall of arteries and arterioles is controlled by the autonomic nervous system and also by endocrine secretions. In **hypertension** (high blood pressure), often associated with stress or aging, the peripheral arterial vessels show increased tonus.

Arterioles

Arterioles are small vessels with a diameter of 0.5mm or less.
They consist of three basic layers:

- ***Tunica intima*** with endothelium alone (no subendothelial layer) and a very thin inner elastic limiting membrane
- ***Tunica media*** with only 4-5 layers of smooth muscle
- ***Adventitia*** that is fairly thin

Metarterioles

These are small vessels that are on the border between arterioles and the capillary bed. They can act as sphincters and cut off the flow of blood into the capillary bed.

Arteriovenous anastomoses

These represent direct connections between arterioles and venules. When there is no need for blood flow in the capillary bed these permit direct blood passage (arterial-venous-shunt). Arteriovenous anastomoses are very common in the dermis of the skin.

Anatomical and Functional End Arteries

Anatomical end arteries are vessels whose terminal branches do not anastomose. In the event that these vessels become blocked (atherosclerosis, blood clot) the tissues will be deprived of oxygen and an "infarct" develops (e.g. coronary arteries, kidneys, brain).

Functional end arteries have anastomoses and in the event of blockage, alternative routes for blood and oxygen are available.

Arterial pathology has major clinical importance in medicine. Common arterial disorders include: atherosclerosis (fatty deposits and occlusion), arteriosclerosis (hardening of the arteries), hypertension, aneurysms (ballooning of the vessel). Cardiac infarct and cerebral infarct resulting from occlusion of the lumen of arteries are major causes of morbidity.

Veins

The veins constitute a **low-pressure system** of vessels. The return of blood to the heart from the capillary beds of the tissues follows a route of **small venules**, **small veins** and **large veins**. As the venous vessels near the heart they become large and with thicker walls. The route of the veins is in parallel to that of the arteries.

Characteristics of veins:

- more numerous than arteries
- diameter of vessels is larger than that of adjacent arteries
- walls of veins are thinner and less elastic or distensible than arteries. (As a result in histological preparations the lumen often appears collapsed or irregular)
- the relative numbers of *vasa vasorum* are greater in the veins (necessary as the vessels have much less oxygenated blood)
- valves are found in veins.

Veins are classified as large, medium or small veins.

Veins have three layers (***Tunica intima***, ***Tunica media*** and ***Adventitia***), however the borders between these layers are much less distinct than in arteries.

In veins the smooth muscles of the *T. media* are all **circular muscles**, grouped in bundles, whereas muscles present in the adventitia are **longitudinal**.

The movement of blood in veins is passive. The muscles play a role in tonus. Most of the muscles in veins are present in the adventitia and are longitudinal. **Valves** are present in veins, especially in those that transport blood against the force of gravity, such as in the legs. The valves are composed of folds of the *Tunica intima* (endothelium and connective tissue). The valves prevent the backflow of blood. Weakness in the walls of veins can result in **varicose veins** and improper closure of the valves.

Venules

These have a very small diameter (20-50 m). In total preparations such as from the mesentery, the venule wall is so thin that the erythrocytes are visible, whereas they are not seen in the adjacent and parallel arterioles.

Post-capillary venules

In lymphatic organs, such as the lymph nodes, the post-capillary venules have high or cuboidal endothelium. These specialized venules permit the recirculation of lymphocytes (especially T-lymphocytes) from the blood to the lymph.

Umbilical blood vessels

The umbilical blood vessels are atypical. The single vein is unusual in that it is very muscular. The paired umbilical arteries are also unusual. The *T. intima* is composed only of endothelium. The *T. media* is composed of two muscle layers: an inner longitudinal layer and an outer circular layer. Elastic fibers are present throughout the media.

Blood portal systems

In typical configurations an artery or arteriole carrying oxygenated blood enters the capillary bed, where there is exchange of oxygen and metabolites, and the vessel exiting the capillary bed is a venule or vein with deoxygenated blood.

Portal systems describe situations where the blood vessel leaving the capillary bed is of the same category as the blood vessel entering the capillary bed. (vein - capillary bed - vein or artery - capillary bed - artery).

In a **venous portal system** (such as in the liver) a vein (hepatic portal vein) enters the capillary bed and a vein (hepatic vein) exits the capillary bed. A similar portal system is found in the hypothalmus-hypophysis.

An example of an **arterial portal system** is found in the renal cortex. Afferent arterioles break up into the capillary bed of the glomerular tufts of the renal corpuscle and the blood exits in efferent arterioles.

LYMPH VASCULAR SYSTEM

Functions of the Lymph Vascular System

- The lymph vessels return to the blood extracellular fluid from connective tissue spaces. This system ensures the return of water, electrolytes and plasma proteins to the blood.
- The lymph vascular system plays a kerole in homeostasis of the volume of extracellular fluid.
- The lymph vascular system also returns lymphocytes from the lymph nodes to the blood.
- The system also transports immunoglobulins (antibodies) from the lymphnodes to the blood.

Lymph capillaries

Lymph is a fairly clear, transparent fluid that flows (passively) in small lymph capillaries. These are found in most organs close to blood capillaries (an exception is the CNS). The lymph capillaries begin as small blind-ending tubes.

- Typical lymph capillaries have a diameter of 10-50 m only. Lymph vessels have very thin walls. The wall of the lymph capillary is composed of a single layer of endothelium (about 0.3 m thick). Lymph vessels are hard to detect in histological preparations as the lumen tends to collapse.
- Lymph capillaries (unlike endothelial cells of blood vessels) lack a basal lamina.
- No pericytes or adventitial cells afound.
- They lack marginal folds (as seen in ultrastructure of blood capillaries).
- The lumen is usually free of cells (in comparison with blood capillaries where erythrocytes and other blood cells are common).

Movement of lymph in the lymph vessels is entirely passive. Valves, constructed from endothelial cells, prevent backflow of lymph. The lymph capillaries eventually drain into larger lymph vessels, with large lumina and thin walls and these ultimately drain into two large lymphatic ducts in the area at the base of the neck (**thoracic duct**, **right lymphatic duct**). These vessels return the lymph to the blood.

Lymph vessels drain into lymph nodes (each node has several afferent vessels, but only a single efferent vessel) and in passing through the node the lymph is filtered.

HISTOLOGY OF THE INTEGUMENT

- the skin and all of its derivatives

Components

- skin (epidermis, dermis, hypodermis)
- derivatives (sweat glands, sebaceous glands, mammary glands, hair, nails, claws,hooves, horns, antlers, combs, wattles, and feathers)

Functions

- protection - from drying out, from invasion by microorganisms, from UV light and from insults (mechanical, chemical or thermal)
- sensation - for touch, pressure, pain and temperature
- thermoregulation - decreases heat loss in cold temperatures; increases heat loss in hot temperatures
- metabolic functions - energy stored in fat deposits; synthesis of vitamin D

Structure of the Skin

- Three distinct layers can be seen in the skin:
  - Epidermis - consists of keratinizing stratified squamous epithelium
  - Dermis - consists of fibroelastic connective tissue
  - Hypodermis - consists mostly of white adipose tissue (sometimes referred to as the subcutis)

Epidermis

- **Layers of the Epidermis**
  - in order from outermost (surface) to innermost (deepest):
- **Stratum corneum** - consists of the remains of keratinocytes; mostly composed of the protein, keratin
- **Stratum lucidum** - present only in very thick skin; pale-staining layer of cells between the stratum corneum and stratum granulosum in which the dying keratinocytes contain a lot of keratin but are not completely replaced by it
- **Stratum granulosum** - consists of keratinocytes containing large numbers of granules that contribute to the process of keratinization
- • **Stratum spinosum**- consists of large, polyhedral keratinocytes that are actively
- synthesizing keratin which is inserted as tonofibrils into the area of the plasma membrane
- beneath desmosomes that connect adjacent cells together. These "connections" or
- desmosomes between cells in this layer help hold them together and result in the "spiny"
- appearance of the cells that gives this layer its name.
- • **Stratum basale** - consists of keratinocytes undergoing mitosis to produce the constant
- supply of keratinocytes needed for replacement of the dead and dying cells in the more
- superficial layers of the epidermis

Dermis

- **Two zones of the dermis:**
- • **papillary zone** - consists of loose areolar connective tissue containing collagen and fine
- elastic fibers; connects the epidermis to the thicker and denser reticular zone of the
- dermis
- • **reticular zone** - contains dense, irregular and coarse collagen fibers and thick elastic
- fibers interspersed with fibroblasts and blood vessels and nerves

Glands in the skin

- Several different types of glands are located in the dermis of the skin serving a variety of
- functions.
- • Sebaceous glands
- • Apocrine sweat glands
- • Merocrine (= eccrine) sweat glands

Sebaceous Glands

- The epithelium of this gland is an outgrowth of the external root sheath of
- the hair follicle and the gland empties its oily product directly into the follicle itself. The glands
- are of a branched acinar type and produce a lipid product called sebum that serves to reduce the
- entry of microorganisms into the body through the skin, to lubricate the hair and preventing it
- from drying out. The secretory cells die and become part of the product; a holocrine mode of
- secretion. These glands are not found in hooves, foot pads, claws or horns.

Apocrine Sweat Glands

- These glands are coiled, tubular glands with a large lumen and a duct
- connecting it to an adjacent hair follicle. These glands secrete a viscid, milky product and are
- analogous to odiferous glands of many mammals. Once thought to use the apocrine mode of
- secretion, it is now known that their mode of secretion is more like that of the merocrine sweat
- glands. These glands are the primary sweat gland of domestic animals and are especially
- prominent in the horse.

Merocrine Sweat Glands

- These glands are unbranched tubular in form and appear as a mass
- of tubules in cross section. They are plentiful in the upper regions of the fatty hypodermis. They
- secrete a watery product that is hypotonic to the plasma. It is the evaporation of this secretion on
- the surface of the skin that aids in thermoregulation. These are sometimes called eccrine sweat glands.

Hair

- **General structure of hair and associated structures:**
- **Hair shaft:** the part of the hair above the surface of the skin
- **Hair root:** the part of the hair below the surface
- **Bulb:** an enlarged, hollow portion at the base of the root
- **Hair papilla:** projection of dermis into center of the bulb
- **Follicle:** the indentation in the skin within which the root lies

Layers of Hair

- **Cuticle:** the outermost layer. Single layer of flattened, keratinized cells. Overlap like shingles, with free edge distally.
- **Cortex:** the thickest, intermediate layer.
  Consists of several layers of keratinized cells containing hard keratin.
- If hair is colored, these cells contain pigment.
  Cells held together by desmosomes.
- **Medulla:** central core; loosely packed cuboidal cells. The structure and organization of the cuticle and medulla cells are species-specific.

Hair follicles

- The hair follicle is the structure that anchors the hair in the dermis and produces the hair itself. It
- is composed of **five layers** of epithelial cells arranged concentrically
- The inner three layers form the hair shaft through a process of keratinization while the outer two layers

  form the hair sheath.
- cells in the innermost layer form the **medulla** of the hair or core of the hair shaft
- cells in the next layer form the **cortex** that makes up most of the hair
- cells in the third layer form the **cuticle** on the surface of the hair

- 4. cells in the fourth layer make up the

  internal root sheath

- 5. cells in the fifth or outermost layer form a layer called the **external root sheath** that does
- not take part in hair formation

- The external root sheath is separated from the surrounding connective tissue by a thick

- basement membrane known as the glassy membrane.

Types of follicles

Hair follicles can be classified in two ways: based on their size, i.e., diameter and based on their organization.

- **Based on size (diameter):**

- **Primary hair follicle:** large having sweat gland, sebaceous gland and arrector pili muscle; ex. overcoat or guard hairs in dogs

- **Secondary hair follicle:** smaller, lacking sweat glands and arrector pili muscle; ex. underhair

- **Based on Organization:**
- **Simple follicle:** a single hair from one follicle
- **Compound follicle:** cluster of several follicles with several hairs emerging from one opening onto surface of skin

The Hoof

- The equine foot includes the hoof, dermis, first, second and third phalanges and associated
- structures. The hoof itself is the cornified layer of the epidermis, lacking the stratum granulosum
- and stratum lucidum. It is important to understand the histology of the hoof because a disease
- involving the epithelium of the foot, called *laminitis,* is the most devastating clinical disease of the foot.

- The peculiar histology of the hoof is formed from special relationships between the dermis (or corium) and the overlying epidermis.

- In some places, the dermal papillae and epidermal pegs are

- confluent forming apparent layers, i.e., they are *laminar* or consist of *lamellae*; in other places they are more typical.

- It is this lamellar interaction between the epidermis and dermis that gives the hoof its strength.

- The wall of the hoof is that part of the hoof
  which is visible when the foot is on the ground, and
- it can be divided into three layers. From outside to inside, they are the *stratum externum*
- (tectorium), the *stratum medium*, and the
  stratum internum (lamellatum).

The Wall of the Hoof

- The *stratum externum or tectorium* is an extension of the perioplic epidermis and is composed of cornified epithelial cells which appear as a soft, white, shiny material. This tissue attaches the hoof to the epidermis of the skin of the foot.
- The *stratum medium* or coronary epidermis is composed of prominent tubular and intertubular horn, and this layer comprises the bulk of the wall of the hoof.
- The *stratum internum* or *stratum lamellatum* is the epidermis in the laminar region.

Stratum Lamellum

- This layer is made of nontubular horn which fuses with the stratum medium and helps hold the
- wall to the foot. In this region, the dermal papillae and epidermal pegs form elongated ridges
- oriented perpendicular to the ground. These ridges are formed from primary and secondary
- laminae - the secondary laminae being oriented at close to a right angle to the primary laminae.

- There are about 600 primary laminae and about 100-200 secondary laminae for each primary lamina.
- This system of interdigitating primary and secondary laminae provides the tight bond between the wall of the hoof and the underlying dermis.
- Thus, damage to the laminae leads to disruption of this interdigitating system which results in separation of the hoof wall from the dermis and phalanx beneath it.
- Laminitis (acute laminar degeneration) is an inflammation of the laminae within the hoof.
- Many pathophysiologic mechanisms are thought to cause laminitis, among them vasoconstriction within the digit, perivascular edema, arteriovenous shunting of blood at the level of the coronary band, venoconstriction and microthrombosis.
- These lead to less than normal perfusion of blood to the digit resulting in ischemia, edema and eventually necrosis of the laminae.